Research Unit
Self-assessment document

EVALUATION CAMPAIGN 2019-2020
GROUP A

GENERAL INFORMATION

Name of the unit concerned by the current contract: Biochimie et Physiologie Moléculaire des Plantes	
Name of the unit concerned by the next contract (if different):	
Acronym of the current contract:	**BPMP**
Acronym of the next contract (if different):	
Scientific field (name two fields if interdisciplinary evaluation):	**SVE**
Scientific sub-domains (in Hcéres' nomenclature) in descending order of importance:	**SVE1, SVE2**
Director for the current contract:	**Alain GOJON**
Director (or project leader) for the next contract:	**Christophe MAUREL**

Type of application:

Identical renewal ☒ Fusion, scission, restructuring ☐ *Ex nihilo* creation[1] ☐

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[1] Units created *ex nihilo* will be evaluated based on a project.

Academic institutions and affiliated organisms:

List of Institutions and Organisms supervising the Research Unit **for the current and next contract**:

Current contract:	Next contract:
- CNRS	- CNRS
- INRA	- INRA
- Univ Montpellier	- Univ Montpellier
- Montpellier SupAgro	- Montpellier SupAgro

Choice of the research unit's interdisciplinary evaluation (or of one or more in-house teams):

Yes ☐ No ☒

Clinical research activities:

Yes ☐ No ☒

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RESULTS

1- Presentation of the unit

Introduction

History, localisation of the unit
Structure of the unit (teams or themes)

The UMR "Biochimie et Physiologie Moléculaire des Plantes" (BPMP, *https://www1.montpellier.inra.fr/wp-inra/bpmp/*) is located on the INRA/Montpellier SupAgro Campus ("La Gaillarde") and gathers from 110 to 150 people (127 on 01/06/2019), including permanent staff from four institutions (CNRS, INRA, Montpellier SupAgro and University Montpellier), and students, post-docs and non-permanent technical assistants from various origins (14 different nationalities on 01/06/2019).

BPMP directly originates from the "Laboratoire de Biochimie et Physiologie Végétales" (BPV), founded in 1971 by Prof. Louis Salsac on the Campus La Gaillarde, with the physiological and biophysical aspects of mineral nutrition in higher plants as its main scientific focus. In the following years, BPV was associated with the four institutions to which BPMP now belongs, and from 1981 was directed by Prof. Claude Grignon. The current UMR BPMP was created in 1999. This was simply a modification of the name of the unit, without any change in staff or in scientific activities. BPMP was successively directed by Prof. Claude Grignon (until 2006), Dr. Jean-François Briat (2006-2012) and Dr. Alain Gojon (2012-).

BPMP is constituted of 11 research teams created between 1999 and 2019 (https://www1.montpellier.inra.fr/wp-inra/bpmp/en/research/the-teams/), one administrative team, one logistics team, and several technological platforms or common facilities (see the organization chart below). All teams, platforms and facilities are located in a single building (named "Institut de Biologie Intégrative des Plantes"), shared since 2005 with the UMR LEPSE ("Laboratoire d'Etude des Plantes sous Stress Environnementaux") with which BPMP has long standing collaborations. BPMP has another minor location in the Faculté des Sciences (Campus Triolet, University of Montpellier), which corresponds to the rooms used by the University staff of BPMP in connection with their teaching activities.

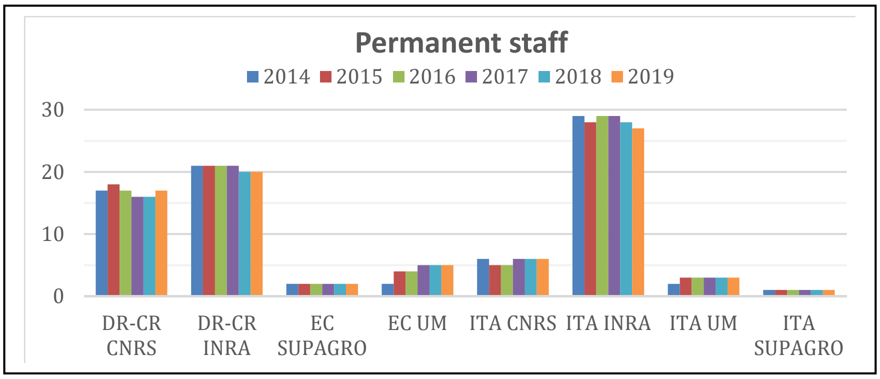
A key characteristic of BPMP is that it has kept over nearly 50 years a strong common focus on the mechanisms involved in the water and mineral nutrition of plants, and in the response of plants to abiotic factors.

Unit's workforce and means

Unit's Workforce

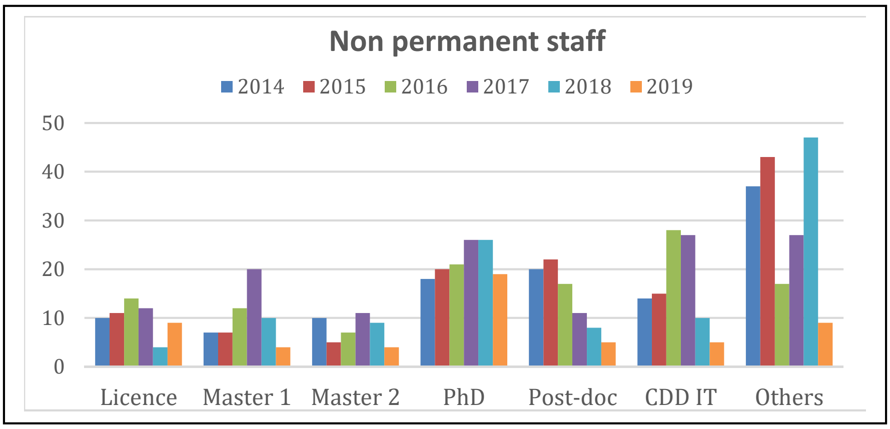
Permanent and non-permanent staff

Between 2014 and 2019, BPMP's workforce relied on around 80 permanent staff (50% scientists, 50% technical and administrative assistants), and a variable number of non-permanent staff (between 30 and 70 are present at any time, depending on the period of the year). The total number of permanent staff remained stable during the 2014-2019 period (see graph below), with the exception of the University staff, which increased from 4 to 8, as a consequence of our marked efforts to strengthen our links with the University (see section below).



In more details, 13 permanent staff left the unit during the current contract (6 retirements and 7 outgoing mobilities), and these departures were more than fully compensated by the arrival of 15 newcomers (4 recruitments and 11 incoming mobilities).

More pronounced changes occurred among the non-permanent staff. In particular, the number of post-docs and technical assistants has markedly decreased since 2015 or 2016, due to a decrease of ANR projects funded in the unit. The number of graduate students increased, whereas no real significant trend was noticed for the other categories. **During 2014-2019, BPMP hosted 446 non-permanent staff in total**, among which 67 visiting scientists and students, 37 post-docs, 46 graduate students, 46 non-permanent technical assistants, and 250 undergraduate students. All these numbers are higher than during the previous 2008-2013 reporting period. This is especially true for Master students (96 in 2014-2019, as compared to 53 in 2008-2013).



General organization

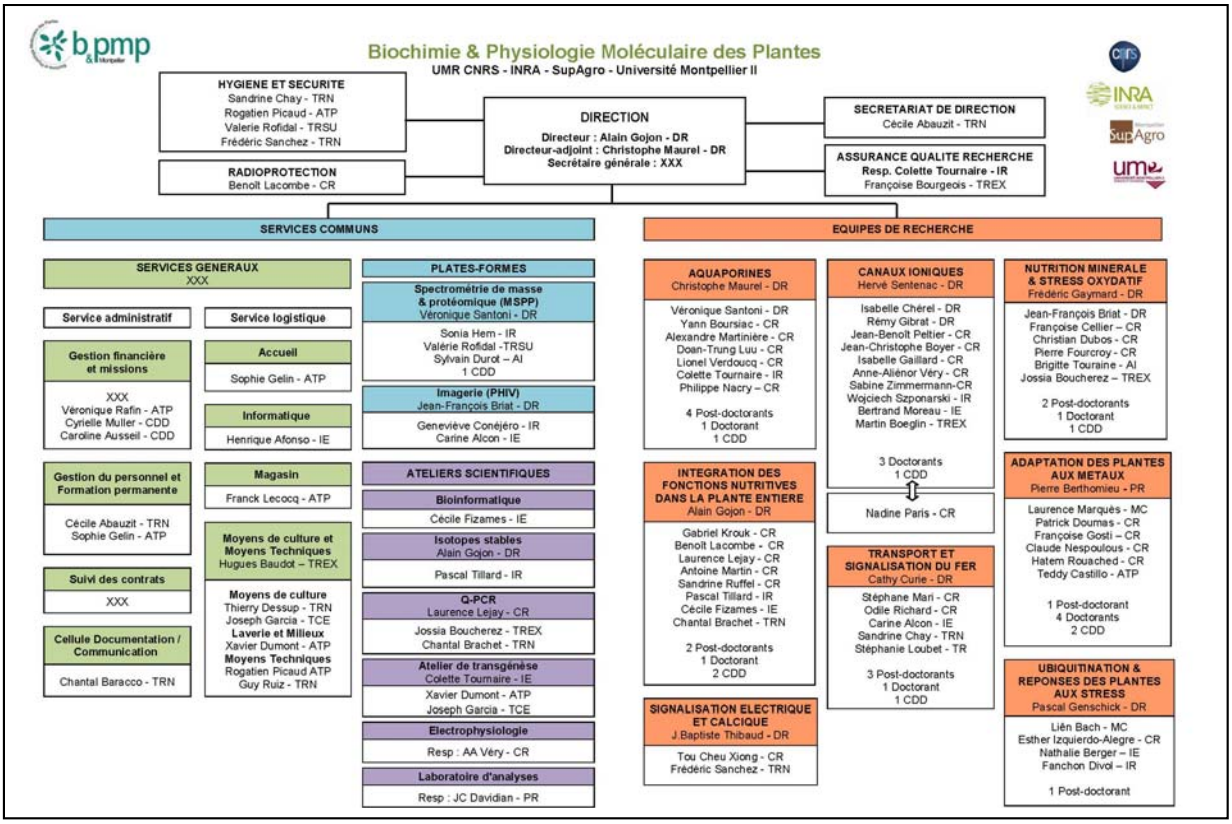
Whereas BPMP shows a well-identified and focused research topic, its scientific organization relies on individualized research groups having both a significant size and strong scientific and financial autonomy. In our view, this organization provides the best compromise between the needs for a collective efficiency and international visibility, and a recognized creativity and autonomy of scientists. On one hand, the significant size of the groups improves their credibility, allows ambitious strategies, and partly solves transient funding problems of individual researchers. On the other hand, the autonomy of the groups permits the development of original and innovative projects. One consequence of this organization is that BPMP has no formal transversal scientific structure. Common themes or joint projects are encouraged between teams, but are not mandatory, as they may limit the freedom of these teams.

Another key characteristic of the scientific organization of BPMP is that it is not determined by the institutional affiliation of the staff (CNRS, INRA, SupAgro, University). All groups mix people from several of these institutions, making BPMP a true "Unité Mixte de Recherche" (Joint Research Unit) where the scientific objectives of each group are examined and validated by all institutions.

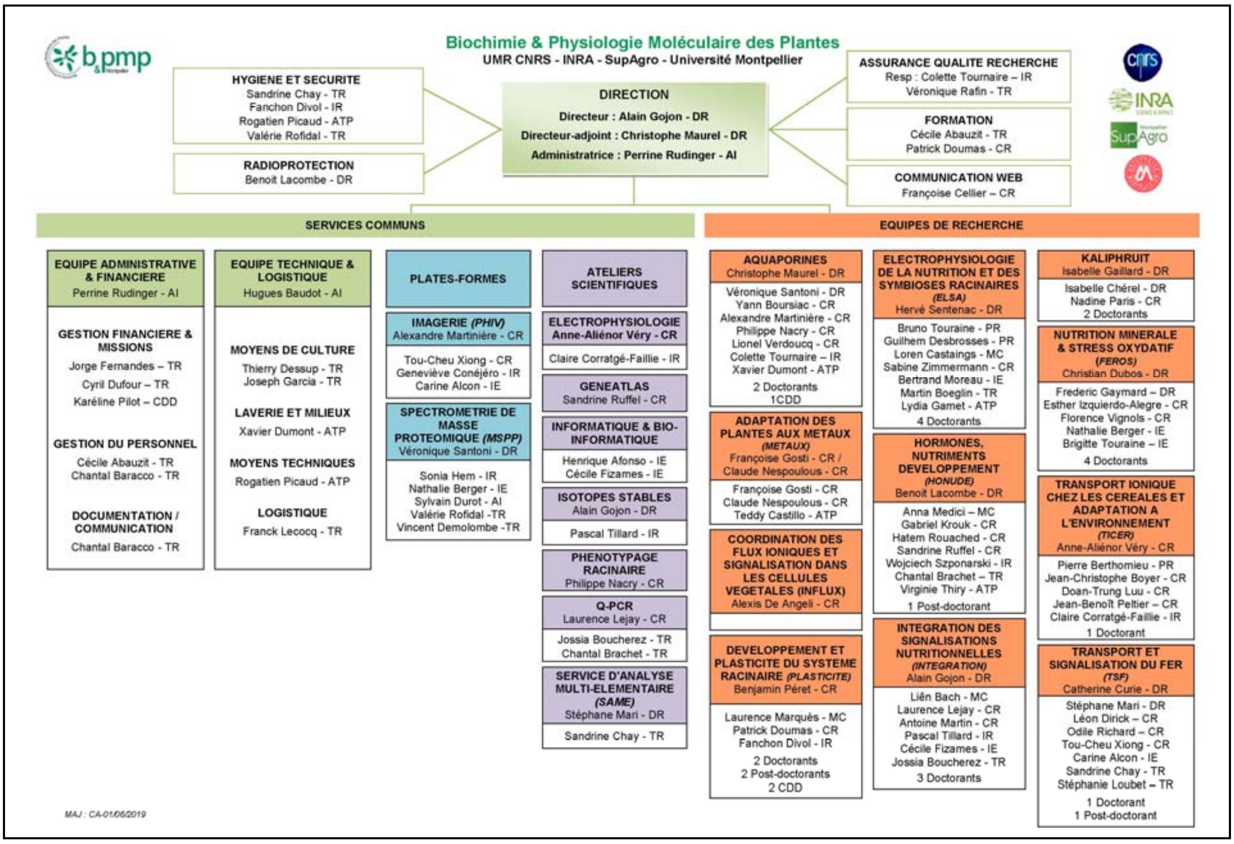
Although the principle of research team autonomy was preserved during the whole 2014-2019 period, a genuine general strategy was implemented at the whole unit level. In particular, the structuration and organization of the unit was significantly changed during the current contract period. The changes mostly resulted from events that occurred immediately before this period (2012-2013), from the recommendations made by the AERES Committee which performed the previous evaluation (2014), and from our own dynamics to conduct the project proposed during this evaluation.

This evolution is visualized by the comparison between the BPMP organization charts of 2014 and 2019 (see below).

2014 Organization chart



2019 Organization chart



The most significant changes and associated actions are listed below.

At the strategic and scientific levels:

- *The creation of 5 new research teams in 2015* (ELSA, HoNuDe, Integration, KaliPHruit and TICER), resulting from the split of two previous larger teams (Ion Channels and Integration). The objective was to strengthen the scientific dynamics of BPMP through the emergence of new independent projects and to ensure a significant renewal of research team leaders. Both aims were clear recommendations of the AERES Committee in 2014. This also allowed to favor projects centered on crops as the KaliPHruit and TICER teams focus their studies on grapevine and cereals, respectively.

- *The integration of 2 new research teams* (Plasticity and Influx, in 2015 and 2019, respectively) created *de novo* by young scientists who joined BPMP with grants dedicated to the emergence of junior research teams: ERC Starting grant for B. Péret (Plasticity), and ATIP-Avenir for A. de Angeli (Influx).

- *The closure of the "Ubiquitination and responses of plants to stresses" team* (UbiStress) in 2016, due to the mobility of the head of this team (P. Genschik) to the IBMP Institute in Strasbourg. The other permanent staff of this team joined other teams of BPMP.

- *A strategy to increase the staff from Montpellier University*. This was a recommendation of the AERES committee of 2014 and also a strong priority we put forward in our 2014 project. Between 2014 and 2017, we doubled the number of permanent University staff working at BPMP (from 4 to 8), due to the move of 3 colleagues (including 2 full professors) from another unit in 2015, and the recruitment of an assistant professor in 2017.

At the organizational level:

- *A sustained policy to support and develop common technological facilities in BPMP (see Annex 3)*. This had several aspects at both human resources and financial means levels: (i) a strong financial effort for the acquisition of scientific equipment (approximately 1.7 million €) including 3 major investments ("heavy equipment") for a confocal microscope, a proteomic mass spectrometer and a stable isotopes mass spectrometer, (ii) the integration of our proteomics and imaging platforms (MSPP and PHIV, respectively) into dedicated institutional mutualized organizations of the Montpellier area ("Pôle Protéomique Montpelliérain" and "Montpellier Ressources Imagerie", respectively, see Annex 3), (iii) the total renewal of the scientific and technological staff of the Electrophysiology platform (EHEV), a unique facility in the French plant science community, and (iv) the *de novo* creation of three new common facilities for transcriptomics (GeneAtlas), elemental analysis (SAME platform) and high-throughput root phenotyping (HIRROS) (see the organization chart above).

- *An in-depth reorganization in 2015 of the "General services" team*. This was one of the recommendations of the AERES committee of 2014 as this team had faced various difficulties during the previous contract period. The team was split in two distinct teams (Administrative and Logistics, respectively), with new leaders at their head. In addition, and thanks to a major support from both CNRS and INRA, the staff of the Administrative team was significantly renewed in 2014-2015 (3 new permanent positions). We now consider this problem to be solved since both teams largely demonstrated their ability to conduct their tasks satisfactorily.

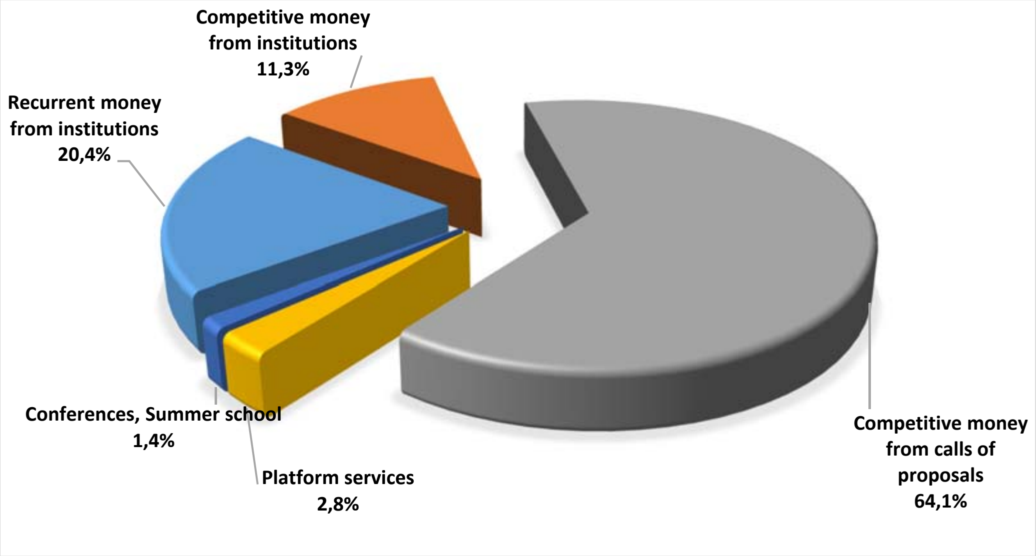
At the human resources level:

- *A significant turn-over of the permanent staff.* This was due to the arrival of 30 new permanent staff between 2012 and 2015 from various origins (external recruitments, people coming from other units, integration of the INRA MSPP proteomic platform previously located in another unit of the campus), but also to the departure of 14 permanent staff over the same period (for various individual reasons). This obviously had strong consequences during the current reporting period. The successful integration of the new colleagues and the functional replacement of those who left our unit constituted a challenge.

- *The human resources action plan* elaborated for reinforcing the 7 new research teams and the MSPP proteomic platform (to fulfil our principle of significant size of the teams). In addition to the people initially involved in these creations, our plan led to the integration of 12 new permanent staff in these teams between 2016 and 2018 (mostly by internal move within BPMP, but also from external recruitments). To date, this action plan is nearly completed, with the exception of two requests for new permanent positions (to CNRS and INRA) for the KaliPHruit and Influx teams.

Financial means

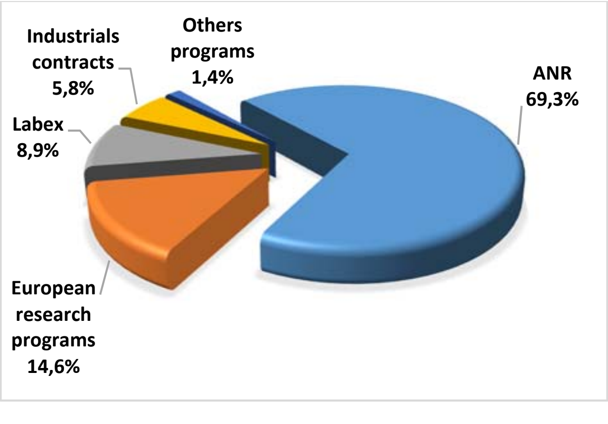
Concerning the financial means of BPMP, the 2014-2018 period was globally highly favorable but with a markedly decreasing trend. The Excel file "Current contract" displays the total funds spent by the unit between 2014 and 2018. Note that these data somewhat underestimate the actual operational budget of BPMP: they do not take into account several grants obtained by BPMP and for which the funds were handled by other institutions (AgreenSkills grants, PHC grants, SATT contracts, etc…). Altogether, the actual operational budget amounted to 11.25 million € for the whole 2014-2018 period (1.99 million € expected in 2019). It gathered the recurrent support from the four institutions to which BPMP belongs (20.4% of the total, see Figure below), the competitive grants from these institutions (11.3%, mostly for investment in scientific equipment), the competitive money from local, national or international calls of proposals (64.1%, mostly for research projects), the income resulting from services of our platforms (2.8%), and the resources we obtained for organizing conferences and summer schools (1.4%). This budget does not include most PhD grants (because the money was directly provided to the students).



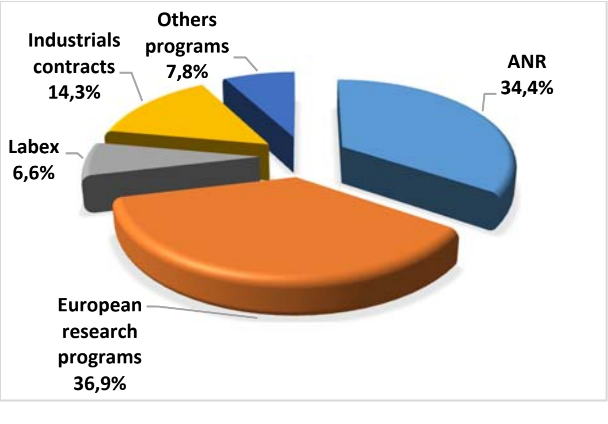
This mean annual budget of around 2.2 million € for the current reporting period represented a nearly 50% increase as compared to the previous period (2008-2013). Because the recurrent support from the institutions only increased from roughly 400 to 450 k€/year between the two periods, this shows that BPMP has been quite efficient in its quest for competitive grants. **However, the origin of the competitive money markedly evolved between the two periods (see Figures below). In 2011-2012, the main provider of competitive grants was by far the ANR (70% of the total), whereas it only represented 34% of the 2014-2018 budget.**

Origin of the competitive money from calls of proposals

2011-2012



2014-2018



This evolution was due to a very significant and constant decrease during the current period in our success rate to the ANR calls for proposals. This decrease also affected a large majority of research units focused on fundamental plant biology. In more details, we had 6 ANR projects accepted in 2014-2016 (out of 54 proposed), whereas only 2 were accepted in 2017-2018 (out of 49 proposed). The contribution of the Labex AGRO (created in 2011) also decreased during the current period as compared to the previous one. This reflected an increasing priority of the Labex for scientific and technological actions promoting the agronomical development of Southern countries. These actions are clearly outside the scope of BPMP. These abrupt changes in funding created a challenging situation for the teams of BPMP. However, they collectively reacted and were able to increase their funding from the European Union, from private partners and from other various sources. In particular, BPMP was awarded 3 ERC grants since 2014 (1 starting grant to B. Péret, and 2 advanced grants to P. Genschik and C. Maurel), which correspond to a total budget of 4.4 million € (the grant of P. Genschik was only partly carried out in BPMP, from 2014 to 2016). Also noticeable is our success to the 2018 call for proposals of the Montpellier iSITE (MUSE, created in 2017), with a federative pluridisciplinary project (eCO_2THREATS, 360 k€). These successes did not compensate, however, the general decrease in funding from the ANR and the Labex. Thus, the yearly amount of competitive money available from research projects decreased from 1.64 million € in 2014 to 0.91 million € in 2018 (see current contract Excel file).

The use of the operational budget is defined by the following rules:

- Each group has an independent budget, which is fueled by the competitive money (non-permanent staff salaries, small equipment and consumables) that it receives from funded projects. Collectively, the individual budgets of the research teams correspond to 70-75% of the total operational budget of the UMR. A "tax" amounting to 15% of the money dedicated to consumables is levied by the unit on all contracts, except (until 2018) those specifically devoted to young researchers (e.g., ANR JCJC, ERC Starting). Each group uses its budget according to its own priorities.

- The general common budget (not distributed to the groups) is constituted of the recurrent allocations from the Institutions, plus the 15% tax taken from the projects. This budget usually represents about 25-30% of the total operational budget, and is used to pay all infrastructure expenses, maintenance costs of all significant equipment and common facilities, expenses related to hygiene and safety, quality assurance, training, communication, scientific animation, and student (Masters) gratifications. As a consequence, almost all common facilities are free of cost for all the research teams (at the exception of proteomics, imaging, elemental analysis and stable isotopes platforms). Furthermore, the money left overs are used at the end of the year for common equipment, following a collective discussion.

- Rules for financial solidarity have been defined in 2012, in order to provide minimal funding to the groups that would transiently lack their own competitive funds. These rules were discussed in the various lab committees (see below) and were formally approved by the Unit Council.

Scientific policy

BPMP can be defined as an Integrative Biology research unit. **Our general objective is to understand how plants ensure their water and mineral nutrition, and respond to environmental factors (mostly abiotic) that may impair their nutrition and growth. The activity profile of the unit is predominantly directed towards basic research, with a significant involvement in higher education**. We mostly aim at identifying fundamental molecular and cellular mechanisms, using the most relevant models and approaches for reaching a high quality scientific standard and maintaining our visibility at the international level.

All research teams investigate various functional and/or developmental processes determining the water and mineral status of the plants. **Many of these groups have a strong focus on membrane transport processes involved in the acquisition of water and mineral nutrients by roots, and their distribution between various organs.** As a consequence, the study of membrane transporters and channels, of their integration into the whole plant and of their regulation in response to environmental factors, has been the first pivotal axis of BPMP's research projects. In addition to water, most main macro- and micronutrients are investigated (potassium, nitrogen, phosphorus, iron, zinc, manganese, etc…).

The second main scientific axis of BPMP is to elucidate the signaling mechanisms that allow plants to perceive abiotic constraints, and to develop relevant adaptive responses. This topic encompasses a wide palette of responses, from genome-wide transcription reprogramming or large-scale proteome modifications to adaptive changes in growth and development. This second topic is not disconnected from the first one (transport), because it also involves the determination of how the transport machinery is modulated to maintain an optimized nutrition under environmental fluctuations. However, a significant evolution during 2014-2019 was to strongly focus on signaling mechanisms triggering root developmental responses. As a consequence, the root system has become a privileged model for most projects of BPMP. The abiotic factors under study are mostly the external availability of water and mineral nutrients or metals, light, CO_2 and temperature. As a general rule, the levels of biological organization addressed by the research projects go from the molecules to the whole individual plant, which is the most integrated level investigated in BPMP (populations are outside of our scope).

The basic research made at BPMP clearly addresses major socio-economic challenges. In the context of sustainable agriculture and global climate change, we believe that the questions related to nutrient and water use efficiency by plants will be of increasing strategic importance. Furthermore, the abiotic factors investigated correspond to major environmental stresses that already strongly hamper crop production world-wide, such as drought, flooding, saline stress, nutrient starvation or metal toxicity. During the recent period, we also had an increasing interest in investigating the responses of the plants to the main abiotic factors of climate change (high temperature and elevated CO_2), as well as in studying beneficial biotic interactions (PGPR bacteria and symbioses) that may be relevant for ecological intensification of agriculture.

Finally, training is also a major objective for BPMP, in direct connection with its basic research activity. We contribute to the general teaching in Plant Sciences at both Montpellier University and Montpellier SupAgro. Staff from these two institutions is present in the laboratory, and researchers from both CNRS and INRA significantly participate in training. Furthermore, our unit has a long-standing experience in supervising French and foreign Master and PhD students, as well as post-doc researchers or temporary technical assistants. Fifty to 100 of these non-permanent people are joining BPMP each year.

Following the previous evaluation by the AERES committee (2014), several weaknesses were identified and BPMP received recommendations dealing with both its scientific strategy and organization. As detailed below, most have been taken into account since then:

- *The committee recommended that BPMP should develop more ambitious actions to better exploit its scientific leadership*. We took this remark as an important issue and addressed it somewhat successfully along two main lines. The international visibility of BPMP was enhanced by organizing three major international congresses, and its attractivity and ability to develop more ambitious projects were validated by hosting three ERC projects conducted by BPMP group leaders.

- *The committee pointed out that several leaders of the former research teams will retire or will be close to retirement before the end of the current 2014-2019 period, and that a strategy to favor the emergence of new research groups should be developed.* This has been a key priority as early as 2014 and resulted as stated above in the creation of 7 new research teams since 2015. Overall, this period has seen a major renewal of the research team leaders (6 new leaders out of 11).

- *We were strongly encouraged to get more involved in the training at Montpellier University, and to attract more staff from the University.* This goal was reached with success as the University staff doubled during the period (from 4 to 8), and because the number of Master students from the University hosted at BPMP was significantly increased as compared to the previous period (93 as compared to 53).

- *We were encouraged to better communicate on the scientific strategy of the unit towards technical and administrative staff.* To address this point, various aspects of BPMP's strategy were discussed during an increasing number of meetings of the Unit Council (see the "Organization and life of the unit section"). In addition, all creations of new research teams by incoming team leaders were preceded by an internal seminar to inform all BPMP staff of the corresponding project. Finally, the scientific project of each new team was discussed during Unit Council meetings.

- *The committee recommended that BPMP should be more active in widening its collaborations with private partners and other local research Units focusing on ecophysiology and soil science.* Several significant actions were conducted to sustain valorization (research contracts with Syngenta, Solvay, Imperial Tobacco and the local SATT). These efforts proved successful as the total funding from private partners increased from 384 k€ during the 2008-2013 period to 1,030 k€ during the 2014-2018 period. However, partnership with private companies remains an aspect where BPMP has still decisive progresses to make. Efforts to involve BPMP into more integrated projects with ecophysiologists and soil scientists were also successful, in particular through a main collaborative project with soil scientists coordinated by BPMP and funded by the iSITE MUSE ("eCO_2THREATS" project), and through our partnership with ecophysiologists within the ERA-CAPS "Root Barriers" project.

2- Presentation of the unit's research ecosystem

Although the institutional context of BPMP has remained stable with respect to the four organizations to which the unit belongs (CNRS, INRA, Montpellier University, Montpellier SupAgro), the local context was significantly modified at several levels, which had consequences for BPMP.

The Labex AGRO to which BPMP belongs was created in 2011 to replace the previous RTRA (Réseau Thématique de Recherche Avancée). The Labex AGRO, which performed most of its funding and structuring actions during the 2014-2019 period, has predominantly focused on scientific questions related to the agricultural development in Southern countries. Therefore, it has been, and still is, a challenge for BPMP to appear as a key player in this Labex. Most of our project proposals were rejected, because of their supposedly lack of impact, and the overall funding from the Labex significantly dropped as compared to the previous RTRA period. BPMP obtained only ca~2% of the total funding provided by the Labex between 2011 and 2018. When normalized to the number of scientists working in different units belonging to the Labex, BPMP funding was up to 8-times lower than in similar plant biology UMRs, but working on tropical plants. Nonetheless, support from the Labex was significant for the two international congresses organized by BPMP in Montpellier, and for our collaboration with Solvay. Noticeably, together with the support from the CNRS, our continuous exchanges with the Direction of the Labex finally allowed to set up a Federative project on "Fundamental Plant Biology" (2019-2022) funded by the Labex (600 k€) that BPMP will lead together with the LGDP Unit (CNRS/University of Perpignan) (see project).

University of Montpellier (UM) was created in 2016 from the merge of the previous Montpellier 1 and 2 universities. The UM has been significantly supporting BPMP through a new assistant Professor position in 2017 (in addition to the 3 other University staff who joined BPMP in 2015) and the funding of several projects for scientific equipment (120 k€ in total). Conversely, and in connection with our general strategy to strengthen the links with the University, BPMP has been a main contributor of the building and functioning of the Scientific Department "Biology-Agrosciences" of the new Montpellier University. Indeed, the Director of this Department (Prof. B. Touraine) is a member of BPMP, and the Director of BPMP (A. Gojon) is a member of the Council of this department.

The iSITE project MUSE (Montpellier University of Excellence) to which BPMP contributes was launched in 2017. Its main objective is to structure the Life Sciences community of Montpellier. MUSE gathers 120 research units and focusses on scientific questions related to three main research communities that are strongly represented in the Montpellier area: (i) Animal and Medical sciences, (ii) Ecology and Environment, and (iii) Plant Sciences and Agronomy. Despite its recent creation (2017), MUSE already funded around 70 competitive research projects, among which 10 major ones in 2018. One of the major projects (360 k€) is coordinated by BPMP, highlighting our visibility in the iSITE.

The former Region "Languedoc-Roussillon" merged with its neighbour ("Midi-Pyrénées") to give birth to the new "Occitanie" Region. As compared to the former one, the new "Occitanie" region has a significantly different strategy for funding research, notably concerning PhD grants that are now easier to obtain as soon as the PhD project is relevant for the Regional economy. This in particular provides an important opportunity for our projects on grapevine, which already benefited from two half PhD grants since 2016.

Finally, two additional important players in BPMP's local ecosystem are the Biocampus unit, and the GAIA Doctoral School. Biocampus is a technological structure (UMS CNRS/INSERM/UM) gathering a large number of mutualized platforms for medical and biological research in Montpellier. BPMP is both a contributor and a beneficiary of Biocampus because our imaging (PHIV) and proteomic (MSPP) platforms are integrated into the large imaging (MRI) and proteomic (PPM) platforms of Biocampus, respectively (see details in Annex 3). Concerning GAIA, BPMP is contributing to its functioning (A. Gojon is a member of the GAIA Council, B. Lacombe is a member of the evaluating committee for PhD grant allocation), and has also been very successful in obtaining PhD grants from this Doctoral School (11 over the period, taking into account that 57 research units are associated to GAIA, and that only around 120 PhD grants have been delivered since 2014).

3- Research products and activities for the unit

Scientific track record

Scientific production

The total number of publications during the Jan 2014-June 2019 period (5.5 years) is 237 articles (excluding BioXriv), among which 222 appeared in journals having an impact factor (see Table below and Annex 4).

Name of the Journal	Number of articles	Impact factor 2017		Name of the Journal	Number of articles	Impact factor 2017
Science	2	41,058		Mol. Microbiol.	1	3,816
Cell	1	31,398		Plant Sci.	2	3,712
Nat. Genet.	1	27,125		Int. J. Mol. Sci.	4	3,687
Physiol Rew.	1	24,014		BBA - Gen. Subjects	1	3,679
Nat. protoc.	1	12,423		Front. Plant Sci.	20	3,677
Nat. Commun.	3	12,353		Environ. Exp. Bot.	3	3,666
Trends Plant Sci.	8	12,149		Ann. Bot.-London	1	3,646
Nucleic Acids Res.	1	11,561		Mol. Plant-Microbe Interact.	1	3,588
Nat. Plant.	4	11,471		Plant Mol.Biol.	1	3,543
P. Natl. Acad. Sci. USA	6	9,504		Proteomics	1	3,532
Mol. Plant	8	9,326		Plant Soil	1	3,306
Curr. Biol.	1	9,251		Planta	1	3,249
Plant Cell	7	8,228		J. Integr. Plant Biol.	1	3,129
eLife	3	7,616		Molecules	1	3,098
New Phytol.	9	7,433		Rice	2	3,039
Curr. Opin. Plant Biol.	4	7,349		Febs Lett.	1	2,999
Compr. Rev. Food Sci. F	1	7,028		Biochem.	1	2,997
Sci. Signal.	2	6,378		J. Biol. Inorg. Chem.	1	2,952
Plant Biotechnol. J.	1	6,305		Reg. Envir. Chang.	1	2,872
Plant Physiol.	19	5,949		J. Plant Physiol.	4	2,833
Plant J.	8	5,775		Environ. Sci. Pollut, Res.	1	2,800
PLoS Genet.	2	5,540		Mycorrhiza	1	2,778
Plant Cell Environ.	6	5,415		PLoS one	4	2,766
Development	2	5,413		Plant Physiol. Biochem.	2	2,718
J. Exp. Bot.	19	5,354		MicrobiologyOpen	1	2,682
Crit. Rev. Biotechnol.	1	5,239		BioMed Res. Int.	2	2,583
Mol. Cell. Proteomics	1	5,236		Physiol. Plantarum	1	2,580
Environ. Microbiol	1	4,974		J. Cereal. Sci.	1	2,302
Plant Methods	1	4,269		Transgenic research	1	2,197
Mol. Plant Pathol.	1	4,188		Curr. Genomics	1	2,172
Sci. Rep. - UK	9	4,122		Trees-Struct. Funct.	2	1,782
Metallomics	1	4,069		Biol. Plantarum	1	1,424
Plant Cell Physiol.	4	4,059		Adv. Bot. Res.	6	1,388
J. Biol. Chem.	2	4,040		Arid Land Res. Manag.	1	0,970
Front. Microbiol.	1	4,019		J.Agric.Sci.Technol.	1	0,890
BMC Plant Biol.	2	3,930		Int. J. Agric. Biol.	1	0,869
Aquat. Toxicol.	1	3,884		Chil. J. Agric. Res.	1	0,775

These 222 articles correspond to a weighted mean journal impact factor of 6.13, with 122 (55% of the total) of these articles published in journals with an impact factor higher than 5. **Furthermore, 44 articles (20% of the total) were published in top ranking journals** (*Science*, *Cell*, *Nature Genetics*, *Physiological Reviews*, *Nature Protocols*, *Nature Communications*, *Trends in Plant Sciences*, *Nucleic Acids Research*, *Nature Plants*, *PNAS*, *Molecular Plant*, *Current Biology*, *Plant Cell*).

Compared to the previous reporting period (see table below), **our current data illustrate a significant quantitative improvement (27% increase in publication rate per full-time permanent scientist) without loss in quality**: BPMP's production between Jan 2008 and Jun 2013 (5.5 years) was 169 articles in journals with a mean impact factor of 6.15 (IF 2011). Furthermore, this increase in scientific production is in continuation of a long term trend recorded since 2004 (see table below). It should be emphasized that these progresses occurred despite the fact that about half of the current research teams were created in July 2015, and that several of them did not have time yet to fully publish the results of their new research project.

Long-term evolution of the scientific production of BPMP in journals with impact factor

Period	Total number of articles	Mean number/year/Full-time permanent scientist	Mean impact factor
2004-2008 (4.5 years)	120	0.81	5.40
2008-2013 (5.5 years)	169	0.82	6.15
2014-2019 (5.5 years)	222	1.04	6.13

The vast majority (>95%) of the 237 articles published over 2014-2019 is directly connected with the scientific topic of BPMP. The remaining articles mostly result from the open activity of our technological platforms, which provided a methodological support to scientific projects from other research units (see Annex 3).

Furthermore, considering the 222 articles published in journals with an impact factor, it is noteworthy that:

- **36% include a PhD student of BPMP as an author**,
- **63% are with a member of BPMP as first and/or corresponding author**,
- **40%, 25% and 43% are in co-authorship with French, European and foreign (other than European) collaborators, respectively.**

Finally, 2 patents, 20 books or book chapters, 6 softwares, 25 defended theses and 83 invited talks in national or international conferences have also been recorded during the 2014-2019 period. With the exception of the patents and defended theses, these numbers also show a marked increase as compared with the previous period (3 patents, 9 book chapters, 0 software, 25 defended theses and 65 invited talks between 2008 and 2013).

Main scientific outcomes at the whole unit level

For the last evaluation in 2014, we proposed several priorities for the current contract of BPMP (see our AERES report of 2013). These priorities are listed below:

- *Strengthen our studies on plant water transport and responses to water stress.*
- *Develop more integrated projects connecting root development and function.*
- *Expand our Systems Biology approaches.*
- *Generalize the study of post-translational regulatory mechanisms.*
- *Initiate more prospective genetic approaches.*

Most of these priorities actually translated in strong research projects and led to significant advances, illustrated by original publications in high-ranking journals.

The topic where this was particularly obvious is water transport and responses to water stresses. Indeed, several projects, mostly led by the Aquaporin team, have shed new light onto the regulation of water transport in the shoots and roots of *Arabidopsis*. While the role of aquaporins in stomatal movements had remained elusive, the team elucidated a dual hydraulic and signaling function of aquaporins in guard cells (Grondin et al., 2015; Rodrigues et al., 2017). In particular, tight connection between stomatal closure, hydrogen peroxide-mediated ABA signaling, and aquaporin functions and regulations were established. Another major breakthrough was brought by quantitative genetics analyses of water transport in Arabidopsis roots (Shahzad et al., 2016; Tang et al., 2018). Based on its unique capacity to phenotype root hydraulics at high throughput, the group was able to isolate several genes involved in water transport regulation. In particular, a new signaling pathway that mediates root responses to hypoxia, in relation with K^+ availability was uncovered.

Work on the responses of root development to abiotic signals was strongly reinforced since 2014. A first set of studies addressed the sensing/signaling mechanisms of nitrate and their impact on integrated functional and developmental properties of roots. In particular, studies on the NRT1.1/NPF6.3 transceptor and its connections with auxin signaling were pursued and amplified (HoNuDe and Integration teams). These projects reached their full maturity in terms of scientific production, and unraveled novel connections with phosphate, ABA and cytokinin signaling (Mounier et al. 2014, Bouguyon et al. 2015, Léran et al. 2015, Medici et al. 2015, Bouguyon et al. 2016, Ristova et al. 2016, Poitout et al. 2018, Medici et al. 2019). Second, investigations on the developmental plasticity of the root system were initiated in several other groups (Aquaporins, ELSA, FeROS, Metal toxicity), leading to new information on root responses to other nutrients (e.g., iron and zinc), water stress, or rhizospheric bacteria (Reyt et al. 2015, Poitout et al. 2017, Bouain et al. 2018, Rosales et al., 2019). Third, BPMP was able to strengthen its positioning in the field of the environmental control of root development by assisting the creation in 2015 of the "Development and Plasticiy of the Root System" team (B. Péret, see above). In addition to its work on Arabidopsis, this group has introduced lupin cluster roots as an additional model (Gallardo et al. 2019). Fourth, another important milestone was the development of an automated robot for root architecture phenotyping (HIRROS). Even before 2014, our capacity to conduct refined analyses of root system architecture at sufficient speed had been identified as a crucial bottleneck. Our high-throughput phenotyping device is currently fitted for fast acquisition of root system pictures on plants grown *in vitro*. The prototype was fully developed in house, and will soon be fully operational as a platform for BPMP teams and external users, thanks to the planned recruitment of a technical assistant in 2019.

Systems biology approaches expanded into two directions. First, frontline developments were carried out on algorithms used to handle large-scale genomic data (HoNuDe), in collaboration with applied mathematicians (Krouk et al. 2015, Carré et al. 2017, Val et al. 2017, Carré et al. 2018). This effort was associated with software developments (See annex 4) that may now have an innovation potential (project funded by SATT). Second, systems biology tools were progressively integrated into more classical biological approaches to infer gene regulatory networks and identify new regulators of plant mineral nutrition (Integration, HoNuDe, KaliPHruit, Metal toxicity), often within the frame of collaborations with other teams in the USA and Chile (Alvarez et al. 2014, Ristova et al. 2016, Pal et al. 2017, Varala et al. 2018, Brooks et al. 2019). In a broader sense, mathematical modelling approaches have been initiated by several other groups, but with different aims. For instance, the Aquaporin group has been integrating elementary water flows into whole root systems to model the root hydraulic architecture of different *Arabidopsis* genotypes.

Posttranslational regulatory mechanisms are now routinely investigated in almost all teams of BPMP. Not surprisingly, this led to the identification of protein phosphorylation and protein-protein interactions as key mechanisms for modulating water or ion transport, and nutrient signal transduction (Ronzier et al. 2014, Bouguyon et al. 2015, Grondin et al. 2015, Léran et al. 2015, Bellati et al. 2016, Lefoulon et al. 2016, Shahzad et al. 2016, Corratgé-Faillie et al. 2017, Prado et al. 2019). Our strategy on this topic also included a strong support for both personnel reinforcement and technological upgrade to our proteomic platform (MSPP, see Annex 3).

Quantitative genetics has recently emerged as a highly successful approach in BPMP (Aquaporins, Metal toxicity), allowing several breakthroughs in our understanding of complex mechanisms associated with responses of plants to abiotic constraints (Shahzad et al. 2016, Bouain et al. 2018, Kisko et al. 2018, Tang et al; 2018). One consequence is that GWAS can now appear as a major approach in the recent proposals concerning federative projects coordinated by BPMP (eCO$_2$THREATS and Flagship project CalClim).

Beyond the above priorities identified in our 2014 project, several other important developments occurred during the last years. These developments will undoubtedly structure the scientific strategy of BPMP in the coming years:

- A renewed interest on transcriptional reprogramming in response to abiotic signals. Several projects focusing on signaling mechanisms and gene regulatory networks triggering responses to nutrients identified a handful of transcription and chromatin factors as key players of these responses (Alvarez et al. 2014, Khan et al. 2014, Medici et al. 2015, Pal et al. 2017, Bellegarde et al. 2018, Bellegarde et al. 2019, Medici et al. 2019, Tissot et al. 2019). Therefore, the mechanisms of transcriptional regulation, now including chromatin dynamics, have been increasingly investigated by several teams (HoNuDe, Integration, FeROS).

- Convergent data from several teams highlight the general importance of ROS signaling and redox processes in governing water and nutrient (N, Fe) homeostasis. Although this was generally not the objective, the pivotal role of these processes emerged from several independent projects (Reyt et al. 2015, Wudick et al. 2015, Rodrigues et al. 2017, Bellegarde et al. 2019, Martinière et al. 2019).

- Significant efforts were devoted to interactions between nutrient signaling pathways, in the context of combinations of abiotic constraints (N and P, N and C, N and water, P and zinc, etc…). These studies resulted in many original discoveries, showing that multi-stress situations often unravel emerging properties: plant responses to combined stresses rely on specific mechanisms that are not the simple addition of the responses to each individual stress (Khan et al. 2014, Medici et al. 2015, Li et al. 2016, Shahzad et al. 2016, Kisko et al. 2018, Medici et al. 2019).

- Several groups (Aquaporins, Influx, KaliPHruit, TSF) expressed an increasing common interest in technological developments concerning cutting-edge imaging techniques and the use of biosensors (pH, iron, ROS, hormones, calcium, nitrate, …) adapted to *in vivo* studies (Xiong et al. 2014, Hosy et al.

2015, Martinière et al. 2018). As a consequence, dedicated projects were developed by our imaging platform (PHIV) that are expected to provide many novel tools for both transport and signaling studies.

Finally, the investigation of membrane transport, the historically strong topic of BPMP, has remained very active in several teams. As in the past, some studies aimed at the functional characterization of *Arabidopsis* transporters for major mineral nutrients (Nieves-Cordones et al. 2014, Léran et al. 2015, Taochy et al. 2015, Castaings et al. 2016, Alejandro et al. 2017). However, a significant number of new projects has been addressing more original questions, such as the transport of organic solutes associated with iron nutrition (Fourcroy et al. 2014, Grillet et al. 2014, Fourcroy et al. 2016), Na^+/K^+ transporters involved in saline stress tolerance in cereals (Ben Amar et al. 2014, Tounsi et al. 2016, Nguyen et al. 2017), K^+ channels and transporters determining grape berry acidity (Nieves-Cordones et al. 2019), or transport systems ensuring nutrient transfer or electrical signaling in root symbioses (Garcia et al. 2014, Charpentier et al. 2016, Guerrero-Galan et al. 2018, Wang et al. 2019).

As during the previous reporting period, a large part of the research conducted at BPMP has been performed within international collaborations. There is no specific geographical focus since, during 2014-2018, most of our collaborative programs targeted many countries located in 4 continents: Europe (many different countries), Africa (essentially Maghreb), America (mostly USA and Chile), and Asia (China, Japan, Taiwan and Vietnam). Beyond the high percentage of common publications with foreign collaborators (see above), the vitality of our international collaborations can be evidenced by: (i) the number of visiting scientists and students from abroad (61 in total), and (ii) the significant proportion of international projects (33%) among the major projects (36 in total) funded in BPMP from various local, national or international sources (Labex, iSITE, ANR, LIA, ATIP, ERC, H2020, Marie Curie-Sklodowska, NSF).

Key events

Beyond a simple list, it is important to detail the significance of these key events with regard to the scientific strategy of BPMP. Therefore, these events were grouped and linked, when relevant, to the scientific priorities put forward by BPMP in 2013, or to the recommendations of the AERES Committee in 2014. Also, we only refer to scientific key events here, as those associated with the organizational changes of the unit were previously detailed in the "Unit's workforce and means" section above.

National and International visibility and attractivity of BPMP

- *The organization of three major international conferences*: (i) Nitrogen2016: the 3rd International Symposium on the Nitrogen Nutrition of Plants (EMBO conference also supported by the NSF), (ii) IPMB2018: the 12th Congress of International Plant Molecular Biology, and (iii) IPSB2018: the 1st International Plant System Biology Meeting. These three conferences provide an unprecedented demonstration of BPMP's renown in international scientific communities at the heart of our research field.

- *The creation of a LIA* (Laboratoire International Associé) between BPMP and the Center for Genomics and System Biology of New York University (USA). Based on a long standing collaboration, this LIA played a strong structuring role in the development of our systems biology approaches.

- *The creation of two new teams led by young scientists moving from other research units.* BPMP succeeded in attracting two promising colleagues, Benjamin Péret (CR CNRS) and Alexis de Angeli (CR CNRS) who selected our unit to run their projects supported by an ERC starting grant and an ATIP-Avenir grant, respectively. These achievements assess our strategy to provide an attractive scientific and material environment to external team leaders.

Water transport and response to water stress

Recent work of BPMP on root water transport represents a major breakthrough with respect to previous studies in the field of plant water relations. The work relied on an innovative quantitative genetics approach that was centered on root hydraulic conductivity, a key trait that had not been explored before. This work has had several impacts:

- It led to two high-rank publications (Shahzad et al., Cell, 2016; Tang et al., 2018, Nature Commun.) and a third publication to come (Shahzad et al., Plant Cell, in revision).

- In recognition of this outstanding *work*, the Aquaporin team was awarded two prizes from the French Academy of Sciences (Major French Advances in Biology 2017 to Z. Shahzad; Georges Morel Prize to C. Maurel in 2018).
- The results provided a robust ground for the ERC Advanced grant obtained by C. Maurel in 2017. This grant will allow the Aquaporin team to explore the many facets of root hydraulic architecture of maize plants under water stress.
- Finally, the press release related to the work by Shahzad et al. (Cell, 2016) led to several interviews in public broadcasts (France, Switzerland) and the national press.

Integration of functional and developmental responses of the root system

Convergent studies made by HoNuDe and Integration teams (both arising from the split of the former Integration team) provided major insights into nitrate signaling pathways, and in particular the pathway triggered by the NRT1.1/NPF6.3 nitrate transceptor (transporter-receptor). In a series of publications (Mounier et al. 2014 *PCE*, Bouguyon et al. 2015 *Nature Plants*, Léran et al. 2015 *Sci Signal*, Medici et al. 2015 *Nature Commun*, Bouguyon et al. 2016 *Plant Physiol*, Medici et al. 2019 *Plant Cell*), these teams unraveled the far reaching functions of NRT1.1/NPF6.3 in governing a large palette of physiological and developmental responses of the plant to nitrate, and demonstrated that NRT1.1/NPF6.3 also participates in a signaling cascade integrating nitrate and phosphate signals to modulate both N or P transport and root growth. Altogether, these publications constitute a unique comprehensive study of nutrient sensing mechanisms, which in turn coordinate functional and developmental responses of the root system.

See press releases:

- Les stratégies des plantes pour optimiser l'utilisation des nitrates
- Détection des ions minéraux par les plantes : du nouveau pour le nitrate et le phosphate
- « Une phosphatase comme régulateur positif du transport et de la perception du nitrate chez les plantes » (http://archives.cnrs.fr/insb/article/2015/b-lacombe)

See also the 2015 INRA report on key scientific events at the national level (pp 19-20): http://institut.inra.fr/Reperes/Documents/Rapports-d-activite/Rapport-d-activite-2015

Other scientific key events

- 3 ERC grants have been hosted in BPMP.

- The *de novo* sequencing of the white lupin genome was completed in 2018. This large scale project was headed by the Plasticity team and involved a large consortium of a dozen of research teams from various institutes.

Interaction with social, economic and cultural environment

The 2014-2018 period has seen a marked improvement of our interactions with private companies, through 3 major projects running in part or in totality over the period, and corresponding to a significant level of private funding for the BPMP teams (Syngenta: 600 k€, Solvay: 300 k€, and Imperial Tobacco: 300 k€).

BPMP also enhanced its interaction with the social environment by participating in various initiatives open to the public. These include the "Fascination of Plants" day organized by the EPSO, and our yearly "Open doors" day for the "Fête de la Science" during which we receive the Preparatory Classes to the Engineer Schools of Agronomy.

Training

A key aspect is the long-standing continuation of the MISTRAL international summer school (Montpellier international school on ion and water transport in plants), which is fully managed by BPMP. Created in 2012, MISTRAL aims at providing a high-level research training on both theoretical and practical aspects of ion and water transport in plants. MISTRAL supports our strategy to position BPMP as an internationally leading Institute in the field of water and mineral nutrition. Each session of this bi-annual summer school is limited to around 15 PhD students, post-docs or senior researchers with an already significant background on the topic. The summer school runs over two full weeks, with both lectures (one week) and practical training on the various technological platforms of BPMP (one week). During the evaluation period, the 3 sessions, run in 2014, 2016 and 2018, gathered 43 attendees in total (68% from abroad), from 13 different countries.

4- Organisation and life of the unit

Steering, life, organisation in the unit

In complement to the autonomous scientific functioning of the research teams, the organization and life of BPMP rely on three main pillars:

- *The «Internal Regulations Statement» («Règlement Intérieur»)* of BPMP. This document establishes all formal rules related to main aspects of our individual and collective functioning, such as «Hygiene and Safety», «Informatic chart», «Working hours and organization of work during nights or week-ends»…. Any newcomer at BPMP receives this document and attests its reading by a signed statement.
- *The functioning of various executive or non-executive management committees (detailed below).*
- *Several supporting teams and groups with administrative and logistical tasks (detailed below).*

Management committees

BPMP is currently managed by a direction team including Alain Gojon as a Director, Christophe Maurel as a Deputy-Director, and Perrine Rudinger as an Administrative Manager.

The Unit Council, where the various worker categories are represented according to the legislation which applies to our laboratory size, is the only official and obligatory council. It meets on average 4 times a year and can give advice on all issues related to the laboratory life, with emphasis on those concerning the general strategy of the unit, hygiene and safety, financial resources, human resources and management of common services. Since 2013, the role of the Unit Council was reinforced regarding major scientific orientations. This resulted from a recommendation of the AERES committee that suggested that new communication means on these issues should be set up between the direction and the technical staff. In practice, two main procedures were developed. First, the rules and criteria for validating the creation of a new research team (or promoting someone as a team leader) were detailed in a written chart, which was discussed and validated by the Council. Second, each creation of a new research team was discussed in the Council, which in most instances expressed its opinion by a formal vote.

The Team Leaders Committee meets once a month and helps the Direction to take decisions related to the scientific policy and human resources management, such as defining the scientific priorities, creating or closing teams, elaborating the resource requests to our four Institutions, and making investments for scientific equipment. This committee is composed of the research team leaders, but also of the administrative and logistics team leaders, and of the scientific leader of the proteomic platform. All these people (14 in total) exert a direct hierarchical responsibility on permanent staff downstream of the Director.

In addition, two other collective structures participate in the organization and life of the unit:

The Laboratory Technical Committee is composed of one member per team and of the Direction. It is in charge of elaborating solutions to any technical or practical problem that may arise in the unit (with the exception of plant cultures, see below). It also makes recommendations for ameliorating «BPMP's life» on a day to day basis. It meets once a month and has its own budget (10 k€) to be distributed collectively.

The Plant Growth Facilities Committee is also composed of one member per research team, and of the technical assistants involved in managing the plant growth facilities (greenhouse, grown chambers, growth cabinets). This committee has a crucial role in ensuring a smooth and efficient collective use of plant growth facilities, by setting rules for attributing growth space to the research teams and by defining protocols for plant growth (management of various species, environmental conditions, pest management, etc…). This is particularly important because most plants grown in the unit are GMOs. In addition, the increasing number of plant species investigated in BPMP results in an increasing difficulty to satisfy all needs of the various teams. This requires a strict organization for the use of our facilities.

Finally, *the General Assembly* of all the people working at BPMP takes place once a year, generally in January. This meeting gives a good opportunity for the Director to detail the main aspects of the unit's life during the previous year, and the prospects for the incoming year. In addition, specific meetings between the Direction and each college of staff (permanent scientists, permanent technical and administrative assistants, and non-permanent staff) are organized each year.

Supporting services

In addition to well-structured technological platforms and common facilities (see Annex 3), BPMP research activities are supported by several teams and groups with both administrative and logistical tasks (see organization chart above).

The Administrative and Financial team is headed by the administrative manager (P. Rudinger). It is in charge of the reception of visitors and human resources management, the accounting follow-up, the organization of the business travels, and the communication media (at the exception of the Website). Concerning financial aspects, the team (two full-time administrative assistants) establishes a forecast budget and an analytical budget management, integrating all the funding resources of BPMP. This difficult task is achieved by means of the Geslab CNRS software using a specific procedure developed by the team. This enables the Direction of BPMP and all the research groups to follow on line their purchases and invoices and to be informed almost in real time of the status of their financial resources. Concerning the human resource management, BPMP hosts each year 50 to 100 non-permanent people (undergraduate and graduate students, post-docs, visiting scientists …) from France or abroad, for periods from few months to three years. We have therefore established a "welcome procedure" for these new-comers, which facilitates their arrival and integration. A full-time administrative assistant (with the help of another part-time administrative assistant) is in charge of human resources and professional training for the whole BPMP staff. Finally, a part-time administrative assistant is in charge of the communication and of the scientific and technic information.

The Technical and Logistics team (6 full-time technical assistants) is headed by the technical manager (H. Baudot). It maintains our highly developed setup of plant growing facilities (https://www1.montpellier.inra.fr/wp-inra/bpmp/en/platform/plant-culture/), runs the plant cultures in the green house, and ensures a strict regulatory monitoring of the transgenic plants produced in the Unit. This team also performs building maintenance, and inventories the equipment (including scientific apparatus), while monitoring its removal when out of order. Finally, the team takes care of the dishwashing and the preparation of media. It receives all deliveries by external suppliers, and runs the internal store where all teams can pick up most consumables needed (more than 600 different items in stock).

In addition to these two fully-dedicated teams, several groups take care of the Health and Safety (see specific section below), of the Research Quality Assurance (see specific section below), of the in house training of BPMP staff, and of the Website monitoring. These groups are predominantly constituted by volunteers who perform the related tasks in addition to their main activity in a research or support team.

Main actions concerning the organization and life of the unit

As stated in the "Unit's workforce" section above, these teams and groups have been significantly reorganized during the current period, with the exception of the Hygiene and Safety group (compare 2014 and 2019 organization charts above). This allowed the development of several important actions, mostly led by our new administrative and technical managers, and improving the general organization and life of BPMP. These actions are listed below.

On the technical aspects:

- *Changes in space allocation within the building*. These changes result frommajor structural changes in many research teams and in the two support teams, and are connected with the integration of the proteomic platform in 2013. Overall, a general reallocation and layout of nearly half of the building space was decided in 2015. This involved the move of almost all staff. Our aim was that, when possible, each team present in the building (currently, 14 including the proteomic platform, as compared to 9 in 2014) can be located within a unique and dedicated area. This plan also required specific funding to modify the building infrastructure for hosting the proteomic platform that moved from another building (84 k€).

- *Upgrade of the plant growth facilities and energy saving plan*. Due to the needs of the LupinRoots ERC project on the one hand, and on the recommendation of both the Unit Council and the Team Leaders Committee on the other hand, an ambitious upgrade of our growth chamber facilities was carried out by the technical manager. This work required the building of three brand-new growth chambers, the installation of three high-tech phytotrons for refined environmentally-controlled experiments, the replacement of the regulatory systems of three older growth rooms, and the doubling of air conditioning systems for all growth rooms (to prevent any of these to be out of function for more than one day).

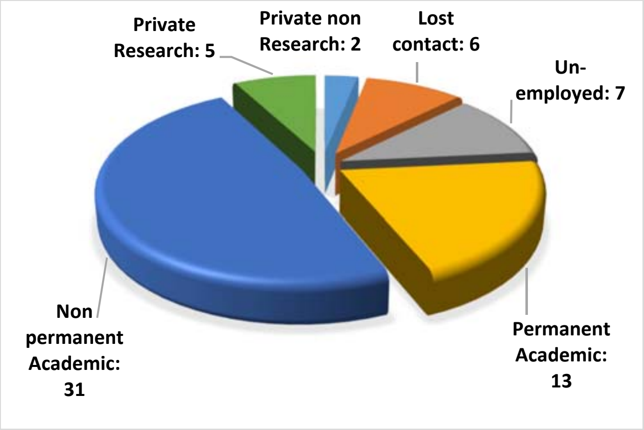
Altogether, this ambitious upgrade required a 572 k€ budget. In addition, we decided to replace all former lighting devices by new LED systems for energy saving purpose (still ongoing)

On the human resources aspects:

- *Help in the career evolution of administrative and technical staff*. Upon her arrival in 2015, our administrative manager proposed to set up a procedure for supporting the non-scientific staff of BPMP in its career evolution. The writing of annual activity reports is supported, and trainings for oral examination during professional selections and contests are proposed. This initiative fills a gap in the strategy of the Unit on these aspects. The general record of promotions of BPMP permanent staff (including scientists) is provided in the table below.

Type of promotion	Number of people promoted	Type of promotion	Number of people promoted
ATP2 >ATP1	1	IE>IR	1
ATP1>TCN	2	IR1>IRHC	1
TCN>TCS	3	CR2>CR1	5
TCS>TCX	1	CRCN>CRHC	4
TC>AI	1	CR>DR2	3
AI>IE	1	DR2>DR1	1
IECN>IEHC	2	MDC>PU	1

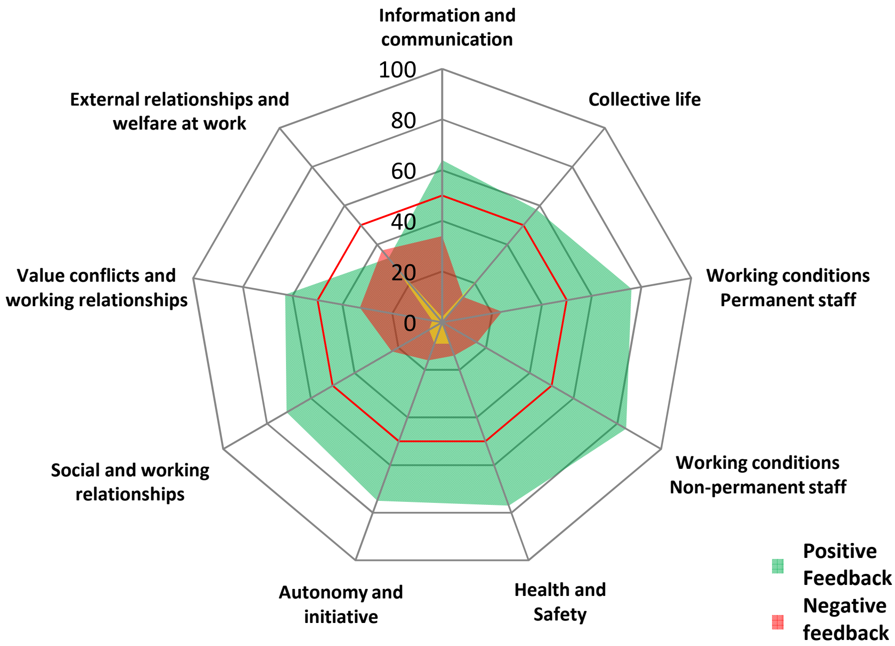
- *Mentoring of the non-permanent staff and its fate*. Another clear lack that was identified in BPMP was the absence of formal assisting procedures for the large number of students and non-permanent staff. Many actions that cannot be presented in detail here were undertaken to correct this problem. In brief, we focused our efforts on improving the communication towards this category of staff by organizing mandatory information meetings for all newcomers and dedicated collective meetings with the Direction team. We also initiated a systematic monitoring of the fate of the PhD students, the post-docs, and the long-term (> 1 year stays) non-permanent technical or administrative assistants. We considered crucial to understand how their work at BPMP helped them to find a job thereafter. The graph below summarizes the current outcome of this monitoring, considering the 64 people who left BPMP between 2014 and 2019. It shows that among the 58 colleagues we have kept contact with, 49 (85%) have presently a job related to research or training in plant sciences (either in public academic institutions such as research organizations or universities, or in private companies). Among the 13 people having a permanent academic position, 2 were recruited at BPMP. Finally, only 7 people are presently unemployed and 2 have a job unrelated to plant sciences.



- *Communication strategy*. Another area where significant actions were pursued is the internal and external communication of BPMP. On this aspect, the main event of the period was the total renewal of our Website by a group headed by the Administrative manager, with both extranet (https://www1.montpellier.inra.fr/wp-inra/bpmp/) and intranet pages. We defined a procedure and installed a dedicated team for ensuring a continuous update of the website. We included in the intranet numerous resources, services and applications (agenda, scientific protocols, administrative guidelines, safety information, reports of Councils meetings, room and equipment booking, etc.) that are crucial for running all common facilities and optimizing the work of all staff. In addition, we carried on publishing a weekly electronic information letter, which sorts all the scientific, technological, administrative and organizational information useful to the whole unit. All the letters (since 2006) are stored on the intranet and can be browsed for a specific topic.

Most importantly, BPMP had a unique opportunity in 2018 to evaluate all aspects of its organization and life. This resulted from the national initiative of INRA to monitor the psychosocial risks and the quality of life in its research units. Since 2016, the various research units of the Montpellier INRA centre are subjected to this action (3-4 units/year). Therefore, all BPMP staff (regardless of his/her employer) was proposed to answer in January 2018 an anonymous survey consisting of more than 100 questions dealing with quality of life and management in the unit. A large majority of BPMP staff (78%) answered the survey, allowing a good feedback evaluation of the Unit.

The graph below shows the synthetic outcome of the survey on the major items investigated. A majority of BPMP staff (between 60 and 80% depending on the items) has a positive judgement (green area) on all items, with the exception of the one dealing with "External" means outside BPMP, mainly at the local administration and employer levels. The latter item somehow reflects that conditions for career evolution are judged unsatisfactory. Concerning information, communication, and collective life, the outcome is also generally positive (around 60% of satisfaction). However, a more refined analysis of the various criteria included in this item (not shown here) indicates that most people in the unit (75%) point out a clear lack of communication and interaction between research teams. This is certainly a consequence of the large scientific independence and functional autonomy of these teams. Of particular interest, the working conditions are judged very good or excellent by all people, especially the non-permanent ones (>80% of satisfaction). This is a strong reward to all BPMP staff that has been involved in supervising the general organization of BPMP, and in particular all people involved in the actions listed above that aimed at improving these working conditions.



The 2018 survey discussed above also led to initiatives for improving social interaction between BPMP teams. Therefore, a "conviviality meeting" open to all is organized every month, following one of our scientific seminars by an external invited scientist. This is an opportunity to further discuss with this scientist, and also to have informal interaction between all BPMP staff. In addition, the 2018 scientific retreat (see below) was an opportunity to propose a more ambitious social program than in previous events. To this end, the Administrative manager applied to the 2018 "Psychosocial risks and quality of life" call for proposals of CNRS, and obtained specific funding for ensuring what happened to be a unique and unprecedented friendly event (games, cocktail, dinner and party).

Scientific animation

The general scientific animation of BPMP first relies on many exchanges during meetings of the various unit committees or in weekly lab meetings of the teams. It is also promoted by a series of specifically dedicated actions, as listed below.

- *Scientific seminars*. All over the year, scientists are invited to give seminars (in general on Thursdays afternoon). Since 2014, 138 seminars were given (https://www1.montpellier.inra.fr/wp-inra/bpmp/actualites/), mostly from scientists outside BPMP, with a high proportion of them coming from abroad (58%). The vast majority of these seminars are open to the whole Montpellier scientific community. A specific group of people in BPMP (the Seminar Committee) has been handling very efficiently these aspects for more than 10 years (collecting the proposals from the research teams, taking care of the trips and housing of the invited scientists, booking of seminar rooms, advertising the seminars). A special budget is allocated to this Committee (about 10 k€/year on average). Each year, all incoming PhD students are also invited to give in house seminars to present their research project to all BPMP staff.

- *Thematic full-day seminars*. To focus more precisely on specific topics that appear important for the scientific strategy of BPMP, 3 full-day seminars have been organized since 2014. Each of these events included a couple of seminars given by invited external specialists of the chosen topic, several seminars by BPMP scientists, and a round-table for exchange and conclusion. The selected topics were: Cell signaling, Quantitative genetics, and Biotic interactions.

- *Scientific retreats*. These events were held twice during the current contract period (2015 and early 2019). The retreats took place outside of Montpellier and gathered for a couple of days all BPMP staff (BPMP building is closed at this occasion to make sure that everyone will attend the retreat). The main aim is to perform an in-depth exchange on the scientific strategy of the unit. Each event included specific seminars by all teams, and thematic round-tables. The 2015 retreat was designed to allow the new research teams created at that date to present their new projects and have them discussed with all BPMP staff. The 2019 retreat was more specifically devoted to the preparation of the unit's project for 2021-2025.

Parity; scientific integrity; health and safety; sustainable development and environmental impacts; intellectual property and business intelligence.

Parity

With 39 women for 42 men, the parity is almost complete within the permanent staff of BPMP. At the management level, a significant evolution occurred in 2015 following the creation of new research teams. Overall, more women have become team leaders. As a consequence, the current team leaders Committee comprises 6 women (43%) as compared to 3 (30%) in 2014. Finally, recruitments or arrivals of permanent staff since 2014 are almost equilibrated, with 9 men and 8 women.

Scientific integrity

These questions are addressed at two different levels. At the unit level, precise guidelines are given to all new comers concerning handling of lab books, storage and saving of raw data, traceability of all biological materials, etc…This is done through a "welcome book", and by a mandatory information meeting. At an upper level, all PhD students are requested to follow a specific training on this topic, organized by the Doctoral school.

Health and Safety

The health and safety care is a crucial activity in BPMP. Therefore, it has been decided since 2012 that this should be taken in charge by a strong team of volunteer staff. This team currently comprises 5 people (including radioprotection, see organization chart), who have official letters of assignment validated by the Director and the four Institutions to which BPMP belongs. As a consequence, the amount of work performed by this team is important, and most aspects of health and safety care are performed at the BPMP level, most often without the support of corresponding services of the Institutions.

The work of this team is guided by precise procedures. First, health and safety rules have been explicitly defined in an annex of our Internal Regulations Statement. Second, we use the INRA system, and in particular the corresponding computer application OPPI (Outil Principal de Pilotage de Prévention de l'INRA), which provides rigorous and comprehensive methods to deal with these problems. The OPPI also generates all relevant warnings when the needed actions are not performed in due time. Third, we have set up our own procedures, which involve: regular meetings between the Safety team and the director (at least 4/year), the elaboration of an annual report and an annual action plan, a dedicated meeting of the Unit Council for discussing this report and action plan, mandatory health and safety information meetings for all incoming staff and student (in French and in English), a mandatory re-education training for all permanent staff (every 4 years), specific documents and clearance procedure for allowing the work out of regular working hours and during week-ends. We also have identified all required financial investments (the overall annual budget is around 10 k€/year), and interact with the research campus direction for ensuring safety within the building or relevant medical monitoring of the staff.

Collectively, these team, actions and procedures have proven their efficiency. Fortunately, no serious safety incident has been recorded over 2014-2019, and most importantly, among the main items considered for the psychosocial risks and quality of life survey, health and safety displays the second highest satisfaction score (close to 80%, see figure above). This clearly demonstrates the efficiency of our health and safety team.

Sustainable development

Two main actions towards reducing wastage have been developed since 2014. First, an ambitious energy saving plan has been implemented to replace by LED systems all the lighting devices of our growth chambers (nearly 80 k€ budget). Second, the Technical team has defined very efficient methods for eliminating, through appropriate means, all out of order equipment and of all plastic waste.

Intellectual property and Economic Intelligence

One of the team leaders (C. Curie) is specifically in charge of following BPMP projects with private partners or organizations aiming at supporting innovation (SATT, Pôle de compétitivité). Nevertheless, most of our activity on these topics relies on the support provided by the corresponding services of the institutions to which BPMP belongs (CNRS, INRA, SupAgro, University). In particular, our main focus on basic research does not require that we develop on our own an ambitious and specific Intellectual Property strategy. Also, the rationale governing our partnerships with private companies predominantly relies on their scientific relevance, and not on their potential for generating financial resources. Thus, we did not feel the need for a specific plan for Economic Intelligence. However, our increasing interest in crops as plant models may change these views in the future.

Research Quality Assurance

Significant efforts were devoted to improve our procedures for Research Quality Assurance. Besides actions targeting the research teams (reliability and traceability of genetic resources, seed storage, correct handling of lab books), the dedicated committee conducted an ambitious update on the inventory and monitoring of all technical and scientific equipment (more than 700 items). Furthermore, work on Research Quality Assurance was markedly increased in our platforms and common technological facilities (ISO9001 certifications for MSPP and PHIV, and award of the “Fiabilité des mesures” certification to the Stable Isotopes Analysis facility, see Annex 3). The emphasis put onto Research Quality Assurance is further illustrated by the recent (2019) reinforcement of the dedicated committee with a full-time administrative assistant (see organization chart above).

FIVE-YEAR PROJECT AND STRATEGY

1- SWOT analysis

Strengths ✓ A broad expertise in transport and stress physiology and biophysics ✓ A renewed set of research teams ✓ A diversified and upgraded set of equipments and platforms ✓ A good national attractivity and international visibility	***Needs for improvements*** ✓ Integration of biotic and other soil components in plant nutrition ✓ Links with private partners
Opportunities ✓ Diversification of plant models and technological developments for crop studies ✓ Structuration of the Montpellier site (I-SITE) ✓ Pressing needs for solutions to climate change	***Threats*** ✓ Uncertainties of fundamental research funding results in large resource variations and heterogeneities between teams ✓ Local policy favoring studies on tropical crops

2- Structure, workforce and scientific orientations

1) WORKFORCE AND COLLECTIVE ORGANIZATION

1) Elaborating the project:

The main lines of the present project were elaborated through several rounds of concertation run over the last 12 months. A first step was, from spring 2018 on, the identification of the future director and management team, based on an intention letter and prospective discussions during a general assembly. A scientific retreat gathering all BPMP staff over 2 days in January 2019 provided a unique opportunity for exchange and strategic reflection. This event included oral presentations of individual team projects, round-tables on six prospective topics, each being prepared and animated by a pair of BPMP scientists, and a plenary discussion of BPMP organization and scientific perspectives. Prior to these discussions, BPMP platforms had presented their activities and perspectives in the frame of weekly seminars. Later on, a series of 5 thematic meetings between group leaders allowed finalizing the strategic orientations described below. The main lines of this project were also presented to the laboratory council in May 2019.

2) Strengths and overall organization

The present project reflects the marked scientific reorganization of BPMP over the last few years. Our laboratory was able to attract external teams (Plasticity, Influx), and let emerge a new generation of internal teams and leaders (HoNuDe, TICeR, KaliPHruit, FeROS). Also, it was agreed that, because of a lack of leader, two former teams (ELSA, Metal Toxicity) would stop their activity, their members being redeployed in other teams. Thus, the BPMP project will be structured around nine scientific teams, the technical organization remaining essentially unchanged (see last paragraph). While the great scientific and budget autonomy of teams ensure their creativity, we will mobilize collective strength on transversal and institutional challenges that go beyond single team objectives.

2) SCIENTIFIC STRATEGY

Plant biology has experienced a continuing revolution over the last decades. Molecular and cellular mechanisms at work throughout plant life can now be described with a great accuracy, mostly in model plant species, and BPMP has contributed to this effort. Also, ever more powerful genomics methods such as deep genomic sequencing, GWAS, and genome editing are now generalized to numerous plant species including major crops. Thus, there is virtually no limitation for developing fundamental mechanistic studies in these species, and explore their biological originality or relevance to applied objectives.

While BPMP was founded nearly 50 years ago, its chance and major challenge have been that its historical research field, the mineral and water nutrition of plants under abiotic constraints, has constantly remained a highly disputed scientific topic. From the beginning, the long-term societal demand directed to research institutes such as ours has been to improve crop yield with a minimal and sustainable use of inputs.

On top of this challenge, climate change has recently been raising increasing emotion and political concerns in our societies. From a biological perspective, climate change exacerbates the environmental constraints experienced by plants through a primary increase in atmospheric CO_2 and temperature, which in turn induce more extreme climatic events. More unexpectedly, elevated atmospheric CO_2 (eCO_2) and high temperatures alter the plant nutritional status through as yet unknown mechanisms. Thus, in conjunction with a steady rise in human population, climate change puts agriculture and food security under strong pressure.

In view of these pressing challenges, BPMP wants to mobilize its efforts on exploring and proposing solutions to many facets of climate change:
- To secure plant productivity under environmental constraints and degraded environments
- To preserve the nutritional value of plants, and therefore food quality
- To work on solutions for carbon storage in the soil.

For this, our strategy will be to strengthen the exploratory potential of BPMP in fundamental plant biology. Through this, we aim at developing high standard studies in plant biology, using the Arabidopsis thaliana model when it is the most relevant, but with a stronger emphasis on model crops than in the past. Although it is a narrow way, we believe that it is the best strategy to operate a relevant transfer of knowledge from the study of biological mechanisms (our primary mission) to applied objectives (our ultimate contribution).

3) MAIN SCIENTIFIC OBJECTIVES

BPMP has prioritized four major fields of investigation in which significant scientific contributions can be brought during the next years. Note that the long-standing interest and expertise of BPMP teams in membrane transport will underlie and consolidate most of this research.

1) Root functions

Studies on root functions have been and will remain central at BPMP. This research addresses a wide range of topics, mostly investigated through the lens of plant nutrition. These include the mechanisms of water and nutrient uptake and their coordination with adaptive responses of root growth and development to environmental signals (Aqua, HoNuDe, Integration, KaliPHruit, Plasticity). Also, studies on interaction of roots with beneficial (myccorhizae, diazotrophic bacteria) or pathogenic microorganisms will be further developed (TICeR, FeROS). Root growth is also an important target for improving carbon storage in the soil (Integration).

With respect to our previous activities, a specific attention will be brought to specialized root structures and types such as root hairs, cluster roots (lupin), and nodal roots (cereals). On a longer perspective, root functions will have to be considered in relation to the soil. Specific programs on root adaptation to calcareous soils, on the mode of action of bio-fertilizers or on root exudation may be adequate to address these difficult issues.

2) Environmental stress signaling

The mechanisms of environmental signaling will be investigated, both at the cell level and over long-distances, mostly using roots as a model of choice.

First, these studies will address how cells are able to perceive multiple stress-related signals (nutrient or water availability, plant hormones), with complex roles of receptors, membrane transporters or transceptors. These signals (*e.g.* NO_3^-) and the downstream role of various second messengers (H^+, Ca^{2+}, ROS) will be addressed using specific biosensors (Aqua, HoNuDe, Influx, Integration, KaliPHruit, MeMo). Understanding how signals are transduced through membrane dynamics with protein complexes being mobilized in nanodomains or endocytic vesicles will also be a key aspect (Aqua, TICER). Signal transduction also involves master protein kinases/phosphatases that are extensively studied by several teams (Aqua, HoNuDe, KaliPHruit, MeMo). Our aim will be to understand how these molecular actors serve in the regulation of specific targets such as transcription factors or membrane transport proteins.

Second, long-distance signaling related to water or N availability will be investigated using original split root systems (Aqua, HoNuDe). Multiple types of signals (miRNAs, peptides, major phytohormones, ROS) are potentially involved. Their identification will rely on a fine combination of physiological, transcriptional and genetic analyses.

We note that many of our studies point to a major signaling role of ROS. Because of the generalized involvement of these molecular species in stress responses and nutrient homeostasis, characterization of their function and more generally of the plant redox status will represent a major transverse axis of BPMP research.

3) Genome expression reprogramming

Our aim is to analyze how environmental stimuli induce profound plant transcriptional reprogramming by acting on gene regulatory networks. More than one half of the research teams (Integration, HoNuDe, FeROS, Plasticity, KaliPHruit, Aqua) develop projects with such questioning, thereby using a set of complementary approaches.

Systems biology, which offers a robust theoretical frame to address these issues, is now more widely used at BPMP. This approach will be further developed by implementing epigenetic and chromatin accessibility data (FeROS, Integration), in addition to more classical gene expression or protein interaction data. Frontline studies on cell-type specific epigenetics and inter-individual expression variability will also be strengthened in the Integration team.

Besides, BPMP will further enhance its capacity to study the function of transcription factors per se. In complement to chromatin interaction studies, specific protein regulation mechanisms that involve post-translational mechanisms (see MSPP platform) or interaction with protein partners with multiple functions (e.g. chromatin remodelers) will be investigated (FeROS, Integration, MeMo). These mechanisms can play a key role in linking environmental perception and signaling to the function of master transcriptional regulators.

4) Integrated responses of plants to complex environmental stresses

BPMP teams want to move their focus from plant responses to punctual stresses in simplified experimental systems to a comprehensive analysis of plant stress responses under more realistic contexts.

One first aim of these studies will be to understand how plants can perceive and respond to combined stresses. GWAS and systems biology have proved to be highly efficient for revealing emerging signaling mechanisms and response networks that had been missed in earlier studies and may be of major agronomical importance. A typical context is nutrient stress interaction (Zn vs. P vs. N) which provokes unanticipated responses in Durum wheat and Arabidopsis (HoNuDe). In relation with climate change, BPMP teams will also investigate how high temperature interferes with plant nutrition in calcareous soils (MeMo and others).

A second aim will be to analyze integrated responses of plants to complex stresses. One example is how environmental stresses like drought, salinity or high CO_2 alter directly or indirectly the functioning of osmocontractile cells such as stomatal guard cells (Influx TICeR)) or cells determining leaf rolling in cereals (TICeR)). Beyond plant growth, BPMP teams will also investigate the plant nutrient status as it ultimately determines food product quality. Several BPMP teams address the mechanisms that underlie the negative effects of eCO_2 and high temperature on plant nutrient assimilation or grape K^+/pH homeostasis, respectively (Integration, FeROS, KaliPHruit, MeMo).

4) SCIENTIFIC AND TECHNICAL APPROACHES

To reach its scientific objectives, BPMP will engage strong efforts in the following approaches.

1) Articulated work on model and cultivated plant species.

In recent years, BPMP has moved from exclusive studies on Arabidopsis to projects on an expanding set of plant species with two main objectives: (1) to explore novel biological systems with questions that cannot be addressed in Arabidopsis, (2) to open new routes to translational biology. Time has come to a more rational and focused planning of such developments with several complementary goals.

(i) The impacts of climate change on plant nutrition as a key target of translational biology approaches. BPMP wants to cover main issues related to drought (Aqua, Plasticity), eCO2 (FeROS, Integration, MeMo), and high temperatures (KaliPHruit, MeMo). Our aim is to explore solutions to the impacts of these threats on water and nutrient uptake, and their allocation to the edible parts of the plants, using relevant crops (maize, rice, grapevine, tomato), or by parallel studies on model and cultivated plant species (*e.g.* Arabidopsis *vs.* Durum wheat).

(ii) Fundamental and long-term studies in crops potentially leading to new openings in translational biology. Efforts of BPMP in this direction will mostly rely on cereals. The teams will address some specific classes of transporters (HKT; TICeR), their highly differentiated root systems (Aqua), or their pronounced response to composite stresses (HoNuDe).

(iii) Arabidopsis as an intermediate step in translational biology approaches. This approach represents a strength and originality of the BPMP teams. For instance, the Plasticity team would like to introduce cluster roots in new plant species, based on its fundamental work in lupin. Here, Arabidopsis may be used as a model to explore issues related to species barriers. While work by KaliPHruit aims at improving grape quality, this teams routinely establishes the functionality of grapevine K^+ transport systems using Arabidopsis expression.

Because of the important issues and difficulties of these approaches, we have been keen on securing them in large scale projects (ERC HyArchi) or in collective projects ensuring a robust partnership (Flagship Programs CalClim and eCO2THREATS; LabCom SOLSTIS). Overall, sharing efforts and experience on crops will represent a major axis for future cooperation between teams. After an initial phase of exploration and diversification, our aim would be to constrict the number of crop species investigated at BPMP. Nevertheless, a challenge for the plant growth facility team will be to secure the simultaneous production of plant materials from these species.

2) Interdisciplinary research and method developments

In support of its historical expertise in membrane transport and stress physiology, BPMP will further develop interdisciplinary approaches as a key component of its integrative projects.

(i) Mathematical modelling of solute and water flows, from cellular to organismal levels will represent one first direction. These so-called Functional-structural plant models aim at integrating water flows in root system architecture (Aqua), ion transport between guard cell subcellular compartment (Influx), auxin transport in lateral root primordia (Plasticity), ion loading from phloem to mesocarp or cell wall plasticity, both in grape berry (KaliPHruit).

(ii) Imaging techniques will be used to monitor the water and ionic status of complex organs. For instance, elemental imaging allows to access tissue profiles of iron concentration and speciation (MeMo). Several teams will use MRI to monitor the water status of plant tissues in relation to their hydraulic properties (Aqua), local pH (KaliPHruit, Influx) or intracellular NO_3^- and Cl^- (Influx).

(iii) Systems biology will be further developed at BPMP. The discovery power of this approach will be enhanced along two complementary directions (i) improved mathematical analysis based on newly developed machine learning algorithms (HoNuDe) (ii) optimized plant phenotyping to test candidate master-regulators with a high throughput. We believe that BPMP has strong potentialities on the latter point, which will also be instrumental in GWAS. Along these lines, BPMP teams will pursue their efforts in developing new instruments for microfluidics (Integration), pressure chamber techniques (Aqua) or novel microscopic setups (PHIV platform).

Several institutional contexts have been identified for developing these programs: the University of Montpellier and its departments of physics and mathematics and associated LabEx (Numev) offer a privileged environment. Also, INRA has launched a Métaprogramme in Predictive Biology, to which BPMP wishes to contribute. For this, BPMP will have to reinforce its internal strength and organization. This will require the continuing involvement of BPMP researchers in specific interdisciplinary areas together with reinforced partnerships with specialized chemists, physicists and mathematicians. Relevant international collaborations (Chile; CNRS LIA with University of New York) will also be promoted. Finally, we believe that the recruitment of an engineer in computer coding (profile is under discussion) may provide a higher autonomy to the laboratory, and smoother development of projects requiring specific programming.

3) Scientific platforms

These equipments provide a unique competitiveness to BPMP in several main scientific lines of our project.

(i) Root phenotyping. Due to our major focus on roots, we will pursue the development of tools for automated phenotyping, and thereby implement the current HIRROS platform. This will include new device for 2D phenotyping of larger root systems grown on filter paper (PhenoROOT). Image analysis remains a central challenge for tracing the dynamics of root system architecture and monitoring root growth rates. Novel approaches involving machine learning will have to be used. Another challenge for BPMP will be to improve our capacity in root tissular and cellular phenotyping, to track root hairs or lateral or cluster roots under development, for instance. While new imaging equipments may be required, these efforts will open new perspective in functional analysis and genetic screening. The efforts will be developed within a well-identified frame of collaborators specialized in root phenotyping (4PMI, AgroEcology, INRA, Dijon) and we will seek additional support through interdisciplinary CNRS or MUSE programs.

(ii) Imaging. Important challenges in imaging (super-resolution microscopy, biosensor technologies) will require specific efforts. In the latter case, BPMP wants to develop biosensors for ROS and ions (potassium, phosphate, ammonium, nitrate, iron, manganese,…), mostly by expressing in plants sensors, such as ClopH, that have been developed in animals or microorganisms (Influx, KaliPHruit, MeMo). Due to the generalization of this approach, it might also be relevant to enhance our local set of instruments for imaging these sensors. Accordingly, a CNRS technician position was requested, to reinforce the PHIV platform and allow its current engineer to spend more time in method developments.

(iii) Mass Spectrometry Proteomic Platform (MSPP): With respect to proteomics, quantitative analyses of protein modifications (phosphorylation, ubiquitination) will remain a major focus. While the potential demand of BPMP teams in proteomics is important, the synergy between these teams and MSPP can still be improved. Another challenge will be to secure the competiveness of MSPP by renewing its major mass spectrometry instrument in the next two years. A good local positioning within the Montpellier Proteomic Pole (BioCampus)(IBISA calls), but also a support from our national institutions will be crucial.

(iv) Electrophysiology. Based on its robust experience, BPMP aims at further developing in planta measurements, on stomata in particular (Influx, TICeR). A second objective will be to couple electrophysiological and imaging approaches (Influx, TICeR).

(v) Ion and stable isotope analysis. BPMP possesses several key equipment and platforms in this area. For optimization and better visibility at local and national levels, it might be judicious to merge these activities within a common structure. In these respects, and because of an anticipated retirement, the stable isotope analysis activity will need support through a new recruitment. An assistant engineer position has been requested from INRA.

(vi) Bioinformatics. We all face an increasing demand in bioinformatics for analyzing proteomic, chip hybridization, RNAseq or New Generation Sequencing (NGS) data. GWAS also requires specific statistical analysis in relation to complex genotypic data. Due to this increasing and diversified demand, BPMP feels a strong need for better structuring its activities in this field. While some data treatments can be externalized, we will also have to reinforce and continuously adapt our capacity for in house analyses. First, the individual needs of each team and the competencies present in the teams or technical platforms will have to be confronted. Following this step, the training of dedicated staff, and possibly the identification of a new technical recruitment profile and of a dedicated platform can be anticipated.

4) Quality and traceability

A better implementation of these approaches throughout our various activities is important but collectively demanding. First, efforts to better organize our common biological resources and accredit methodological facilities will be pursued. Second, a pragmatic approach will be undertaken using emblematic actions to demonstrate the overall benefits of rational planning and organization of large scale projects (e.g. ERC projects).

5) BPMP IN ITS ENVIRONMENT

Taking into account guidelines of its four institutions, BPMP will articulate its research and teaching activity at various levels

1) To assert the scientific positioning of BPMP within the Montpellier site.

Because of its local policy, our site undoubtedly provides a key level for exploring solutions to climate change from a plant biology perspective. Thus, we will play an active role in scientific animation of local structures such as LabEx Agro, Scientific Department of the University, and the future “Agriculture and Environment” pole of the MUSE I-SITE. Beyond individual team projects, Flagship or collective programs led by BPMP (eCO2THREATS, CalClim) and addressing impacts of climate change on plant production are typical examples of actions to be pursued at this level.

Complementary to this, we will be keen on defending the relevance of our positioning in fundamental plant biology, in both model plants and crops. As in the past, scientific interactions with neighboring laboratories will be instrumental for enlarging the scope of our studies (*e.g.* mathematical modelling, food quality, plant-soil interactions). In particular, the emergence of an Institute of Plant Health in Montpellier may open new cooperation opportunities, within the frame of the LabEx Agro, to develop projects on plant-biotic interactions

Our imaging and proteomic platforms are well-integrated within a multi-institutional technical service consortium (UMS BioCampus), mostly linked to the Medical Biology pole. Yet, the Montpellier site needs a better integration of equipments and platforms that are specific to the Plant Biology and Agronomy community. BPMP is willing to contribute to this evolution by bringing in its centralized and highly visible capacities in root phenotyping, electrophysiology, and ion analyses.

Last but not least, BPMP will pursue its efforts in teaching, at both University of Montpellier and Montpellier SupAgro. Beyond lectures, that are mostly delivered by colleagues from these institutions, all teams can provide a significant offer in research training (Undergraduate and Graduate students). This may be a typical means for reinforcing our links with SupAgro. For now, the positioning of BPMP within the Biology-Agrosciences department is excellent but may be weakened in a few years, due to anticipated retirement of its director (B. Touraine).

2) To contribute to national scientific challenges

At the national level, BPMP will defend its rank in fundamental plant biology and contribute to major challenges carried by the INRA Plant Biology and Breeding section and the CNRS Biological Science Institute. For instance, BPMP will work at finding an original positioning within the promising area of predictive biology, through a dedicated INRA Metaprogramme for instance. Studies on root functions (phenotyping) and plant responses to multiple stresses also fall with the scientific priorities of INRA. In line with the 4 per 1000 initiative, BPMP is also willing to bring original insights into the links between carbon metabolism and root growth. Finally, several BPMP projects clearly fit with the interdisciplinary research agenda promoted by the CNRS.

3) To defend BPMP international visibility.

As in the past, BPMP wants to bring its research activity at the highest international level. Beyond well-established collaborations in Western countries (Europe, USA), the teams now explore with a special emphasis links with Asia (China, Japan). Beyond research collaborations, student training using dedicated instruments (*e.g.* CSC fellowships) or through our bi-annual summer school MISTRAL will be crucial. Our relations to the South will specifically target Maghreb and Vietnam, and a support from the Labex Agro is anticipated for all these international actions.

4) To enlarge BPMP industrial partnership

BPMP has substantially improved its industrial partnership over the last few years, mostly through bilateral projects (Solvay, Syngenta, SATT AxLR). Yet, its activity in this field needs to be consolidated. One direction is to establish a close partnership between BPMP and a private company using a specific ANR call (LabCom). Contacts have been established with Frayssinet, a company selling organic fertilizers. We are discussing the opportunity of establishing a joint public-private research structure, hosted in our institute, to investigate the mechanisms of biostimulation and the role of soil micro-organisms. Beyond its partnership dimension, this project would fill an obvious scientific gap in our activities and strengthen studies on plant soil-interactions. On a longer term, we anticipate that our reinforced efforts on crops will open new partnership opportunities with private companies.

6) HUMAN RESOURCES AND INTERNAL ORGANIZATION

While scientific programming and local positioning represent major challenges for BPMP future, several internal organization items will also require a specific attention,

1) To consolidate the collective functioning of the unit.

Over the last period, BPMP has developed a robust collective organization. The research teams receive efficient support from two well-organized and autonomous common service teams and from a wide spectrum of technical platforms and facilities. Our economic model, which has been challenged by but resisted to a recent drop in research funding, provides a fine balance between individual team initiatives and collective solidarity. Yet, daily life and harmony between all staff categories require a continuous attention. Special care will be put into the animation of our multiple management committees which address key pillars of our collective activity, such as common equipment and plant health and production. In addition, a strong collective mobilization will be required to pursue our energy saving plan.

2) To handle a steady flow of retirements

Due to inevitable ageing of our staff, we anticipate the retirement of about two scientists and one technical assistant per year over the next five years. Thanks to the scientific dynamics of BPMP, and to its attractivity, this should not impede our scientific dynamics. However, a particular attention should be paid on personals of common facilities (plant growth, laundry) and platforms (stable isotopes). Corresponding positions have already been requested at INRA and CNRS. Our future representation at University of Montpellier and Montpellier SupAgro is also one of our weak points. BPMP will have to be a source of proposals when several professor positions have to be renewed in next years.

3) To secure small team development

Our scientific organization and management have been deeply renewed in recent years but are not fixed. Some teams have pointed to the potential emergence of novel group leaders (A. Martin, H. Rouached). The human strength of these teams and other small size teams (Influx, KaliPHruit) will have to be reinforced by targeted recruitments. We note that the arrival of a young CNRS Research associate (S. Cortijo) in the Integration team already contributes to this dynamics.

Finally, we are aware that soil micro-organisms play a key role in plant nutrition and that BPMP activity in this field requires additional strength. Hosting news colleagues or even a new team on this topic will be a strategic priority.

ANNEXES

Annex 1: Letter of commitment

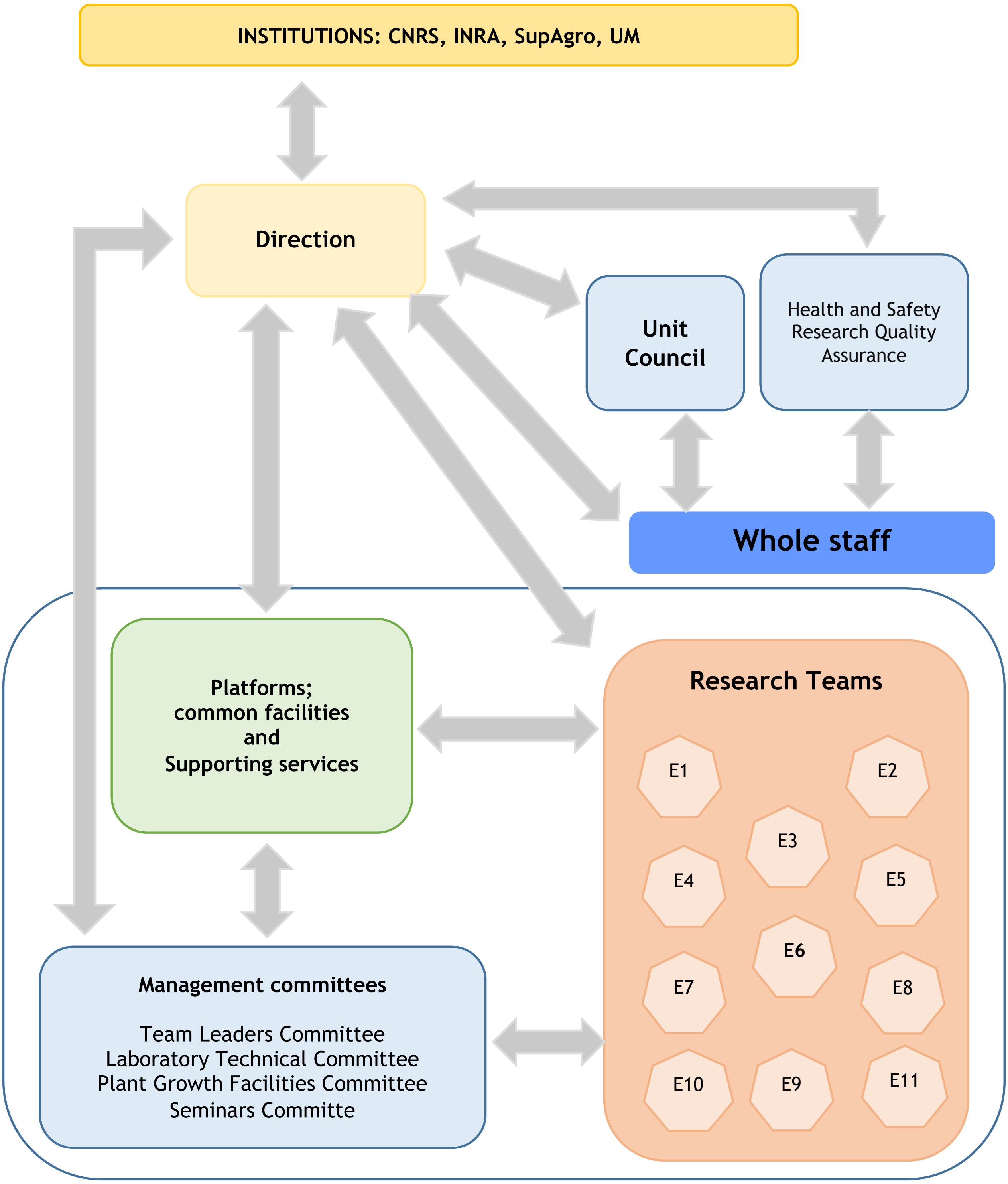
Montpellier, 30 June 2019

I, the undersigned, GOJON Alain, Director of the research unit Biochimie et Physiologie Moléculaire des Plantes certify, by the present letter, the exactitude of all data presented in this self-evaluation file, containing the self-evaluation document, and two Excel files named « Current contract data » and « Next contract date ».

BIOCHIMIE ET PHYSIOLOGIE
MOLECULAIRE DES PLANTES

Alain GOJON
DIRECTEUR

Annex 2 – Functional Chart



Annex 3 – Equipment - Platforms

Introduction

BPMP has been developing a strong organization for collective use of technological facilities, resulting in the creation of several formalized common platforms and workshops (see the organization chart in the self-assessment document, and https://www1.montpellier.inra.fr/wp-inra/bpmp/en/platform/). These platforms and workshop are largely open to all research teams of BPMP, and to external users, either through collaborations, or through charged analytical services.

The list and description of the platforms and workshops/common facilities are provided below, distinguishing between the two platforms that have an official positioning within institutional mutualized organizations at the local or national level (PHIV and MSPP), and the workshops that are specific to BPMP.

Platforms

Histocytology and Plant Cell Imaging Platform (PHIV)

The Histocytology and Plant Cell Imaging Platform (PHIV : http://phiv.cirad.fr/) is a common platform between our research unit (BPMP) at the « La Gaillarde » campus (PHIV-La Gaillarde) and the UMR « Amélioration Génétique et Adaptation des Plantes (AGAP) based at the « La Valette » CIRAD campus (PHIV-La Valette). As early as 2003, PHIV became a founding member of the «Montpellier Ressources Imagerie » (MRI, UMS Biocampus) regional platform which is recognized by IBiSA, the consortium in charge of organizing at the national level the platforms dedicated to biological, health and agronomical sciences. MRI itself is a member of the France BioImaging (http://france-bioimaging.org/) and has received the certification ISO 9001-2015 NFX 50-900.

The missions of PHIV are, (i) to provide equipment from the sample preparation to observation with microscopes, (ii) to maintain all the machines in good running conditions, (iii) to help the users to design their experiments, to train them to use the materials and help them in data interpretation, (iv) to develop new methods of imaging and (v) to communicate on its knowledge and to participate in teaching.

MRI-PHIV-Lavalette and MRI-PHIV-La Gaillarde are managed to be fully complementary, both for materials and competencies. MRI-PHIV-Lavalette is more oriented on molecular histochemical approaches and has a highly valuable expertise in plant anatomy, whereas MRI-PHIV-La Gaillarde is more involved in the development of *in vivo* imaging methods (fluorescence-based methods, especially the development of ratiometric biosensor probes).

Describing where proteins are localized in the plant (at both organ and cellular scales) is crucial to understand their functions. This type of questions is addressed by a majority of BPMP researchers. Therefore, important investment was made at MRI-PHIV-La Gaillarde in this sense, with the acquisition of a Leica SP8 laser scanning confocal microscope and a Zeiss Apotome epifluorescent microscope. In addition, a third of the research teams either use or even develop biosensor probes. These molecules, which can be encoded genetically to drive their subcellular localization, permit to perceive variations in different cellular components like pH, ROS, calcium, Rho-GTPase activity, nitrate, iron, … To follow this growing interest, our ratiometric microscope was upgraded with a new light source. **In addition, we are developing a prototype to combine the usage of bioluminescent biosensors probes with long time course acquisition (several days) on a vertical imaging set up**. This project was funded by the CNRS "défi "instrumentation aux limites" program in 2018.

During the last period (2014-2018), PHIV was associated to 24 publications from BPMP (see Annex 4). PHIV is also in charge of a class on plant cellular imaging dedicated to the Master students at the University Montpellier, and contributes to the MISTRAL summer school (organized by BPMP). PHIV also organizes each year two to three on-site training periods on microscopy, molecular histology (immunocytology and in situ hybridization), image analysis for graduate students and permanent scientists from the various research institutes. PHIV staff belong to several network (GDR imabio (CNRS), Rmui (INRA), BIOCCI (biosensors in Occitanie), and co-organize every year the "Plant Imaging in Occitanie" with colleagues in Toulouse (LIPM/LRSV), Perginan (LGDP) and Banyuls (UPMC), to federate the knowledge in plant imaging in the new region.

Finally, the users of PHIV are not restricted to the BPMP and AGAP research Units. In the future, PHIV plans to federate all the research groups from Montpellier working in the field of plant and agronomical sciences, and which need imaging methods for their research. **Therefore, BPMP and AGAP, through their investment in PHIV and MRI, play an important strategic role in the organisation of the microscopy and imaging facilities opened to the whole plant and agronomical sciences community from Montpellier.**

Mass Spectrometry Proteomic Platform (MSPP)

MSPP presentation

The MSPP (Mass Spectrometry Proteomics Platform) of the BPMP laboratory belongs to the "Pôle Protéome de Montpellier" of the UMS Biocampus (PPM, https://www.ppm.cnrs.fr/). PPM regroups regional technological facilities in proteomics analyses, is recognized as an IBiSA platform at national scale and is certified ISO9001:2015. Its management and coordination are ensured by a scientific manager (P. Marin,) a deputy scientific manager (V. Santoni, DR INRA at BPMP, Head of the MSPP) and a technical manager (M. Séveno) together with executive and steering committees. Strategic decisions are based on recommendations of a Scientific Advisory board made of external experts.

The MSPP staff regroups 1 DR 50%, 1 IR 80%, 1 IE 50%, 1AI 100 %, 2 TR 100%. MSPP disposes of two nano-HPLC (Thermo Fisher Scientific) coupled to mass spectrometers (Maxis HD Bruker and Q-Exactive + Thermo Fisher Scientific). The software offer relies on bioinformatical tools dedicated to protein identification, label-free quantification of proteins and statistical analyses.

Being hosted by BPMP, the objectives of MSPP are to provide proteomic tools in order to fit with the objectives of the laboratory and this includes the capacity to identify and quantify membrane transporters and component of signaling pathways, in their native forms and in their modified forms. Indeed, post-translational modifications contribute to major regulatory mechanisms investigated at BPMP.

Projects handled during 2014-2019

In addition to BPMP users, MSPP is open to external users, including academic laboratories and private companies. Projects submitted to MSPP are selected on the basis of their technical faisability and the presence of adequate funding which must cover the cost of analyses. As a whole, during the period 2014-2019, MSPP handled an average of 10 projects/year: 75 %, 13 % and 12 % from regional, national and european origins, respectively. During this period, the collaborations and services on demand represented 97% and 3% of the projects realized by MSPP, respectively. Mean incomes were 24,000€ per year including grants on internal projects.

Internal projects represented less than 10% of projects realized by the platform during the period and were dedicated to methodological developments including 1) the implementation of a targeted mass spectrometry strategy (Parallell Reaction Monitoring) for the identification and quantification of lowly abundant proteins 2) the proteomic analysis of protein ubiquitination 3) the comparison of softwares for large scale and label-free protein quantification. Developments 1) and 2) were supported by INRA grants (BAP department)

19 papers including at least one member of MSPP were published over the period.

Teaching and formation

During the evaluation period, MSPP was involved in numerous thematic schools and days: - MISTRAL (Montpellier International School on ion and water TRAnsport in pLants, 2014, 2016, 2018) - Inter-organisms school (2014) - Research school of MassProt network (2016) - Technological workshops by Biocampus Montpellier (2014) - Young researchers club SFEAP (2015). MSPP also supervises students, engineers and post-docs as part of internal or collaborative projects (2 masters, 2 post-docs during the period) and ensures internal formation of new members of MSPP (N. Berger and V. Demolombe)

Perspectives

The scientific objectives are to pursue the implementation of mass spectrometry strategies and bioinformatics developments in order to provide the plant scientific community opportunities to address **large-scale** and **targeted proteomics** projects of **native** and **modified** forms of proteins with particular emphasis on the **quantification of ubiquitination**.

SWOT analysis: Strength Good momentum of MSPP (new technologies)/ Weakness: Only one mass spectrometer adequate to most of projects and that may soon become out of date / Opportunities: Purchase of new equipment (Biocampus, IBISA, INRA, CNRS)/ Risks: Less calls for tenders available due to national and regional restructuring.

Workshops/common facilities

Plant culture facilities

Since our move to our current building in 2005, BPMP has been consistently developing its plant culture facilities to satisfy the increasing demand from its increasing scientific and technical staff. These facilities now include: 13 growth chambers (around 6-10 m^2 each) for growth of plants on soil, hydroponics or *in vitro* under standard conditions, 7 smaller growth cabinets for growth and experiments under specific non-standard conditions, 3 growth cabinets with sophisticated environmental control for specific experiments under tightly controlled conditions, and one S2 greenhouse of 350 m2 (see details on all these facilities at: https://www1.montpellier.inra.fr/wp-inra/bpmp/en/platform/plant-culture/).

Heterologous Expression and Electrophysiology Platform (EHEV)

Molecular analysis of membrane transport of water and mineral ions in plants requires the identification of proteins responsible for these different transports. A plant has several hundreds of different proteins performing this function, often specific (e.g., for potassium, nitrate, iron, water, etc ...). Therefore, one must be able to isolate them and study them separately.

The heterologous expression and electrophysiology platform of BPMP is a unique device in France dedicated to this type of work for plant proteins. It allows in particular characterizing in detail the activity of membrane transport proteins by measuring the electrical signature of their activity, due to the fact that most nutrients are transported in an ionic form. This platform, which gathers seven recording set-ups for various analyses in heterologous context or in native plant cells, is largely open to outside users.

EHEV has hosted during the last period (2014-2019) 10 PhD students and researchers from other French Labs or foreign Institutions (e.g from Italy, UK, Sweden, Tunisia, India) to run their research projects. In addition, EHEV was involved in several trainings. These include the practical module of the thematic school “ion transport in plants” for Master students from the University Montpellier (every year), the practical module of the electrophysiology “E2M” thematic school INRA-CNRS-INSERM (in 2018), and practical modules of the MISTRAL International summer school (organized by BPMP in 2014, 2016 and 2018).

During the last period, the EHEV platform was associated to 20 publications.

Stable Isotopes Analysis (AIS)

The Stable Isotopes Analytical workshop (AIS: Atelier des Isotopes Stables) was created in 1990 and located in BPMP to provide an INRA national facility for stable isotopes analysis. It aims at analyzing the isotopic content (mostly ^{15}N and ^{13}C non-radioactive isotopes) of samples coming from INRA laboratories but also from other laboratories, including foreign institutions. It also supports the research projects of BPMP investigating the mechanisms of the nitrogen and carbon nutrition of plants, with a particular focus on transport and metabolism of N and C compounds. Improving mineral nutrition of plants, especially N nutrition, is a major issue for modern agriculture that must find solutions to increase fertilizer use efficiency. This is an absolute requirement for limiting nitrate pollution in the environment, without lowering crop production. The AIS contributes to this goal through the development of methodologies allowing quantitative assays of N uptake and utilization by plants. To do so, the AIS is equipped with two dedicated on-line Isotope Ratio Mass Spectrometry systems (On-line IRMS, Elementar). The AIS has the certification “Fiabilité des mesures” since 2015.

Multi-Elemental Analyses Service (SAME)

SAME, created officially in July 2016, is devoted to the atomic quantification from plant samples (roots, leaves, fruits, seeds, cellular fluids…) as well as from soil material. Basically, the samples (dry material) are digested by acidic hydrolysis that disrupts molecular structures to release their atomic content (phosphorus, calcium, magnesium, sodium, iron, zinc…). After this hydrolysis step, the chosen elements are quantified by atomic emission spectrometry. The service proposed by the platform includes the hydrolysis of the samples, the choice of elements and the quantification by spectrometry. The equipment is composed of a microwave with 2x24 reactors for the acidic digestions and a microwave-plasma atomic emission spectrometer (MP-AES, Agilent) equipped with an autosampler.

Root Phenotyping Platform

The Root Phenotyping Platform, created in 2017, is dedicated to kinetic and non-destructive analysis of seedling Root System Architecture of many species including Arabidopsis, rice, tomato, rapeseed, and Medicago grown in vitro. The main objective of the platform is to develop approaches, tools and methods to characterize the impact of different growth environments on root growth and development. This includes osmotic stresses, nutrient deficiency or excess or biotic interactions alone or in combination. These analyses provide the basis for further genetic and molecular physiology approaches.

Seedling are grown *in vitro* on 120x120mm (Small plates: SP) or 245x245mm (Large plates: LP) Petri dishes. The phenotyping facility includes a self-contained imaging unit that can handle up to 72 LP and 200 SP at once with imaging frequency ranging from 2 to 24 hours.

The imaging automat is located in a dedicated growth chamber allowing temperature, hygrometry, photoperiod, light intensity being adjusted according to user's requirements. It is equipped with a 16 Mpixel linear camera, a telecentric objective and collimated LED backlight for images at 10µm resolution with high contrast (even for thin and almost translucent Arabidopsis roots). Collected images are stored on long term on a secure data storage system.

Images are currently analyzed through semi-automated. A fully automated pipeline integrated in the OpenAleaLab suite (https://team.inria.fr/virtualplants/software/) is under development. The Root Phenotyping Platform is backed to B&PMP research programs but is open to the scientific community through local, national and international collaborations.

GeneAtlas® Transcriptomic analysis platform

BPMP has set the GeneAtlas System from Affymetrix Company in 2014. This system allows performing, quickly and on site, all steps necessary for transcriptome data acquisition. Gene expression data are acquired by hybridization of microarrays (organized by indivisible strips of 4 microarrays). To date, the system provides access to gene expression of model plants such as *Arabidopsis thaliana*, Medicago, soybean and rice. For example, Arabidopsis microarrays provide expression data for more than 28.000 genes, including precursors of miRNAs.

The simplicity of the system allows everyone to investigate plant transcriptomes whenever needed. The protocol leading to the synthesis of labeled cDNA is a classical molecular biology experiment. Hybridization of microarrays takes place in a dedicated 'Hybridization Station'. A first robotic device called 'Fluidics station' allows washing and staining of microarrays. Then, an 'Imaging Station' is used to image the 4 microarrays in parallel. Finally, a computer dedicated to data analysis can be used. On this computer, the Expression Console software enables to assess the quality of the microarrays. The software also controls a Transcriptome Analysis Console (TAC) which allows data normalization followed by an analysis leading to the identification of differentially expressed genes when comparing treatments 2 by 2 (Statistical analysis using t.test).

This tool allows to bypass some limitations: the budget required for hybridization of a microarray is reduced, the system is less complex and avoids relocating individual experiments on external platforms (control of protocols, timing, data, etc …). With the widespread use of Systems Biology approach, involving an iterative and systematic approach in which each transcriptomic analysis feeds a regulatory network model, this tool allows BPMP teams to efficiently unravel signaling and gene regulation networks that control homeostasis of nutrients in *Arabidopsis thaliana* and other model plants.

Self-assessment documents of the individual research teams

Aquaporin team
Self-assessment document

Name of the team concerned by the current contract: Aquaporins
Name of the team concerned by the next contract (if different): Water, signaling and hydraulic architecture
Acronym of the current contract: Aqua
Acronym of the next contract (if different):
Team leader for the current contract: Christophe Maurel
Team leader for the next contract: Christophe Maurel

RESULTS

1- Presentation of the team

Introduction

The *Aquaporin* team was initiated as an ATIP junior research group in 1999 and has continued since 2002 as one of constitutive research groups of BPMP. The team has a long-standing experience and recognition in the study of membrane water transport in plants.

Team's workforce and means

The team currently gathers 6 permanent scientists (Y. Boursiac, CR INRA; C. Maurel, DR CNRS, A. Martinière, CR CNRS, P. Nacry, CR INRA, V. Santoni, DR INRA, L. Verdoucq, CR CNRS) and 1 research engineer (C. Tournaire-Roux, IR INRA). While three of these scientists (YB, AM, PN) have joined the group between 04-2013 and 01-2014, one of our early colleagues (Doan-Trung Luu, CR CNRS) moved in 2017 to another BPMP team (TICER) to pursue his studies on rice. Since long, the team has been lacking of a specific technical support (see previous AERES report). Although it is not yet satisfactory, the situation has been slowly improving. Since 2016, Xavier Dumont, a technical assistant of the Technical and Logistic team, dedicates 25% of his working time to the team, and contributes to plant genetic transformation and characterization. In May 2019, an INRA technical engineer (AI) will be recruited to work at 50% on the team research programs, while spending the other half of his/her working time on the root phenotyping platform.

Since 2014, 6 post-docs from 5 nationalities (J. Bellati; M. Calvo Polanco, M Rosales Villegas, Z. Shahzad, N. Tang, L. Rishmawi; 180 months in total) and 6 PhD students from 4 nationalities (M. Bellanger, C. Champeyroux, H Chu, V. Protto, O. Rodrigues, M. Smokvarska) have worked or are still working in the group. We also have hosted two professors on sabbatical leave (M. Cappadocia, Canada; T. Romeis, Germany) and have trained 18 master students and 10 undergraduate students (BTS, L3).

The *Aquaporin* team has received substantial support from our national research agency (PRC: HydroRoot 2012-2015; PRCE: MIRGA 2016-2020; PRCI: ABAqua 2019-2022), from the EU (2 Marie-Curie Fellowships : ORYZAQUA 2012-2014, DROUGHTROOT 2016-2017; ERA-CAPS: RootBarriers 2014-2017; ERC Advanced: HyArchi 2018-2023), from a private partner (Syngenta: Aquapartner, HydroRoot) and through competitive grants from the CNRS (Turgomap), INRA (EauDissect, iRhoBot, AgreenSkills) and the Labex Agro (Watermarkers). While all these supports have contributed to the scientific and human dynamics of the team, the partnership with Syngenta has been exemplary: we have received long-term support and confidence from our private partner to develop fundamental research on root hydraulics and aquaporin regulation. Outputs from one of these programs (HydroRoot) have been critical for a subsequent ERC application. As many other research groups of our department, we have suffered in 2017-2018 from a transient lack of support which has slowed down the human dynamics of our group. With recent success from ANR (ABAqua) and ERC (HyArchi), we now have clear perspectives for the next five years.

Scientific policy

Because of their continuous growth and development in ever changing and sometimes challenging environmental conditions, higher plants have to constantly maintain their water status. The control of water flow in the soil-plant-atmosphere continuum is crucial in these respects. The *Aquaporin* group studies the fundamental mechanisms that mediate the transport of water in plant tissues, and its regulation in response to environmental or developmental cues. Aquaporin water channels which mediate most of transmembrane water transport play a major role in these processes.

From initial studies on the function and regulation of aquaporins in *Arabidopsis*, the team has enlarged his scope over the years with two main objectives:

1) to embrace a broader and more integrative view of plant water relation.

During the last five years, this diversification has taken two main directions:

- *to study aquaporin regulation in the context of cell signaling.* Using a bottom-up approach, we have investigated the molecular and cellular mechanisms that act upstream of aquaporins. These studies allowed us to address fundamental aspects of plant signaling under water deficit (hyperosmotic stress, ABA) or excess (hypoxia).
- *to enlarge our view of plant hydraulics.* First, we have developed genetic approaches to discover novel molecular components which, besides aquaporins, govern tissue hydraulics. Second, we have investigated in closer details the significance for water uptake of the root system architecture, in conjunction with hydraulics. Third, we have initiated mathematical modelling approaches to integrate all these components.

2) to diversify the plant species under study,

During its early phase (2000's), the team has put much effort to implement a large set of physiological and biophysical approaches for studying the multiple facets of water transport in the model plant Arabidopsis. In more recent years, the group has tried to apply its expertise to a larger set of plant species, and crops in particular. While openings to translational biology represent one main reason, we also believe that a move towards crops may be beneficial, as it could open original perspectives in fundamental biology (see project). From 2012, we initially focused our efforts on rice as a model cereal and an emblematic species for the Montpellier plant science community. Paradoxically, we failed in receiving significant grants for such studies (in particular from our local Labex). More recently, we explored maize, as a foundational model in genetics and plant water relations. Support from ANR and ERC has now established this species as a key model in our team for exploring the concept of root hydraulic architecture.

2- Research products and activities for the team

Scientific track record

We report here on main results, along the two main directions (see above) explored by our team.

Cell signaling and aquaporin regulation

1) Aquaporin interactomics *(Bellati et al., 2016, Champeyroux et al. 2019)*

A screening approach based on co-immunoprecipitation (co-IP) and quantitative proteomics has revealed that the whole interactome of *At*PIP2;1, one of most abundant plasma membrane aquaporins in *Arabidopsis* roots, contains at least 400 detected interactants. Targeted biochemical and functional validations have revealed the putative role of lipid metabolism (phospholipases D) and protein kinase receptors in regulating aquaporins [1]. A special focus was also put onto CASPLBs and CASPLDs, two subclasses of homologs of CASP proteins. CASPs are membrane proteins that are specifically expressed in a membrane domain delineating the Casparian strip of endodermal cells. While a CASPL was found to regulate *At*PIP2;1 in Xenopus oocytes, no significant role in root hydraulics could be detected [2].

In relation with our early work on leaf hydraulic regulation [3], we also have investigated molecular interactions that are dependent on the phosphorylation status of *At*PIP2;1 C-terminal tail. We showed that two14-3-3 proteins of *Arabidopsis* (GRF4, GRF10) can physically interact with *At*PIP2;1 and modulate the function of *At*PIP2;1 in *Xenopus* oocytes, with more pronounced effects on phosphomimetic than on phosphodeficient forms of the aquaporin [4]. The significance of this interaction was validated in the context of circadian regulation of rosette hydraulics (see below).

2) Aquaporin phosphorylation *(Vialaret et al., 2014 ; Li et al., 2015 ; Prado et al., 2019)*

Our earlier studies have shown that phosphorylation represents a major mechanism for swift aquaporin regulation. Our concern here was to further understand the integrated role of this post-translational modification in plant hydraulics and water relations. As to leaf water transport, we showed that the phosphorylation level of *At*PIP2;1 and the rosette hydraulic conductivity (K_{ros}) oscillate in parallel during several days under various light regimes. Overall, this work establishes an original circadian mechanism, mediated through post-translational modifications of proteins [4]. Remarkably, this control is dependent on 14-3-3 proteins (including GRF4 and GRF10; see above).

Another set of studies deals with stomatal responses to ABA and a bacterial elicitor (flg22), which are strictly dependent on *At*PIP2;1 function [5, 6]. We could show that the OST1/SnRK2.6 protein kinase which plays a major role in plant response to ABA and flg22 is able to phosphorylate *At*PIP2;1 at a unique site of its first cytosolic loop. Using mutated forms of *At*PIP2;1, we further showed that this phosphorylation is required for activation by ABA and fgl22 of water and H_2O_2 transport in the guard cell membrane and, furthermore, for the associated stomatal closure response (see text below).

In conclusion, we have established the functional role of individual phosphorylation sites in physiologically distinct contexts. This was made possible by the expression in transgenic plants of phosphodeficient or phosphomimetic forms of the prototypic *At*PIP2;1 aquaporin. In addition, we have identified new classes of protein kinases that phosphorylate aquaporins (RLKs, SnRK2s). In contrast, the calcium-dependent protein kinase CPK7 regulates root hydraulics without acting directly on aquaporins [7].

3) Cell Biology of aquaporins and osmotic signaling *(Hosy et al., 2015; Wudick et al., 2015; Wang et al., 2015; Chu et al., 2018; Martinière et al., 2019; Platre et al., 2019)*

Previous work from the team showed that root epidermal and cortical cells respond to stimuli such as salt or salicylic acid by internalization of surface-expressed PIPs through a signaling pathway involving reactive oxygen species (ROS) [8]. This hydrogen peroxide (H_2O_2)- induced process was described in more detail [9] using Fluorescence Correlation Spectroscopy (FCS) or evanescent wave microcopy (Single Particle Tracking). In addition, we showed that H_2O_2 induces a transfer of *At*PIP2;1 from the plasma membrane, through early endosomes, until late endosomes/ prevacuolar compartment. The whole process corresponds to a cellular sequestration rather than a targeting towards degradation.

An improved super-resolution microscopic technique called single particle tracking Photo-Activated Localization Microscopy (sptPALM) was used for the first time in plants to monitor the lateral mobility of proteins at the plasma membrane (LTi6a, *At*PIP2;1) and tonoplast (*At*TIP1;1). In contrast to the other membrane proteins, *At*PIP2;1 showed a low lateral mobility, was confined to membrane microdomains and its mobility was enhanced upon a hyperosmotic stress [10].

The molecular bases of ROS production under hyperosmotic stress and the primary cellular processes that they target were further investigated in the Arabidopsis root [11]. It was shown that a RESPIRATORY BURST OXIDASE HOMOLOG (RBOH) pathway and an additional pathway involving apoplastic ascorbate and iron can both account for ROS production upon hyperosmotic stimulation. sptPALM further showed how ROS control specific diffusion and nano-organization of membrane cargo proteins. In particular, ROS generated by RBOHs initiated clustering of *At*PIP2;1 and its removal from the plasma membrane. This process is achieved in part by clathrin-mediated endocytosis [11]. Our knowledge in sptPALM was also used to study the molecular dynamics of small GTPases, which are an essential component of osmotic signaling pathways [12].

In conclusion, work by our team has allowed describing with an unprecedented resolution the diffusion modes of aquaporins at the surface of plant cells and the processes involved in their endocytosis, in both normal and stress conditions. These results have a broader significance as they identify some of earliest membrane responses of plant cells to osmotic or oxidative stress.

4) Aquaporins and stomatal functions *(Grondin et al., 2015; Rodrigues et al., 2017)*

Surprisingly, the role of aquaporins during stomatal movements had remained hypothetical until recently. This topic was addressed by a reverse genetic approach. We showed that epidermal peels of *pip2;1* knock-out (KO) plants showed defects in stomatal closure in response to ABA or fgl22, whereas stomatal responses to other stimuli (light, CO_2) were normal [5, 6]. This role is associated to an *At*PIP2;1-dependent increase in guard cell water permeability due to phosphorylation at Ser121 (see above). Based on the information that some plant aquaporins including *At*PIP2;1 can transport H_2O_2, we investigated a putative role of this aquaporin in ROS signaling. An ABA- or fgl22-induced accumulation of ROS was monitored using a dye (DCF) or a genetic reporter (Hyper). Transgenic expression in *pip2;1* plants of a phosphomimetic form of *At*PIP2;1 at Ser121 indicated that activation of the protein is required for H_2O_2 influx.

In conclusion, this work shows that *At*PIP2;1 plays a dual hydraulic and signaling role in guard cells. In addition, we uncover a novel role of aquaporins in plant responses to biotic stress. A related work had shown the contribution of *At*PIP1;4 to PAMP-triggered immunity [13].

5) Aquaporins and plant reproduction *(Wudick et al., 2014; Novikova et al, 2014; Beauzamy et al., 2016)*

Whereas most plant aquaporins show broad expression patterns, reproductive organs can express organ-specific isoforms. Here, we have investigated the expression and function of two pollen-specifc TIP isoforms, *At*TIP1;3 et *At*TIP5;1. Using fluorescent protein reporters, we established the exclusive expression of *At*TIP1;3 and *At*TIP5;1 in the vacuolar membrane of the vegetative cell and two sperm cells, respectively. The maturation of the vacuolar apparatus of these small cells had never been addressed with such accuracy. Noticeably, concomitant genetic inactivation of *AtTIP1;3* and *AtTIP5;1* altered pollen viability, specifically under stress conditions [14]. In addition, we have finalized a work developed in cooperation with Russian colleagues (International programme of the CNRS), on aquaporin function in *Vicia faba* [15]. A role of vacuolar aquaporins during germination and more specifically during embryo growth was proposed.

Tissue hydraulics

If one excludes punctual work on leaf hydraulics (Prado *et al.*, 2019; Sade *et al.*, 2014; see above), most of the work presented in this section was performed in roots. Our aims were (i) to identify the multiple root components that determine water uptake and (ii) to understand their broad regulation by environmental stimuli.

1) Stimulus-induced regulation of root hydraulics *(Li et al. 2016; Sanchez-Romera et al., 2014)*

To search for possible interactions between the transport of water and two major nutrients, potassium (K^+) and nitrate (NO_3^-), we have performed an extensive phenotyping of KO mutants for channels or transporters carrying each of these elements. The most striking result was a marked reduction of root hydraulic conductivity (Lp_r) in *nrt2.1* mutants that lack the high affinity transport system for NO_3^- absorption. A thorough characterization of this mutant and of the effects of external nitrate on Lp_r led us to propose that signaling processes that depend on NO_3^- accumulation in shoots control root hydraulics [16]. The role of NRT2.1 in these processes remains elusive.

2) Endodermal barriers *(Pfister et al., 2014 ; Wang et al., 2019)*

This work was developed in the frame of a European network coordinated by Prof. D. Salt (Univ. Nottingham, UK). We took the chance of evaluating the Lp_r of various genotypes altered in the differentiation of endodermal barriers (*horst1*, *esb1*, *Shengen3*) [17, 18]. To our surprise, mutant phenotypes could not be directly interpreted with respect to their specific alteration in lignin or suberin deposition. We rather revealed a novel regulatory mechanism whereby sensing of barrier integrity in mutant plants leads to a rebalancing of water and mineral nutrient uptake, compensating for breakage of Casparian strips [19]. This rebalancing involves both a deactivation of aquaporins and a deposition of suberin to limit ion leakage. Thorough phenotypic analysis of an extended set of mutants grown in soil under control, drought or salinity conditions has also revealed the effects of root conductivity and selectivity on growth of aerial parts in these stressing conditions (Calvo-Polanco et al., submitted).

3) Quantitative genetics of root hydraulics *(Shahzad et al., 2016; Tang et al., 2018)*

Since several years, we have been exploring *Arabidopsis* natural populations as a genetic resource for unraveling novel facets of root water transport [20]. From 2012, we embarked, in collaboration with the group of Olivier Loudet (INRA Versailles), in the ambitious project of cloning genes controlling root hydraulics in *Arabidopsis*. Our first strategy was to use biparental recombinant populations. In brief, we identified 14 hypothetical QTLs, of which 4 could be confirmed using NILs. Two of these were selected by positional cloning. This work was performed by Dr Z. Shahzad, a young scientist with exceptional tenacity and scientific qualities.

The first gene, named *Hydraulic Conductivity of Root 1* (*HCR1*), encodes a new RAF-type MAP3K protein kinase [21]. In brief, HCR1 was shown to mediate root responses to hypoxia, in relation with K^+ availability, thereby acting as a negative regulator of Lp_r and root aquaporins. The molecular mode of action of HCR1 was dissected: HCR1 specifically accumulates in the presence of K^+ and absence of oxygen (O_2). HCR1 enhances the abundance of RAP2.12, a key ERF VII transcription factor that is stabilized under O_2 starvation and triggers the core anaerobic transcriptional response. We have proposed that HCR1 phosphorylates RAP2.12 in planta and thereby potentiates the most early plant responses to O_2 starvation [21].

The second gene that was isolated encodes Snf1-related protein kinase 2.4 (SnRK2.4), a homolog of OST1/SnRK2.6 (see above). A dual function of SnRK2.4 was uncovered. Under resting conditions, SnRK2.4 stimulates Lp_r probably by direct phosphorylation of PIPs on their conserved B loop site. In contrast, SnRK2.4 mediates the inhibition of Lp_r induced by ABA, by promoting C-terminal dephosphorylation of PIPs by an unknown mechanism. More generally, SnRK2.4 mediates part of ABA effects on root growth and stomatal movements. While SnRK2.4 was initially thought to be specifically responsive to osmotic stress, our work reveals a new role in ABA signaling (Shahzad et al., in revision).

In view of our positive results with recombinant mapping populations, we turned to Genome Wide Association Studies (GWAS) as a more direct approach for isolating genes controlling Lp_r. Phenotyping a Hapmap panel of > 300 natural accessions pointed to two significant associations, which could be validated using knock-out mutants [22]. One of these genes encodes XYLEM NAC DOMAIN 1 (XND1), a transcription factor that acts as a negative regulator of xylem differentiation and as a consequence of Lp_r. In relation with these effects, XND1 enhances drought stress tolerance. Interestingly, XND1 also mediates the inhibition of xylem formation by the bacterial elicitor flagellin and counteracts plant infection by the root pathogen *Ralstonia solanacearum*. Thus, genetic variation at *XND1*, and xylem differentiation contribute to resolving the major trade-off between abiotic and biotic stress resistance in Arabidopsis.

In conclusion, these new genetic approaches have been very efficient in identifying novel genes controlling Lp_r. It is of note that, although our genetic screen was performed under normal conditions, it has revealed mechanisms that allow plant adaptation to combined or opposing environmental stresses.

4) Modelling and MRI *(Buy et al., 2018)*

The modelling approach aims at understanding how local hydraulic conductivities (whether radial or axial) and/or the architecture of the root system determine the integrated water transport properties of the whole root system. A first model of *Arabidopsis* root hydraulics was developed in collaboration with C. Godin's team (CIRAD-Inria, Montpellier). This model, named HydroRoot, relies on the integration throughout root architecture of elementary hydraulic components. Each component integrates local radial and axial water flows. The architectural reconstruction of the root system is based on a tridimensional multi-scale tree graph (MTG). The model is parameterized using architectural, tissular and physiological data that were experimentally determined in our group. One major finding is that Lp_r computed from the model is dependent on root architecture. In parallel, mutant plants altered in xylem formation (*irx3*, *esk1*) or aquaporin function (*pip2;1* × *pip2;2*) were phenotyped for their root hydraulics and architecture. Our ultimate goal is to explain, by means of the HydroRoot model, the primary alterations that generate the hydraulic phenotype of these plants.

A second model named EauDissect and developed in collaboration with Prof. D. Felbacq (CNRS/University of Montpellier) specifically addresses the process of radial water transport. This 2D model uses partial differential equations to determine the preferential trajectories of water molecules in a digitized, realistic root cross-section. While classical composite models draw a strict parallel between apoplastic and transmembrane paths, the model points to a profound interconnection of the two paths.

Finally, we took advantage of a Flagship project (APLIM) of the Labex Agro to evaluate the use of Magnetic Resonance Imaging (MRI) for monitoring water transport in plants. While our attempts to use various kinds of nanoparticles as contrast agents have been unsuccessful, we contributed to developing a novel method for measuring sap flow [23].

5) Root development *(Boudon et al., 2014 ; Lobet et al., 2015 ; Rosales et al., 2019)*

Water uptake is determined by both the architecture and hydraulic properties of the root system, which overall define the root hydraulic architecture. The team has addressed the integration at whole root level of mechanisms that determine the root hydraulic architecture, and its regulation in response to water stress. *Arabidopsis* plantlets, grown in vitro or under hydroponic conditions under different levels of water stress (PEG8000) revealed a double response of root growth to water stress: an increase in lateral root formation and elongation was observed under mild water stress, whereas these parameters were reduced with higher stress treatments. A similar dual response was observed for Lp_r. Analysis of both root architecture and hydraulics under exogenous-ABA treatments and in ABA-biosynthesis and signaling mutants revealed that ABA acts as an integrator of root responses to water stress (Rosales et al., 2019).

The response of root growth to water deficit was also investigated in maize, within an ANR project led by C. Salon (INRA, Dijon) using plant grown in newly developed rhizotrons (rhizotubes). Our group is now focusing on the kinetic response of roots to drought, using a transcriptomic approach and derived gene networks. The aim is to find master regulator genes which will be tentatively validated by analyzing the function of their Arabidopsis homologs. Work on maize is pursued in the frame of an ERC Advanced grant named "HyArchi: Targeting Root Hydraulic Architecture to Improve Crops under Drought". This projects aims at identifying novel molecular and genetic components that govern the root hydraulic architecture of maize plants under water stress (see below).

Finally, our group contributed to improving the root phenotyping capacity of our department. P. Nacry supervised the development of a high throughput phenotyping platform which is dedicated to in vitro grown plants. This platform will be instrumental for exploring the genetic bases of root adaptive responses to a large array of environmental factors. Since automated image analysis remains the bottleneck in this kind of approach, P. Nacry also contributed to its improvement in the frame of multipartite collaborations.

Key events

Quantitative genetics of root hydraulics

Work of the team on this topic represents a major breakthrough with respect to previous studies in the field of plant water relations. The causes of this success are multiple:
(i) an innovative approach that was centered on Lp_r, a key trait of plant roots that had not been explored before. This trait has become accessible thanks to the development of a pressure chamber device for semi-automated measurements (our initial article relies on the phenotyping of ~ 50,000 plants over 4 years)
(ii) a long term public-private partnership (>4 years) involving ANR and our private partner, Syngenta
(iii) an exemplary collaboration with geneticists (Dr. O. Loudet, INRA, Versailles)

This work has had broad scientific and public impacts. It led to two high-rank publications (Shahzad et al., Cell, 2016; Tang et al., 2018, Nature Commun.) and a third publication to come (Shahzad et al., Plant Cell, in revision). In recognition of this outstanding *work*, Dr Z. Shahzad was awarded a special prize from the French Academy of Sciences (Major French Advances in Biology 2017). Finally, our press release led to several interviews in public broadcasts (France, Switzerland) and the national press.

ERC Advanced grant 2017.

Beyond a sign of international recognition, this project provides two main openings for our research:

To explore the many facets of root hydraulic architecture. HyArchi provides a unique opportunity, in terms of duration, budget and human resources, to investigate how this architecture evolves in time and space by integrating local and systemic signals that communicate water availability (see project).

To move to a cultivated species. This project is based on significant proofs of concept in the model plant *Arabidopsis*. It is of note that, in the early 2000's, the *Aquaporin* team has pioneered molecular and physiological studies of plant hydraulics in *Arabidopsis* by adapting biophysical techniques initially developed in crop species. Our advances have opened novel avenues for forward and reverse genetic analysis of plant hydraulics. We are now excited by the challenge of applying our knowledge to maize to explore novel genetic strategies for its improvement. In addition, cereal root systems display remarkable differentiation and adaptive capacities that open promising avenues for fundamental research.

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FIVE-YEAR PROJECT AND STRATEGY

1- SWOT analysis

Strengths	**Weaknesses**
- A broad range of approaches from molecular to whole plant levels - Unique expertise in plant hydraulics and related genetic analyses - High national and international recognition - Strong financial support with high recruitment capacity	- Lack of full autonomy in key approaches: quantitative genetics, mathematical modelling - Lack of technical support
Opportunities	**Threats**
- Capacity to work on two complementary plant models - Long term support for developing fundamental biology in a cultivated species - Strong partnership for multidisciplinary research	- Multiple tasks of the group leader - A possible lack of internal leadership in pole 1 (Cell signaling)

2- Structure, workforce and scientific orientations

A new team's name and organization that reflects enlarged scientific objectives.

After 19 years of existence, the *Aquaporin* team has progressively moved from exclusive studies on the function and regulation of plant aquaporins to a broader field dealing with water transport and environmental signalling in plants. Our new team's name reflects this evolution: "Aqua: Water, signaling and hydraulic architecture".

Our main objective will be to understand how the availability of water in the environment determines its uptake and conservation by the plant.

More specifically, we will adress the following questions:
- How do plants perceive water availability and transduce this signal using ROS or hormones (ABA)?
- What are the adaptive changes of plant architecture and hydraulics to water availability ? How are they coordinated and contribute to maintaining the plant water status?

In relation to these two set of questions, the team will be structured in two main poles named "Perception and molecular signaling" and "Root hydraulic architecture", respectively. We anticipate, however, important syngergies between individual projects, which will be positionned along a continuus spectrum of keywords such as cell signalling, aquaporin regulation, tissue hydraulics, long distance signalling and root development. The main (but not exclusive) focus of the two poles will be on roots.

Two plant models, Arabidopsis plants and young maize seedlings carrynig an embryonic root system, will be used in our studies. Whereas Arabidopsis will remain a model of choice for cell signalling studies, we expect much about the complementarity of the two plant models for studying root hydraulic architecture.

In addition to all current members, the Aqua team will be reinforced by two new permanent staff: an INRA technical engineer (AI) to be recruited in May 2019 and a CNRS scientist, Enric Zelazny, who will be moving from I2BC (CNRS, Gif-sur-Yvette) to BPMP by the end of 2019.

Perception and molecular signaling

We will adress the following continuum of signaling processes triggered by water availability.

The spatio-temporal origin of water stress in roots, perceived as a change in turgor pressure or osmotic potential, will be investigated by a combination of biophysical measurements (cell pressure probe), mathematical modelling and molecular markers [TurgoMap project (2018-2019); Yann Boursiac with PhD student: Yunji Huang, ongoing CSC application].

The signal transduction of hypertonic stress, which leads to a rapid (<10 min) accumulation of ROS in the Arabidopsis root will be dissected by two complementary approaches. First, we will further characterize a regulatory network that involves the small GTPase ROP6. Second, we will identify new components of osmotic stress signaling using genetic variation observed in Arabidopsis natural populations. Furthermore, the dynamics and organization of signaling nanodomains will be determined by a combination of interactomics and super resolution microscopy [CellOsmo ANR application; Alexandre Martinière, with PhD student: Marija Smokvarska].

The role of membrane microdomains in the control of water uptake will be addressed using the HIR (Hypersensitive Induced Reaction) family of membrane proteins as a molecular entry. By intertacting with PIPs, HIRs may function as scaffolding proteins allowing the recruitment of PIPs in specific membrane microdomains. Besides, the Arabidopsis HIR protein family will be characterized at the cellular level in terms of localization, dynamics, and microdomain identity to shed new light onto their cellular role and reveal potentially new physiological functions [NUTRIR ANR project (2019-2022); Enric Zelazny with postdoc].

The H_2O_2 signature of plants exposed to combined stresses will be investigated in line with previous work on the role of aquaporins in transmembrane transport of H_2O_2. The scope of our studies will be enlarged by considering the role of redox homoestasis and by phenotyping both the pathogenicity and hydraulics under combined osmotic and biotic stresses (*e.g. Ralstonia solanacearum*) [L. Verdoucq in collaboration with JP. Reicheldt, LGDP, Perpignan, and F. Vailleau, LIPM, Toulouse].

The molecular regulation of aquaporins by ABA will be investigated to answer how the different hormone sensitivities and actions of ABA on root and guard cell hydraulics are realized. Detailed molecular analysis of prototypic ABA signaling modules targeting aquaporins will be employed in combination with phosphoproteomics and expression of deregulated signaling components in Arabidopsis. [ABAqua ANR project (2019-2022); C. Tournaire-Roux, L. Verdoucq, V. Santoni, Y. Boursiac, C. Maurel, with postdoc].

Root hydraulic architecture

In these studies, we will consider three root traits: root system architecture, generated through continuous growth and branching, water transport, and environmental signaling. The first two traits yield the root hydraulic architecture. We will investigate how this architecture evolves in time and space by integrating local and systemic signals that communicate water availability. Our ultimate goal is to understand the genetic and physiological components that determine the integrated strategy of plants for optimizing root water uptake under water deficit conditions. These studies will be performed in the context of ANR Mirga (ends in 2020) and ERC HyArchi (2018-2023).

In a first set of studies, we will describe the combined effects of water deficit on maize root architecture and root hydraulics considering both their time and dose-dependency and differential effects of homogeneous vs. heterogeneous water deficits (V. Protto, ERC PhD). The latter effects will be investigated based on an experimental split root system whereby one half of the root is exposed to water stress whereas the other half is exposed to control conditions. These approaches will be coupled to mathematical modelling (ERC postdoc) and key methodological developments in hydraulic and root architecture phenotyping (Y. Boursiac; P. Nacry).

In complement, we will develop two innovative molecular discovery approaches. First, in line with our previous studies in Arabidopsis (Tang et al., 2018), we will perform GWAS on a maize natural variation panel to identify novel genes controlling root hydraulics (L. Rishmawi, ERC Post-Doc, in collaboration with A. Charcosset, INRA, Gif-sur-Yvette). Second, transcriptomic analyses of split-root systems, to be performed in both Arabidopsis and maize, will be used to identify molecules (*e.g.* hormones, miRNAs) involved in systemic signaling and governing root growth and hydraulics (P. Nacry with M. Maillard, CDD Mirga, and ERC post-doc). Genetic validation in maize will be performed using knock-out mutants generated by genome editing (CRISPR-Cas9) (C. Tournaire-Roux, in collaboration with P. Rogowski, ENS, Lyon).

Ultimately, these studies will enable identifying or engineering genotypes with contrasting root hydraulic architectures. Corresponding full-grown plants will then be evaluated for their short and long-term performance under drought, using high-resolution phenotyping facilities (PhenoArch, LEPSE, INRA).

Conclusion and perspectives

A steady involvement in methodological developments.

It is of note that several team members (V. Santoni, A. Martinière, P. Nacry) are in charge of common plaforms or facilities. This reflects the contribution and efforts of the team in developping novel methods in proteomics (protein modifications, interactomics), imaging (Biosensors such as ORp1 roGFP2; super-resolution microscopy, MRI), and root architecture phenotyping. In addition, the HyArchi project will be supported by key methodological developments, such as a new set of pressure chambers for measurements under highly controlled local root environments (Y. Boursiac). Improved root image analyses will be coupled to mathematical modelling to represent local and systemic effects of water on root hydraulic architecture.

A strong group dynamics and an extended network of collaborations

In the last two years, the team has secured several grants to support the present project. The recruitments expected from these grants and two additional permanent staff arriving in 2019 will augment our working force. In complement, we will have to consolidate a rich network of collaborators in maize genetics and physiology (A. Charcosset, F. Tardieu), mathematical modelling (C. Pradal, D. Felbacq), cell biology and signaling (Y. Jaillais, M. Nollman), ABA signalling (E. Grill) and plant pathology (Y. Saijo, F. Vailleau).

Towards an enhanced autonomy of young researchers

One of long term objectives over the next contract will be to promote the emergence of new research topics and leaders. Indeed, several young scientists of the team have reached a good maturity and need to prepare their future beyond 2025. The team project was elaborated in these respects and delineated in two main poles. Because of the multiple tasks of the group leader who will be mostly involved in pole 2 , a greater autonomy of projects and colleagues in pole 1 will be encouraged.

Annex 4 – Research products and activities

EVALUATION CAMPAIGN 2019-2020

GROUP A

Name the team: Aquaporin
Acronym: AQUA

Theme leader for the current contract: Christophe Maurel
Theme leader for the next contract: Christophe Maurel

I - PRODUCTION OF KNOWLEDGE AND ACTIVITIES CONTRIBUTING TO THE INFLUENCE AND SCIENTIFIC ATTRACTIVENESS OF THE TEAM

1- Articles

Scientific articles

1. **Grondin A., Rodrigues O., Verdoucq L., Merlot S., Leonhardt N., Maurel C. (2015) Aquaporins Contribute to ABA-Triggered Stomatal Closure through OST1-Mediated Phosphorylation. Plant Cell. 27 (7): 1945-1954.**
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13. **Vialaret J., Di Pietro M., Hem S., Maurel C., Rossignol M., Santoni V. (2014) Phosphorylation dynamics of membrane proteins fromArabidopsisroots submitted to salt stress. Proteomics. 14 (9): 1058-1070.**
14. **Hosy E., Martinière A., Choquet D., Maurel C., Luu D.T. (2015) Super-resolved and dynamic imaging of membrane proteins in plant cells reveal contrasting kinetic profiles and multiple confinement mechanisms. Mol. Plant. 8(2): 339-342.**
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2. **Li G., Santoni V., Maurel C. (2014) Plant Aquaporins: Roles in Plant Physiology. Biochim Biophys Acta - Gen. Subjects 1840(5): 1574-1582. (Review)**
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2. Cunnac S., Gantet P., Ghesquière A., Guiderdoni E., Luu D. T. (2016) Le riz, une plante modèle à croquer. Biofutur 380 : 30-34.
3. Shahzad Z., Maurel C. (2017) Adaptation conjointe des racines à l'inondation et aux nutriments du sol. Rôle d'une protéine kinase de type MAP3K M S-Med. Sci. . 33 (4): 383-385. (Commentary)

2- Books

Management and coordination of scientific books / Scientific book edition

1. Maurel C.(Nov 2018) Membrane Transport in Plants. Editor C. Maurel, Series Editor JP Jacquot.. Advances in Botanical Research. 87: 234p. (Elsevier eds).

Book chapters (total number)

Book chapters in English or another foreign language

1. Verdoucq L., Maurel C. (2018) Plant Aquaporins. Adv. Bot. Res. 87: 25-56.

2. Maurel C. (2015) Plant Aquaporins. Cell Biology (S. Assmann & B. Liu ed.) Springer New York:1-23
3. Maurel C., Prado K. (2017) Aquaporins and leaf water relations. In: Plant Aquaporins/From Transport to Signaling, ed Chaumont F & Tyermann S. (Springer International Publishing), pp 155-165.
4. Morot-Gaudry JF, Moreau F, Prat R, Maurel C, Sentenac H (2017) Biologie Végétale – Nutrition et Métabolisme ; Collection « Sciences Sup », Dunod 3ème édition, Paris, pp 1-242.
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3- Production in conferences / congresses and research seminars

Articles published in conference proceedings / congress

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1. **Maurel C.**, Rodrigues O., Prado K., Shahzad Z., Grondin A., Tournaire-Roux C., Santoni V., Verdoucq L. (2016) Environmental and hormonal signaling pathways targeting or mediated by plant aquaporins. (29 June-3July 2016) 27th International Conference on Arabidopsis Research (ICAR2016), Gyeong Ju (Korea).
2. **Maurel C.**, Shahzad Z, N Tang, C Tournaire-Roux, M Rosales Villegas, A Martinière, Y Boursiac, P Nacry, O Loudet (2017) Root hydraulics: a key trait in plant adaptation to stresses. 2nd Workshop on Plant Development and Drought Stress, Pacific Grove, USA. (Plenary lecture)
3. **Maurel C.** (2018) Aquaporins and plant hydraulics as targets and players of environmental and hormonal signaling. 12th International Plant Molecular Biology Congress, Montpellier, France. (Keynote lecture)

4. Di Pietro M., Vialaret J., Li G.W., Hem S., Prado K., Maurel C., **Santoni V.** (2014) Quantitative proteomics unravels novel post-translational regulatory mechanisms of plant aquaporins. (31.08.2014 - 04.09.2014) st INPPO World Congress on Plant Proteomics: Methodology to Biology. Hambourg (Allemagne).
5. **Luu D**, AA Véry (2014) Hydro-mineral nutrition and adaptation to abiotic stresses in rice: Analysis of root aquaporins and Na+/K+ transport systems. Global Rice Science Partnership (GRiSP) meeting, Montpellier, May 15, 2014.
6. **Maurel C.** (2014)Hormonal and abiotic stress regulation of plant aquaporins. (27-29 October 2014) 12th France-Japan Workshop on Plant Science. Tokyo University, Tokyo (Japan). (Plenary lecture)
7. **Maurel C.** (2015) Frontiers in aquaporin research. (18-20 May 2015).NSF Workshop on Vascular Transport in Plants – Research Frontiers and Priorities, Washington DC (USA). (Plenary lecture)
8. **Maurel C.**, Prado K., Li G, Grondin A., Rodrigues O., Verdoucq L., Tournaire-Roux C., Lucas M., Boursiac Y., Santoni V., Luu D.-T. (2015) Regulation of plant hydraulics by hormonal and environmental signals. 3rd International Symposium on Plant Signaling & Behavior, Paris, France (29 juin-2 juillet 2015). (Plenary lecture)

9. **Luu D-T.** (2016). Super-resolved and dynamic imaging in plant cells. (26-30 june 2016) Plant Biology Europe, EPSO-FESPB 2016 congress. Prague (République Tchèque).
10. **Maurel C.**, Shahzad Z., Rodrigues O., Prado K., Grondin A., Tournaire-Roux C., Santoni V., Verdoucq L. (2016) Plant aquaporins as targets and players of cell signalling. (20-23 september 2016) 14th Congress of the Italian Federation of Life Sciences (FISV), Roma (Italy).
11. **Maurel C.**, Shahzad Z., Tournaire-Roux C., Martinière A., Boursiac Y., Loudet O. (2017) Regulation of root water transport under flooding. XIX International Botanical Congress, Shenzhen, (China).
12. **Maurel C.**, Prado K., Rodrigues O., Grondin A., Wudick M., Shahzad Z., Luu D., Martinière A., Verdoucq L., Tournaire-Roux C., Santoni V. (2017) Control of aquaporins and plant hydraulics by environmental and hormonal factors. Mini-Symposium on Plant Stress Biology, Sun Yat-Sen University, Guangzhou, (China). (Plenary lecture)
13. **Maurel C.**, Z Shahzad, N Tang, C Tournaire-Roux, M Rosales Villegas, A Martinière, Y Boursiac, P Nacry, O Loudet (2018) Quantitative genetics dissection of root hydraulics uncovers novel pathways for plant adaptation to stresses. 10th Symposium of the International Society of Root Research, Jérusalem, Israël.
14. **Maurel C.** (2018) Aquaporins and plant hydraulics as targets and players of environmental and hormonal signaling. Plant Adapt 2018, Banyuls, France. (Keynote lecture)

Invited oral presentations in national symposia/congress

1. **Boursiac Y.**, Pradal C., Lucas M., Maurel C., Godin C., Felbacq D. (2015) Building a root hydraulic architecture with a keyboard and pipettes. (9-10 April 2015). Agropolis workshop: Modeling plant development from the organ to the whole plant scale, Montpellier (France).

2. **Santoni V.** Plant aquaporin dynamics - a MRM approach (25/03/2014) Réunion annuelle du Protéome Vert. Paris (France).
3. **Santoni V.** Présentation de la plateforme de spectrométrie de masse protéomique MSPP. (21 mars 2014) Ateliers technologiques de Biocampus. Montpellier (France).
4. **Boursiac Y.** Formation of Plant Biomass. (26 novembre 2015) Workshop Atelier LabEx CheMISyst : "chimie physique de la chimie verte". Montpellier (France).
5. **Nacry P.** (2015) RhizoScan HT A key step towards a high-throughput phenotyping platform dedicated to root development. (9-10 mars 2015) Workshop réseau EFOR (Réseau d'Etude Fonctionnelle chez les ORganismes modèle. Gif-sur-Yvette (France).
6. **Boursiac Y.**, D. Felbacq, AM Velez Cardona, B Metzger, H Lhuissier (2018) Understanding water transport in roots with the help of a keyboard and pipettes. Journées Netbio 2018, Montpellier, France.

Selected oral presentations in international symposia/congress

1. **Rodrigues O**, A Grondin, G Reshetnyak, S Merlot, N Leonhardt, L Verdoucq, C Maurel (2015) ABA and flg22 signaling pathways inducing stomatal closure: role of aquaporins in regulation of water and hydrogen peroxide transport. 26th International Conference on Arabidopsis Research, Paris, France (5-9 July 2015).
2. **Martinière A**, JB Fiche, M Smokvarska, M Nollmann, C Maurel (2016) Reactive oxygene species (ROS) contribute to endocytosis regulation in response to osmotic stress. ENPER 2016, Bordeaux, France
3. **Nacry P**, M Rosales Villegas, C Maurel (2018) Abscisic acid mediates and co-ordinates the developmental and hydraulic responses of plant roots to water deficit. 10th Symposium of the International Society of Root Research, Jérusalem, Israël.

4. **Maurel C.**, Prado K., Grondin A., Rodrigues O., Verdoucq L., Tournaire-Roux C., Boursiac Y., Santoni V., Luu D.T. (2014) Hormonal and abiotic stress regulation of plant aquaporins. (22-26 June 2014) Plant Biology Europe FESPB/EPSO Congress 2014. Dublin (Ireland).
5. **Boursiac Y.**, Felbacq D. Calvo M., Maurel C. (2015) Role of apoplastic barriers in root hydraulics. (2-4 September 2015). International Symposium on Plant Apoplastic Diffusion Barriers: Biosynthesis and Functions. PADIBA meeting, Nantes (France).
6. Bellati J., Di Pietro M., Prado K., Champeyroux C., Vialaret J., Hem S., Maurel C., **Santoni V** (2016) Phosphoproteomics and interactomics reveal novel regulatory mechanisms of plant aquaporins. EPSO/FESPB congress (26th-30th June) Prague République tchèque.
7. **Boursiac Y.**, Velez-Cardona A. M., Calvo-Polanco M.M., Felbacq D., Pradal C., Lucas M., Godin C., Maurel C. (2016) How does water flow through the roots. (2016-05-29 - 2016-06-03) Gordon Research Conferences “Salt & Water Stress in Plants, From Molecules to Crops”. Les Diablerets, (CHE).
8. **Maurel C.**, Rodrigues O., Prado K., Grondin A., Tournaire-Roux C., Di Pietro M., Santoni V., Verdoucq L. (2016) Organ- and stimulus-specific aquaporin phosphorylation pathways for regulation of water and H_2O_2 transport. (5-10 june 2016) 17th International Workshop on Plant Membrane Biology, Annapolis, (USA).
9. **Maurel C.**, Shahzad Z., Tang N., Tournaire-Roux C., Martinière A., Boursiac Y., Loudet O. (2017) Quantitative genetics analysis of root hydraulics uncovers novel pathways of plant adaptation to the environment. VIB Conference 'At the Forefront of Plant Research', Ghent, (Belgium).
10. **Maurel C.**, Luu D., Wudick M., Martinière A., Verdoucq L., Rodrigues O., Grondin A., Shahzad Z., Tournaire-Roux C. (2017) Regulation of aquaporins and plant hydraulics under water and salt stress. XIX International Botanical Congress, Shenzhen, (China).

11. **Rosales MA** (2017) Abscisic Acid Participates in Root Developmental and Hydraulic Responses to Water Deficit in Arabidopsis. Lateral root Workshop, Montpellier, France.
12. **Boursiac Y,** D Felbacq, AM Velez Cardona, B Metzger, H Lhuissier (2018) How does water flow through cells. 1st International Plant Systems Biology Meeting, Roscoff, France.
13. **Smokvarska M** (2018) The Arabidopsis RHO of plants GTP-ASE ATROP6 regulates early signaling events in response to osmotic stress. Plant Adapt 2018, Banyuls, France.
14. **Martinière A**, Smokvarska M, Francis C, Platre M., Fiche JB, Alcon C., Dumont X, Nollmann M., Maurel C, Jaillais Y (2019) Dynamic and nanoscale organization of Rho of Plant/NADPH oxidase complexes during cell osmotic signaling. Royal Microscopical Society, Oxford, UK.
15. **Nacry P**, Rosales M.A., Maurel C (2019) Abscisic acid mediates and co-ordinates the developmental and hydraulic responses of plant roots to water deficit. Keystone Symposia conference Climate change-linked stress tolerance in plants. Hannover Germany

Selected oral presentations in national symposia/congress

1. Di Pietro M., Prado K., Li G.W., Vialaret J., Boudsocq M., Hem S., Maurel C., **Santoni V.** (2015) Quantitative proteomics unravels novel post-translational regulatory mechanisms of plant aquaporins. (15-18 september 2015) Congrès Français de Spectrométrie de Masse et Analyse Protéomique, Ajaccio, (France).

2. Alcon C, Fiche J B, Nolmann M. **Martinière A**, (2016) Multi-scale modeling of water transport in plant roots, GDR MIV, Super résolution sur échantillon végétaux, Bordeaux (Décembre 2016), (France).
3. **Boursiac Y**, Velez-Cardona AM, Calvo-Polanco M, Felbacq D, Pradal C, Lucas M, Maurel C, Godin C (2017) Multi-scale modeling of water transport in plant roots, GDR PHYP, Biophysique et biomécanique des plantes, Marseille (20-21 juin 2017), (France).
4. **Martinière A** (2018) Protein clusteing and lateral diffusion during early phase of plant osmotic perception, Paris (juin 2018), (France).

Posters in international symposia/congress

1. Rosales M.A., Schreurs G., Nacry P., Maurel C. (2015) Regulation of Arabidopsis root architecture and hydraulics during water stress. (29 June – 1 July 2015) International Conference of Plant Abiotic Stress Tolerance III, Vienna (Austria).
2. Shahzad Z., Li G, Sutka M., Tournaire-Roux C., Postaire O., Maurel C. (2015) Identification of genetic determinants of root hydraulics in Arabidopsis thaliana. (5-9 July 2015) 26th International Conference on Arabidopsis Research, Paris (France).
3. Champeyroux C., Bellati J., Maurel C., Santoni V. (2016) Regulation of root water transport by Casparian strip domain-like proteins. EPSO/FESPB congress, 26th-30th June, Prague (Tchec Republic).
4. Smokvarska M., Charbel F., Platre M.P., Alcon C., Maurel C., Jaillais Y., Martinière A.(2018) The arabidopsis RHO of plants GTP-ASE ATROP6 regulates early signaling events in response to osmotic stress. (5 -10 août) 12th Congress of the International Plant Molecular Biology. Montpellier (France).

5. Rodrigues O., Grondin A., Verdoucq L., Merlot S., Leonhardt N., Maurel C. Aquaporins contribute to ABA-triggered stomatal closure through OST1-mediated phosphorylation. Salt and water stress in plants. (3-8 August 2014) Gordon Research Conferences. New York (USA).
6. Rosales M.A., Schreurs G., Nacry P., Maurel C. (2015) Regulation of Arabidopsis root architecture and hydraulics during water stress. (12-15 october 2015) 3rd AgreenSkills Annual Meeting, Barcelona (Spain).
7. Rosales M.A., Schreurs G., Maurel C., Nacry P. (2015) Regulation of Arabidopsis root architecture and hydraulics during water stress. (6-9 october 2015) 9th Symposium of the International Society of Root Research. Camberra (Australia).
8. Martinière A., Gibrat R., Sentenact H., Paris N. (2014) Media acidification and proton gradient in *Arabidopsis thaliana* roots (7-10 septembre) ENPER 2014, Lecce Italie
9. Chu TTH, TG Hoang, DC Trinh, AM Ismail, A Henry, NV Do, P Gantet, C Maurel, D-T Luu (2016) Drought and salt stress tolerance: osmotic adjustment and endosomal trafficking as targets. 14th Internationational Symposium on Rice Functional Genomics, Montpellier, France.
10. Rosales M.A., Schreurs G., Maurel C., Nacry P. (2016). Regulation of Arabidopsis root architecture and hydraulics during water stress. (29 May to 3 June 2016) Salt & Water Stress in Plants - Gordon Research Conference Les Diablerets (Switzerland).
11. Shahzad Z., Canut M., Tournaire-Roux C., Martinière A., Boursiac Y., Loudet O., Maurel C. (2016) Hydraulic Conductivity of Root 1 controls potassium-dependent oxygen sensing to regulate root hydraulic. (December 12th-13th 2016) GARnet Natvar2016'Natural variation as a tool for gene discovery and crop improvement', Cambridge (Royaume-Uni).
12. Calvo Polanco M., Ribeyre Z., Franke R.B., Salt D., Simonneau T., Muller B., Maurel C., Boursiac Y. (2017) Role of endodermal casparian strips and suberin in the hydromineral status of plants under salt stres. 21th Congress of the Spanish Society of Plant Physiology, Barcelona (Spain).

13. Rosales M.A., Maurel C., Nacry P.(2017) Abscisic acid mediates root developmental and hydraulic responses to water stress in *Arabidopsis.* 8th International Symposium on Root Development, (29Mai-1^{er} Juin). Umea (Sweden).
14. Rosales M.A., Gasc J., Maurel C., Nacry P. (2017) Abscisic acid participates in root developmental and hydraulic responses to water deficit in *Arabidopsis.*(June 26-29) 21th Congress of the Spanish Society of Plant Physiology, Barcelona (Spain).
15. Champeyroux C, Bellati J, Rofidal V, Maurel C, Santoni, V. (2018) Regulation of a plant aquaporin by a casparian strip membrane domain protein-like. 12th International Plant Molecular Biology Congress, Montpellier (France).
16. Tournaire-Roux C, Z Shahzad, C Maurel (2018) Biochemical characterization of protein kinases acting in environmental signalling and regulation of plant hydraulics. 12th International Plant Molecular Biology Congress, Montpellier (France).
17. Protto V, L Rishmawi, C Tournaire-Roux, Y Boursiac, P Nacry, C Maurel (2019) The HyArchi project : Maize Root Hydraulic Architecture and its response to drought. Third European Maize Meeting, Montpellier (France).

Posters in national symposia/congress

1. Bellati J., Di Pietro M., Prado K., Champeyroux C., Rofidal V., Hem S., Maurel C., Santoni V. (2016) Phosphoproteomics and interactomics reveal novel regulatory mechanisms of plant aquaporins. Congrès SFEAP, 10th-12th October Chambéry (France)
2. Santoni V. Présentation de la plateforme de spectrométrie de masse protéomique MSPP (14-16 avril 2014) Journée du département BAP. Pont Royal (France).
3. Chu T.T.H. (2015) Cell biology of aquaporins in rice. (9-13 novembre 2015) Doctoriales Transfrontalières, Perpignan (France).
4. Bellati J., Champeyroux C., Hem S., Rofidal V., Krouk G., Maurel C., Santoni V. (2017) Interactomics reveals novel regulatory mechanisms of plant aquaporins. (2-5 Oct. 2017) Congrès Spectrométrie de Masse, Métabolomique et Analyse Protéomique (SMMAP), Marne La Vallée (France).

Invited seminars in foreign institutions

1. Luu D.T. Intracellular trafficking of aquaporins in *Arabidopsis thaliana.* International Rice Research Institute (IRRI). (4 Février 2014) Los Banos (Philippines).
2. Maurel C (2018) “Aquaporins and plant hydraulics as targets and players of abiotic and biotic stress signaling”, Max-Planck-Institut für Pflanzenzüchtungsforschung, Köln, Allemagne, 27 juin 2018.
3. Maurel C. Aquaporins: how water channel proteins favour plant life in an ever changing environment. (30 Octobre 2014) RIKEN Research Center, Yokohama (Japan).
4. Luu D.T. Intracellular trafficking of aquaporins in Arabidopsis thaliana (Novembre 2014) Beijing Forestry University (Chine).
5. Luu DT (2015) Cell biology of aquaporins in Arabidopsis, Institute of Experimental Botany, Prague, Tchek Republic, le 15/07/15.
6. Luu DT (2015) Cell biology of aquaporins in rice, Agricultural Genetics Institute, Hanoï, Vietnam, le 17/12/15
7. Maurel C (2015) Control of aquaporins and plant water transport by multiple hormonal and environmental signals, Helmholtz Zentrum, Münich, Germany, le 14/04/2015
8. Maurel C (2015) Control of aquaporins and plant water transport by multiple environmental and hormonal signals, Syngenta Biotechnology, Raleigh, USA le 21/05/2015
9. Maurel C (2018) "Root hydraulics: a key trait in plant adaptation to stresses", Scuola Superiore Sant'Anna, Pisa, Italy, 18 avril 2018.

Invited seminars in French institutions

1. Verdoucq L. Phosphorylation of PIP2;1 aquaporin by OST1 mediates ABA-dependent stomatal closure in Arabidopsis. (6 juin 2014.) Institut de Biotechnologie des Plantes, Orsay (France).
2. Santoni V. Plant aquaporin dynamics: a MRM approach. Réunion annuelle du protéome vert (2014) Paris (France).
3. Martinière A. pH micro-environment in Arabidopsis. (03 février 2015) UMR AGROECOLOGIE, Dijon (France).
4. Martinière A. Mapping pH in plant endomembrane system. (3-5 juin 2014) Zeiss workshop “Imagerie de la plante”. Montpellier (France).

5. Martinière A, Perception and early response of plant to water stress (2014) Centre de Biologie structurale, Montpellier (29/02) (France).
6. Luu D.T. Intracellular trafficking of aquaporins in Arabidopsis thaliana. (08 avril 2014) Laboratoire d'Agroécologie , Dijon,(France).
7. Luu D.T. Intracellular trafficking of aquaporins in *Arabidopsis thaliana.* (10 Janvier 2014) Laboratoire de Reproduction et Développement des Plantes, ENS Lyon (France).
8. Santoni V. Plant aquaporin interactomics. Ateliers technologiques de Biocampus approches basées sur la spectrométrie de masse et la résonance plasmonique de surface (2014) Montpellier (France).
9. Santoni V. Quantitative proteomics unravels novel regulation of plant aquaporins (2015) Club-jeunes de la SFEAP, Marseille (France).
10. Santoni V. Quantitative proteomics unravels novel regulation of plant aquaporins (2015) Action COST FA 1106, workshop Bordeaux (France).
11. Maurel C. (2016) Le statut hydrique de la plante, cible et intégrateur de contraintes environnementales multiples.(27 mai 2016) Comité d'Animation Thématique, GIS Biotechnologies Vertes. Paris (France).
12. Verdoucq L. Aquaporins facilitate hydrogen peroxide entry into guard cells to mediate ABA- and flg22-mediated stomatal closure. (06 Octobre 2017) Laboratoire Génome et Développement des Plantes, Perpignan (France).
13. Santoni V. Interactomics reveals novel regulatory mechanisms of plant aquaporins. (6 avril 2017) Club-jeunes SFEAP, Montpellier (France).
14. Maurel C. Plant aquaporins as targets and players of cell signaling. (6 Mars 2017) Institute of Plant Sciences Paris Saclay (IPS2), Orsay (France).
15. Martiniere A (2018) Early phase of plant osmotic signaling, Institut Jean-Pierre Bourgin, INRA, Versailles (France)

4- Electronic tools and products

Softwares

Rhizoscan 1:

Diener J., Nacry P., Périn C., Diévart A., Draye X., Boudon F., Gojon A., Muller B., Pradal C., Godin C. (2013) An automated image-processing pipeline for high-throughput analysis of root architecture in OpenAlea. 7th International Conference on Functional-Structural Plant Models, Jun 2013, Saariselkä, Finland. R Sievänen, E Nikinmaa, C Godin, A Lintunen & P Nygren (eds) pp.85-87

sptPALM_Analyser

Martinière A, JB Fiche, M Smokvarska, S Mari, C Alcon, X Dumont, K Hematy, Y Jaillais, M Nollmann, C Maurel (2019) Osmotic stress activates two ROS generating pathways with distinct impacts on plasma membrane protein nano-organization and lateral diffusion. *Plant Physiol.*, 179: 1581-1593.

Databases

5- Instruments and methodology

Prototypes

Platforms and observatories

HIRROS (High Resolution Root Scanner)

Automated root phenotyping platform for in vitro grown plants.
Partnership with local ALCI (Montpellier). Licence DI-RV-18-0055. Innovation Domain: PLANTINNOV

PHIV (Plateforme d'Histocytologie et d'Imagerie cellulaire Végétale)

Development of Vertical Imaging Devise (Biocorp, CNRS project: Défi Instrumentation aux Limites)

6- Other products

Theorised artistic creations, staging, movies

7- Editorial activities

Participation in editorial committees (books, collections, etc.)

Christophe MAUREL:

- Managing Editor of *Plant Physiology* (since 2018)
- Member of Editorial Committee of the following journals: *Plant Cell Environ.*, *Front. Plant Physiol.*, *Front. Plant Traffic Transport*, *J Plant Hydraulics*, *Biochem. J.*
- Member of Faculty of 1000 Biology

Collection and series management

Christophe MAUREL:

- Scientific editor of “Membrane transport in plants” Adv. Bot. Res. 87: pp1-342.

Véronique SANTONI

- co-editor with E Jamet of a Special Issue: 2017 Plant Proteomics

8- Reviewing activities

Reviewing of articles

Yann BOURSIAC:

- 2-3 articles /year in journals such as Front. Plant Sci., Sci. Rep., J. Exp. Bot.

Alexandre MARTINIERE:

- 2-3 articles/year in journals such as Traffic, J. Exp. Bot., PLoS One

Christophe MAUREL:

8–10 articles /year in journals including Nature Genetics, Nature Commun., PNAS, Nature Plants, EMBO J., eLIFE and Plant Cell.

Philippe NACRY:

4-5 articles/year in journals including Plant Cell, Plant Physiol., Planta, J Exp. Bot., BMC Plant Biol.

Véronique SANTONI

2-3 articles /year in Proteomics, J Proteom. Res., Plant Cell Environ., Plant Physiol.

Lionel VERDOUCQ:

5-8 articles/year in various journals including Plant Physiol., Plant J. Planta, IMJS, BMC Mol. Biol..

Grant evaluation (public or charities)

Christophe MAUREL:

Evaluation of 3-4 international grants /year (EU: ERC, ERA-CAPS; Belgium: FNRS; Korea: NRF; Germany: DFG; Israel: ISF; Japan: JSPS)

Evaluation of 2-3 national grants/year (INRA, ANR, LabEx SPS)

Philippe NACRY:

Evaluation of 1-2 international grants /year (Germany: DFG; Belgium: FRS-FNRS, FWO; Netherland: Vici NOW)

Evaluation of 2-3 national grants/year (INRA, LabEx SPS, LabEx Agro, Region Pays de Loire, ECOS, CAPES COFECUB)

Véronique SANTONI

Evaluation of 1 national grant/year (INRA, LabEx SPS)

Reviewing of research institutes

Christophe MAUREL

AERES Evaluation Committee : Institut Jean-Pierre Bourgin, Versailles (2014)

Participation in institutional committees and juries (CNRS, Inserm, etc.)

Alexandre MARTINIERE

Member of one thesis defense *committee*

Member of the evaluation jury of Master1 BFP (2016-2019)

Christophe MAUREL

Nominated member of the 23^{d} section (Plant Integrative Biology) of the Comité National de la Recherche Scientifique (2012-2016)

From 2014 to 2019, member of
- *5 thesis defense committee (France (3), Canada (1), Belgium (1))*
- *2 habilitation defense committees*
- *Member of the Recruitment Jury of the SEVAB Doctoral School (Université de Toulouse III, Paul Sabatier)* (2017)

Philippe NACRY

Elected member of the Scientific Committee of the Plant Biology and Breeding department of INRA (since 2011)

From 2014 to 2019, member of
- *6 thesis defense committee (France (5), Australia (1),*
- *1 habilitation defense committees*

Véronique SANTONI

Member of 2 thesis defense *committee*

Member of the recruitment jury of a professor of the universities to the faculty of ondotology of Montpellier

9- Academic research grants

European (ERC, H2020, etc.) and international (NSF, JSPS, NIH, World Bank, FAO, etc.) grants – coordination

2012 – 2014 ; **ORYZAQUA** (Marie Sklodowska-Curie Actions – Individual Fellowship) ; Cell biology of rice aquaporins ; MAUREL Christophe (Coord.), LUU Doan Trung

2016 – 2018 ; **DROUGHTROOT** (Marie Sklodowska-Curie Actions – Individual Fellowship) ; Plants in search of water: physiological and molecular interplay between root hydraulics and architecture during drought stress ; MAUREL Christophe (Coord.), ROSALES VILLEGAS Miguel

2018 – 2023 ; HyArchi (ERC Advanced) ; Targeting Root Hydraulic Architecture to improve Crops under Drought ; MAUREL Christophe (PI)

This project undoubtedly represents a major achievement and international recognition for the team. It will provide a robust frame for future work on maize.

European (ERC, H2020, etc.) and international (NSF, JSPS, NIH, World Bank, FAO, etc.) grants - partnership

2014 – 2017 ; Root barriers (ERA-CAPS) ; Plant root diffusional barriers: genesis and implications for nutrient efficiency and stress tolerance (Coord. David E Salt, Nottingham Univ., UK) ; BOURSIAC Yann (Participant).

A high level collaborative project which provided significant European networking to the team

Other European grants - coordination

2014 – 2016 ; Agreenskills ; Hormonal Regulations of Aquaporins and Root Hydraulic Architecture: their Role in Water Deficit Tolerance ; CALVO POLANCO Maria Monica

2015 – 2017 ; Agreenskills ; Plant root diffusional barriers and aquaporins: their combined role in water transport and salt stress tolerance ; ROSALES VILLEGAS Miguel Angel

Other European grants - partnership

National public grants (ANR, PHRC, FUI, INCA, etc.) - coordination

2012 – 2015 ; HydroRoot (ANR PRC) ; Genetics and integrative biology of plant root hydraulics ; MAUREL Christophe
This project, which paved the way to our ERC grant, led to major publications: Shahzad et al., Cell, 2016, and Tang et al., Nature Commun., 2018

2018 – 2022 ; ABAqua (ANR PRCI) ; ABA-dependent control of plant hydraulics in plant acclimation to water deficit ; MAUREL Christophe
A promising French-german project on fundamental aspects of ABA signaling

2015 – 2016 ; Eaudissect (Department BAP INRA); Tissular dissection of root hydraulic functioning ; BOURSIAC Yann

2018 - 2020 ; TURGOMAP (CNRS Interdisciplinarity Program) ; Towards a mapping of turgor pressure in plant tissues ; BOURSIAC Yann
A national networking project coordinated by a young researcher of the team

National public grants (ANR, PHRC, FUI, INCA, etc.) - partnership

2016 – 2020 ; **MIRGA (ANR PRCE)** ; Maize root growth and architecture ; Coord. Christophe Salon, INRA, Dijon ; NACRY Philippe (Participant)

Local grants (collectivités territoriales) - coordination

Local grants (collectivités territoriales) - partnership

PIA (labex, equipex etc.) grants - coordination

2015 – 2016 ; Water Potential Makers (Labex AGRO) ; Développement de marqueurs genetiques correlés au potentiel hydrique de la plante (Water Potential Markers) ; BOURSIAC Yann

2018 – 2021 ; eCO2THREATS (iSITE Montpellier) ; Alteration of plant nutrient contents by elevated CO2 : consequences for crop quality, human health and environmental protection ; (Coord. A. Gojon); NACRY Philippe (participant)

PIA (labex, equipex etc.) grants - partnership

2016 – 2019 ; APLIM (Labex AGRO) ; Development of magnetic resonance technologies (NMR,MRI and nanoprobes) to support integrative biology of plant response to abiotic and biotic constraints - Advanced Plant Life Imaging and Metrology ; (Coord. JL Verdeil, CIRAD, and C. Goze-Bac, CNRS), MAUREL Christophe

Grants from foundations and charities (ARC, FMR, FRM, etc.) - coordination

Grants from foundations and charities (ARC, FMR, FRM, etc.) - partnership

10- Visiting senior scientists and post-doc

Post-docs (total number)

Since 2014: 8

Foreign post-docs

Since 2014: 6

Family Name	First name	Contract type & Employer	Nationality	Recruitment period	
Bellati	Jorge	CDD INRA ANR	Argentina	23/05/2012	31/03/2015
Calvo-Polanco	Monica	CDD INRA Agreenskills	Spain	15/04/2015	30/04/2018
Rosales Villegas	Miguel	CDD INRA Agreenskills	Spain	15/03/2015	14/03/2016
Rosales Villegas	Miguel	CDD CNRS H2020	Spain	15/03/2016	14/03/2018
Shahzad	Zaigham	CDD INRA ANR	Pakistan	01/03/2013	31/10/2016
Tang	Ning	CDD CNRS ANR	China	01/03/2014	28/02/2015
Tang	Ning	CDD INRA ANR	China	01/03/2015	30/04/2017
Rishmawi	Louai	CDD CNRS ERC	Germany	01/04/2019	31/03/220

Visiting scientists (total number)

Since 2014 : 6

Foreign visiting scientists

Since 2014 : 6

Family Name	First name	Financing source	Nationality	Date of arrival	Date of departure	Status	University of origin
Cappadocia	Mario	Own salary	Canada	30/09/2013	30/06/2014	Invited Professor	Université de Montréal, Canada
LI	Guowei	Fellowship	China	15/02/2014	14/07/2014	Invited Researcher	Chinese Academy of Science
Markus	Katharina	Own salary	Germany	03/07/2017	31/07/2017	Invited PhD student	Bonn Universität, Germany
Rabelo Schley	Thayssa	Fellowshi	Brasil	21/01/2019	22/02/2019	Invited PhD student	Helmholtz Center, Münich, Germany
Romeis	Tina	Own salary	Germany	22/01/2018	16/03/2018	Invited Professor	Frei Universität Berlin , Germany
Tang	Ning	Own salary	China	01/10/2017	17/11/2017	Invited Post-doc	Univ. Montpellier, France

11- Scientific recognition

Prizes and/or distinctions

Zaigham SHAHZAD (with Christophe MAUREL)

Major French Advances in Biology, French Academy of Sciences (2017)

Christophe MAUREL

Georges Morel Prize, “Grand Prize”, French Academy of Sciences (2018)

IUF members

Chair of learned and scientific societies

Organisations of meetings and symposia (out of France)

Invitations to meetings and symposia (out of France)

Christophe MAUREL:

from 2014- to 2018, 9 invited conferences in international meetings including
2016: 27th International Conference on Arabidopsis Research (ICAR2016), Gyeong
2017: 19th International Botanical Congress, Shenzhen, China
2018: 12th International Plant Molecular Biology Congress, Montpellier, France (Keynote lecture)

II - INTERACTION OF THE TEAM WITH THE NON-ACADEMIC WORLD, IMPACTS ON ECONOMY, SOCIETY, CULTURE OR HEALTH

1- Socio-economic interactions / Patents

Invention disclosures

Filed patents

Accepted patents

Licenced patents

2- Socio-economic interactions

Industrial and R&D contracts

2012 – 2016 ; Aquapartner (SYNGENTA AGRO SAS; Research Contract) ; Investigating the regulation of aquaporins (and more specifically PIP2;1 from the model plant Arabidopsis thaliana) as a critical regulatory pathway of water transport in plants ; Coord. MAUREL Christophe

2013 – 2016 ; HydroRoot (SYNGENTA AGRO SAS ; Research Contract) ; Molecular and genetic dissection of root hydraulics together with the production of an integrated view of root structure and function ; Coord. MAUREL Christophe

These two contracts provided a robust support to our research. They were fruitful and led to significant advances in our understanding of aquaporin regulation and root hydraulics, as shown by resulting publications: Bellati et al., Mol. Cell. Proteomics, 2016; Shahzad et al., Cell, 2016, and Tang et al., Nature Commun., 2018

Cifre fellowships

Creation of labs with private-public partnerships

Networks and mixed units

Start-ups

3- Expertise

Consulting

Participation in expert committees (ANSES etc.)

Christophe MAUREL

Nominated member of the 23^{d} section (Plant Integrative Biology) of the Comité National de la Recherche Scientifique (2012-2016)

Member the Eric E. Conn Young Investigator Award Committee, American Society of Plant Biologists (2014 to 2019)

Philippe NACRY

Elected member of the Scientific Committee of the Plant Biology and Breeding department of INRA (since 2011)

Legal expertise

Expert and standardization reports

4- Public outreach

Radio broadcasts, TV shows, magazines and newspaper

Christophe MAUREL

Radiobroadcasts
Plant Roots and Flooding, 'La Tête au Carré', France Inter (2016)
Plant Roots and Flooding, 'CQFD', Radio Télévision Suisse (2016)
Plant Roots and Flooding, Radio Campus, Montpellier (2016)

Newspapers
How flooded plants adapt, "La France Agricole" (2016)
Flooding: when too much irrigated, plants trigger an emergency plan "Humanité Dimanche" (2016)

Public websites
Handling water stress by plant roots "INRA Entreprise"(2017)
Root architecture discoveries could help breed drought-resistant crops (https://horizon-magazine.eu/)(
How drought affects plant roots (https://cordis.europa.eu/result/rcn/239413_en.html)

Journal articles, interviews, book edition, videos, other popularization outputs, debates on science and society, etc.

Philippe NACRY

« Fascination of Plant Days 2015 » Agropolis, Montpellier(18-22 may 2015)

Participation in a public debate "Feeding 9 billions of human beings in 2050 : a few directions from the laboratories " Faculty of Education Montpellier 7 October 2017.

"Sciences en fête" Animation since 12 years of a workshop for high school students

III - INVOLVEMENT OF THE TEAM IN TRAINING THROUGH RESEARCH

1- Educational outputs

Books

E-learning, MOOCs, multimedia lessons, etc.

2- Scientific productions (articles, books, etc.) from theses

Scientific productions (articles, books, etc.) from theses

Original articles

Grondin A., Rodrigues O., Verdoucq L., Merlot S., Leonhardt N., Maurel C. (2015) Aquaporins Contribute to ABA-Triggered Stomatal Closure through OST1-Mediated Phosphorylation. Plant Cell. 27 (7): 1945-1954.

Bellati J., Champeyroux C., Hem S., Rofidal V., Krouk G., Maurel C., Santoni V. (2016) Novel aquaporin regulatory mechanisms revealed by interactomics Mol Cell Proteomics. 15(11) : 3473-3487.

Rodrigues O., Reshetnyak G., Grondin A., Saijo Y., Leonhardt N., Maurel C., Verdoucq L. (2017) Aquaporins facilitate hydrogen peroxide entry into gu114(34): 9200-9205.ard cells to mediate ABA- and pathogen-triggered stomatal closure. Proc Natl Acad Sci U S A. 114(34): 9200-9205.

Wudick M.M., Luu D.T., Tournaire-Roux C., Sakamoto W., Maurel C. (2014) Vegetative and sperm cell-specific aquaporins of Arabidopsis highlight the vacuolar equipment of pollen and contribute to plant reproduction. Plant physiol. 164(4):1697-1706.

Vialaret J., Di Pietro M., Hem S., Maurel C., Rossignol M., Santoni V. (2014) Phosphorylation dynamics of membrane proteins fromArabidopsisroots submitted to salt stress. Proteomics. 14 (9): 1058-1070.

Wudick M.M., Li X., Valentini V., Geldner N., Chory J., Lin J., Maurel C., Luu D.T. (2015) Sub-cellular redistribution of root aquaporins induced by hydrogen peroxide. Mol. Plant. 8(7): 1103-1114. (doct, eu, etr)

Chu T.T.H., Hoang T.G., Trinh D.C., Bureau C., Meynard D., Vernet A., Ingouff M., Do N.V., Périn C., Guiderdoni E., Gantet P., Maurel C., Luu D.T. (2018) Sub-cellular markers highlight intracellular dynamics of membrane proteins in response to abiotic treatments in rice. RICE. 11 (1): 23.

Prado K, V Cotelle, GW Li, J Bellati, N Tang, C Tournaire-Roux, A Martinière, V Santoni, C Maurel (2019) Oscillating aquaporin phosphorylations and 14-3-3 proteins mediate circadian regulation of leaf hydraulics. *Plant Cell*, 31: 417-429..

Martinière A, JB Fiche, M Smokvarska, S Mari, C Alcon, X Dumont, K Hematy, Y Jaillais, M Nollmann, C Maurel (2019) Osmotic stress activates two ROS generating pathways with distinct impacts on plasma membrane protein nano-organization and lateral diffusion. *Plant Physiol.*, 179: 1581-1593.

Champeyroux C, J Bellati, V Rofidal, C Maurel, V Santoni (2019) Regulation of a plant aquaporin by a Casparian strip membrane domain protein-like. *Plant Cell Environ.*, in press.

Wang P, M Calvo-Polanco, M Barberon, C Champeyroux, V Santoni, C Maurel, RB Franke, K Ljung, O Novak, G Reyt, N Geldner, Y Boursiac, DE Salt (2019) Surveillance of cell wall diffusion barrier integrity modulates water and solute transport. *Sci. Rep.*, 9: 4227.

Review articles

Rodrigues O., Martinière A., Luu D. T., Maurel C. (2014) Plant aquaporins on the move: reversible phosphorylation, lateral motion and cycling. Curr Opin Plant Biol. 22 101-107. (doct) (Review)

Maurel C., Verdoucq L., Rodrigues O. (2016) Aquaporins and plant transpiration. Plant Cell Environ. 39(11): 2580-2587. (doct) (Review)

Book chapters

Maurel C., Prado K. (2017) Aquaporins and leaf water relations. In: Plant Aquaporins/From Transport to Signaling, ed Chaumont F & Tyermann S. (Springer International Publishing), pp 155-165.

Mean number of publications per student (Biology & Science and technology only)

3.1 publications/student (mean from last 7 defended theses) or
2.7 publications/student (mean from last 3 defended theses)

3- Training

Habilitated (HDR) scientists

Since 2014 : 4

Family Name	First name	Position	Employer	Date of habilitation
LUU	Doan Trung	CRCN	CNRS	2017
MAUREL	Christophe	DR1	CNRS	1997
NACRY	Philippe	CRHC	INRA MONTPELLIER	2009
SANTONI	Véronique	DR2r	INRA MONTPELLIER	2006

HDR obtained during the period

Since 2014 : 1 HDR

Family Name	First name	Position	Employer	Date of habilitation
LUU	Doan Trung	CRCN	CNRS	2014

PhD students (total number)

6 PhD students

PhD students benefiting from a specific doctoral contract

Family Name	First Name	Starting date	Defense	Duration (months)	Funding
BELLANGER	Marina	23/01/2017	-	17	CDO
CHAMPEYROUX	Chloe	01/10/2014	29/11/2017	37	CDE
CHU	Huyen	01/02/2015	04/04/2018	38	ETR
PROTTO	Virginia	01/11/2018		36	CDO
RODRIGUES	Olivier	01/10/2011	04/12/2014	38	CDE
SMOKVARSKA	Marija	01/10/2017		36	CDE

Defended PhDs

3 defended PhDs

Family Name	First Name	Date of defense
CHAMPEYROUX	Chloe	2017
CHU	Huyen	2018
RODRIGUES	Olivier	2014

Mean PhD duration

Mean duration: 38 months

Internships (M1, M2)

Master 1: 7

Master 2: 11

Family Name	First Name	University Degree	Period	
Reshetnyak	Ganna	Master 1	03/03/2014	27/06/2014
Artins	Anthony	Master 1	01/03/2016	30/06/2016
Smokvarska	Marija	Master 1	01/03/2016	30/06/2016
Rieu	Philippe	Master 1	29/05/2017	28/07/2017
Hajjaj	Soukaina	Master 1	05/03/2018	27/07/2018

Ndella Thiaw	Marie-Rose	Master 1	05/03/2018	31/07/2018
Mertens	Toon	Master 1	12/03/2018	29/06/2018
Champeyroux	Chloé	Master 2	06/01/2014	27/06/2014
Trinh	Duy Chi	Master 2	17/02/2014	31/08/2014
Schreurs	Gerd	Master 2	01/01/2015	30/06/2015
Flandin	Leo	Master 2	01/03/2015	28/08/2015
Dehors	Jeremy	Master 2	01/02/2016	17/07/2016
Francis	Charbel	Master 2	03/01/2017	03/07/2017
Ribeyre	Zoé	Master 2	15/01/2017	30/06/2017
Sophie	Laïma	Master 2	01/03/2017	31/08/2017
Elion	Lessy Lafoi	Master 2	03/04/2018	30/09/2018
Brandt	Dennis	Master 2	01/04/2019	30/09/2019
Azria	Linh-Lan	Master 2	01/03/2016	31/08/2016

People in charge for a mention or a master's degree course (total number)

Philippe NACRY

- co-organization (25h/year) with Dr. L. Laplaze (IRD, Montpellier) of the « Root Breeding » module HMBA315 (CULTIVAR Labex Agropolis). Module for Master 2 students of University of Montpellier (2016-2018)

People in charge for a mention or a master's degree course with international certification (Erasmus mundus)

Plasticity team
Self-assessment document

Name of the team concerned by the current contract: Development and Plasticity of the Root System
Name of the team concerned by the next contract (if different):
Acronym of the current contract: Plasticity
Acronym of the next contract (if different):
Team leader for the current contract: Benjamin Péret
Team leader for the next contract: Benjamin Péret

RESULTS

1- Presentation of the team

Introduction

The team "Development and Plasticity of the Root System" was created in July 2015 when its group leader joined the research unit (CNRS mobility) after being awarded an ERC "Starting grant" to study white lupin root development. The main focus of the team is to understand the molecular mechanisms controlling root development and how they are altered under environmental changes. The team uses 2 major plant models: *Arabidopsis thaliana* and white lupin (*Lupinus albus*). For the latter, in order to initiate the scientific project, important efforts have been made to establish tools and resources on a plant that had never been cultivated before in the unit.

Unit's workforce and means

The team is composed of 4 permanent staff (**Benjamin PERET** – CNRS Researcher CR - HDR – Group leader since July 2015; **Fanchon DIVOL** – CNRS Engineer IR – Joined in July 2016; **Patrick DOUMAS** – INRA Researcher CR - HDR – Joined in October 2016; **Laurence MARQUÈS** – UM Lecturer MCF - HDR – Joined in October 2016).

The team hosted 8 non-permanent staff on fixed-term contract (Célia CASSET – CDD IE – Oct 2015-2018; Fanny GARCIA – CDD AI – Sep 2016-2018; Barbara HUFNAGEL – Post-doc – Oct 2016-2019; André MARQUÈS – Post-doc – Mar-Sep 2018; Alexandre SORIANO – CDD IE – Feb 2018-2020; Virginia FERNANDEZ – Post-doc –Oct 2018-2019; Laurent BROTTIER – CDD IE – Mar 2019- 2020; François JOBERT – Invited post-doc from Stephanie ROBERT's group (UPSC Umea Sweden) part-time 2 years. Jan 2019-2020).

The team hosted 2 PhD students (Cécilia GALLARDO – Oct 2016-2020 – Supervisor Benjamin PERET; Tamara LE THANH – Oct 2018-2021 – Supervisors Patrick DOUMAS and Laurence MARQUÈS). In addition, the team hosted 13 undergrad students (L3, M1 and M2).

Financial means of the team were provided by the ERC LUPIN ROOT project (2M€), Labex Agro Exploratory ROOT4EVER project (17k€), Labex Agro Soutien ponctuel LATERAL ROOT WORKSHOP project (8k€), Kempe foundation project (2y post-doc to F. JOBERT).

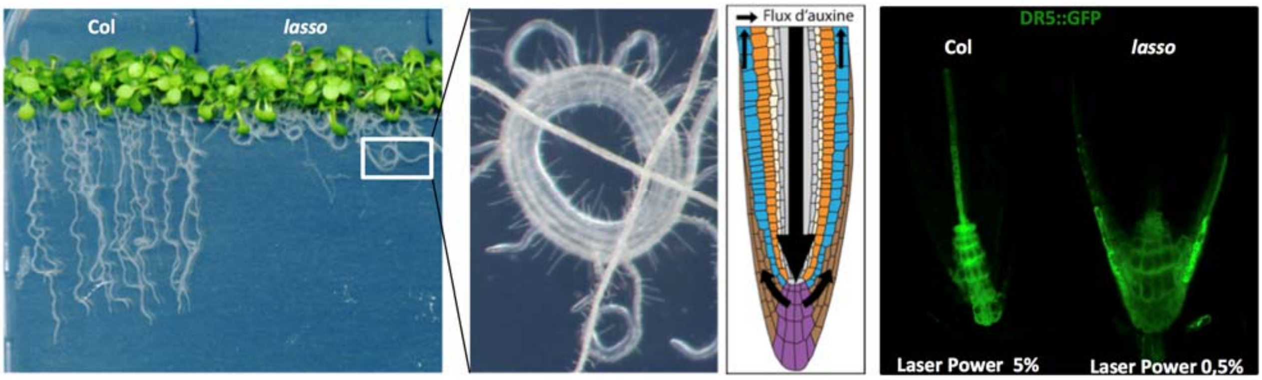
Scientific policy

Plants show a strong level of developmental plasticity that is controlled by a complex combination of perception, integration and response. Root system is a fantastic tool to study this plasticity since the number and position of lateral roots is deeply altered by the environment. We are trying to understand the fundamental mechanisms governing lateral root development and its control by the abiotic environment. Our research focuses on two main biological systems: the model plant *Arabidopsis thaliana* and white lupin (*Lupinus albus*).

From the primary root that is produced during the early stages of embryogenesis, a full root system architecture must developed to acquire water and nutrients, be the site for biotic interactions and allow anchorage for the plant. This is achieved by means of reiterative production of lateral roots (LR), created *de novo* from the pericycle cells. Work performed in Arabidopsis has provided a very detailed description of the successive steps leading to the formation of a new lateral root. It all starts with **pre-initiation** events that predefine future sites for LR production (described as primed or pre-branch sites). Then, **initiation** occurs when two adjacent nuclei migrates towards each other and lead to a first asymmetric division in the pericycle. This gives birth to a lateral root primordium (LRP) that undergoes cellular **patterning** to obtain a typical dome shape. This LRP is a massive structure that is produced deep in the parental root and therefore it has to emerge through the outer tissues; this highly regulated process is defined as **emergence**. Finally, the LRP becomes a newborn lateral root after a process of **meristem activation**. This LR will then proceed to grow by means of cell **division and elongation**, mimicking the primary root indeterminate mode of growth.

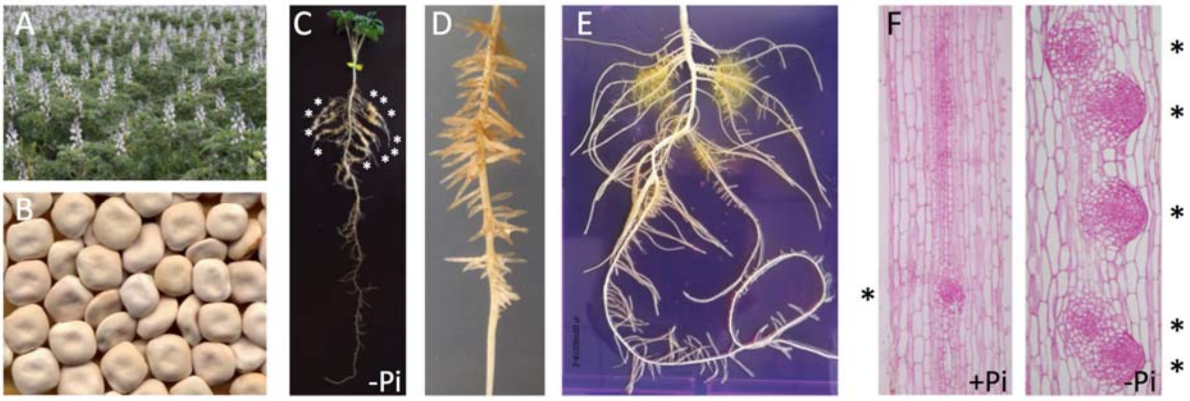
In our team, we are using two complementary models to study the fundamental aspects of lateral root **development** and the **plasticity** of this process that results from a strong interaction with the environment. First of all, *Arabidopsis thaliana* is an obvious choice because of its ease of use and numerous available tools and resources. Secondly, we are using white lupin because of its capacity to produce cluster roots, that are extraordinary developmental adaptations to low phosphate, and for which we use the opportunity of an important source of funding provided by the ERC to generate several resources ourselves.

Our first approach focuses on the mechanisms of pre-initiation and initiation in *Arabidopsis thaliana*. Gravitropic stimulus in this plant induces the formation of lateral roots and has been used as an efficient way to induce LR and synchronize their development. However, if the remodeling of auxin transport during gravitropism is well characterized, leading to an accumulation of auxin in the lower face of the root, it remains a mystery how this induces a LR at the upper face of the gravistimulated root. Our collaborators performed a genetic screen to identify a mutant with altered root gravitropism in salt stress conditions (NaCl). This mutant was termed *lasso* because of the ability of its roots to roll up as lassos and shows an increased number of LR. Using this mutant, we want to understand the links between gravitropic response and LR pre-initiation and initiation; as well as how it is altered by the osmotic environment of the plant.



The Arabidopsis lasso mutant has a strongly agravitropic root system that curls up like a lasso…

Our second approach focuses on the development of cluster roots in white lupin. Cluster roots are specialized root structures that can be found in several botanical families (both dicots and monocots), they are also improperly called proteoid roots because they were originally identified in plants from the Proteaceae family in which they are almost universal (Neumann and Martinoia, 2002; Lambers et al., 2015). White lupin is the only annual crop producing cluster roots and as a result necessitates limited amount of P fertilizers. White lupin cluster roots have been used as a good model to study the physiology of phosphate acquisition (Dinkelaker et al., 1995; Müller et al., 2015) but, strangely, they have been largely overlooked in term of root development despite their amazing phenotype (Watt and Evans, 1999). Indeed, cluster roots are secondary roots that are made of hundreds of tertiary roots called rootlets clustered together and produced as a response to low phosphate. Interestingly, all rootlets primordia are produced from consecutive pericycle cells, suggesting a total absence of longitudinal inhibition between these organs. They become highly active and undergo an "*exudative burst*" of protons, organic acids and enzymes that aim at remobilizing phosphate, and they display high rates of phosphate uptake activity.



White lupin is a crop (A) cultivated for its high protein content seeds (B) that are used as food and feed. Its root system is well adapted to low phosphate (-Pi) conditions (C) by producing numerous cluster roots (D) that will strongly improve P uptake through soil acidification as revealed by pH indicator (E) to remobilize phosphate. Compared to high Pi conditions (+Pi), a strong developmental induction by low Pi (-Pi) is visible (F).

Although the role of auxin is expected in rootlet development, auxin applications are not sufficient to induce fully functional cluster roots, suggesting that another upstream signal triggers both the developmental and physiological responses characteristic of cluster roots. Our hypothesis is that a set of key regulators controls this response downstream of phosphate perception and upstream of auxin signaling. We want to identify these regulators, employing both forward and reverse genetic approaches. We believe that understanding how cluster roots are produced will provide fundamental knowledge on the mechanisms by which plants alter their development in response to the environment, but it will also provide clues to improve crops by means of translational biology.

2- Research products and activities for the team

Scientific track record

- **Characterization of the LBD29 transcription factor that regulates LAX3 during lateral root emergence** (Porco et al., 2016) B. Péret

We identified the role of LBD29 in regulating the induction of LAX3 by auxin during lateral root emergence. This work concerns writing/submitting/editing a manuscript related to work performed in previous laboratories.

- **Characterization of the *lasso* mutant linking root gravitropism and lateral root initiation in salt stress conditions** (on-going work) B. Péret/F. Divol/M. Thomas

Our collaborators identified a novel mutant altered in root gravitropic response in salt stress conditions from a genetic screen (Coll. E. Hrabak, USA). Further studies in the lab showed that lateral root initiation events are also altered in this mutant. There is an increase in auxin accumulation suggested by over-expression of the DR5 reporter in the columella and lateral root cap cells. We believe this mutant can be a useful tool to understand the link between perception of gravity and the initiation of lateral root and how this link is modulated by the osmolarity of the medium.

- **High quality genome sequence discloses important events during domestication of white lupin** (Hufnagel et al., in preparation) B. Péret/P. Doumas/L. Marquès/F. Divol/B. Hufnagel/A. Marquès

In 2018, we generated the first *de novo* sequence of white lupin (*Lupinus albus* cultivar AMIGA) with a high quality assembly (N50=17Mb) and automated annotation (38 258 genes). We assembled an international consortium to analyze this genome in term of repeated elements (for which we performed FISH and immunoFISH analysis), paleogenomics (on genes and repeated elements), synteny, variability and population structure (14 resequenced varieties). This approach allowed us to understand the effect of domestication on various traits such as seed quality and root development, using a wild white lupin relative and an Ethiopian landrace as reference varieties. (Coll. J. Gouzy, LIPM Toulouse; H. Bergès, CNRGV Toulouse; R. Guyot, IRD Montpellier; J. Salse, INRA Clermont; M. Crespi, IPS2 Paris; K. Gallardo, Agroécologie Dijon; PM. Delaux, LRSV Toulouse; M. Nelson, Australia; F. Geu-Flores, Denmark). This work is under preparation for publication (Hufnagel et al.).

- **Anatomical and hormonal description of rootlet primordium development along white lupin cluster root** (Gallardo et al., 2019) B. Péret/P. Doumas/L. Marquès/F. Divol/C. Gallardo/B. Hufnagel/C. Casset/F. Garcia

We produced an anatomical description of cluster root development using classical histology techniques. We confirmed that rootlets originate from the pericycle cells and that divisions in the endodermis and cortex occur concomitantly. We developed a transient genetic transformation protocol (hairy root) suitable for expression studies and used a set of marker genes to identify the various tissues during rootlet development. We also were able to describe the establishment of an auxin maximum in the rootlet.

- **Identification of transcription factors involved in cluster root formation** (on-going work) B. Péret/P. Doumas/L. Marquès/F. Divol/C. Gallardo/B. Hufnagel

We generated two major transcriptomic datasets describing cluster root development based on a spatial (8 points) and temporal (12 points) sampling technique. We performed a Gene Regulatory Network analysis using TDCor (Coll. L. Laplaze, IRD Montpellier) and identified several candidates with interesting expression profiles and strongly connected in the network. We performed functional studies using hairy root transformation that suggests an involvement of 3 Transcription Factors (TFs) in this process. Despite putting important efforts in developing stable transformation, white lupin regeneration is still not possible. Therefore, we developed a small TILLING population (600 M2 families) to perform reverse genetics studies. We plan to screen this population to search for stable mutants affected in these TFs and study their impact on cluster root development.

- **Identification and characterization of constitutive cluster root mutants** (on-going work) B. Péret/F. Divol/F. Garcia/V. Fernandez/L. Brottier

We screened our EMS-mutagenized population in the dedicated growth chambers built in 2015-16. Around 22 000 seedlings from ca. 600 M2 families were grown in repressive conditions (high phosphate) where no cluster roots are formed in wild-type plants. We identified several mutants with constitutive cluster root formation and genetic studies are underway. We have performed crosses for allelic tests and to generate F2 populations (back-cross to AMIGA cultivar and out-cross to FEODORA cultivar). These F2 populations will be harvested in September 2019 and subsequently sequenced to identify the causal mutations of 4 mutants with the strongest developmental phenotype. We believe that this forward genetic approach will lead to identification of major genes controlling cluster root formation.

- **Rootlet emergence necessitate physical remodeling of outer cell layers** (on-going work) B. Péret/F. Jobert

Lateral roots are produced deep inside the parental tissue (pericycle) and have to pass through various cell layers in a process called lateral root emergence. Although it was described in the model plant Arabidopsis, it is not known whether the mechanisms governing emergence are conserved in other plants. Together with Stéphanie Robert (UPSC, Sweden) we obtained a funding from the Kempe foundation for a 2-year post-doc to study the emergence process in white lupin. We believe this is an interesting model because of the numerous cortical cell layers that rootlets have to go through and because of the numerous rootlets produced very closely to each other. We are looking at the expression profile of the 9 lupin auxin influx transporters (AUX-LAX homologs) and we want to study the cell wall properties of the cortical cells facing the rootlet primordium using AFM (Atomic Force Microscopy). We will also generate mutants of the major AUX/LAX genes using our TILLING population.

- **Rootlet determinism is associated with physiological activity** (on-going work) P. Doumas/L. Marquès/T. Le Thanh

Rootlets from white lupin present a determinate mode of development, we observed very quickly after their emergence, that cell divisions stop (observed with a *CYC;B1* marker). Further growth of the rootlet is then achieved mainly through cell elongation (observed with an expansin *EXPA* marker). We also identified that maturation of the rootlets is associated with specific organic acid exudation profiles (mainly citrate and malate) and phosphate uptake activity. This means that cluster roots display some sort of synchronization between their development and their physiological activity.

Key events

De novo **sequence of white lupin genome**

One of the key scientific advances of the Plasticity team was the *de novo* sequencing of white lupin genome that was achieved in 2018. This genomic resource represents a major project in the team that is currently under preparation for publication. It gathered a consortium of a dozen of research teams (several from INRA laboratories) to generate a comprehensive study of white lupin genome (from repeated elements to diversity, from seed quality to root system architecture). We were able to build on this resource to produce high quality transcriptomic datasets and to start functional studies. We also shared this resource with different partners to promote their research, as an example we helped colleagues interested in alkaloid synthesis allowing to find the molecular mechanisms behind a major QTL for sweet lupin seeds. Although this is not the primary focus of the team, we believe that generating this resource was an indispensable pre-requisite for our on-going and future functional studies.

FIVE-YEAR PROJECT AND STRATEGY

1- SWOT analysis

Strength	Opportunities
-Numerous tools/resources developed in white lupin -Original biological model -Identification of mutants with strong phenotypes -Growing network of collaborators	-Ability to focus on functional approaches -Ability to study strong developmental responses -Possibility to identify novel regulatory mechanisms -Possibility to extend our research focus
Weekness	**Threat**
-Reduction of team size (less contracts/retirement of 1 member) -Reduction of budget (end of ERC project) -Difficulties inherent to the white lupin model (few seeds/long lifecycle)	-Risk of being overwhelmed: need to focus on a small number of scientific questions -Risk of lack of funding: need to apply for grants -Delays in publishing our work

2- Structure, workforce and scientific orientations

In the next unit period, we plan to continue to focus our efforts on studying the molecular aspects of Development and Plasticity of the Root System using both Arabidopsis and white lupin as models. The project will be organized in 3 axis (only permanent personnel participations are indicated for the project):

- Pre-initiation/initiation (B. Péret/F. Divol and collaboration with C. Maurel/P. Nacry AQUA team)

Understanding how osmotic stress (either salt or sorbitol) is impacting auxin transport in the Arabidopsis primary root to alter gravitropic response and lateral root pre-initiation and initiation is of major interest. We will keep characterizing the *lasso* mutant for which the causal mutation has been identified (confidential) and we plan to determine how auxin homeostasis is affected in this mutant by measuring gene expression of genes involved in auxin synthesis, transport, conjugation and catabolism. We also anticipate that auxin measurement in the root tip in various conditions will be informative to understand the reduction in the DR5 reporter expression seen upon sorbitol application. We believe that the expertise of our colleagues form the AQUA team will be useful to understand how an osmotic stress can alter plant development.

- Cluster root initiation (B. Péret/P. Doumas/ L. Marquès/F. Divol)

-Identification of the causal mutations in the *ccr* mutants

We have now identified several constitutive cluster root mutants on which we will focus our energy to reveal their causal genes and then perform functional characterization. At the time we are writing this report, it is difficult to anticipate what these functional approaches will be given the unknown nature of the gene(s). However, our speculation was that the genes responsible for the *ccr* phenotype should sit somewhere downstream of phosphate perception and upstream of auxin response. Indeed, we observed that *ccr* mutants not only over-produce cluster roots but also display constitutively high phosphate starvation gene expression. Both responses however remain further induced by low phosphate. We are planning to perform proteomic experiments on wild-type plants and this will help us speed the characterization process once we identify candidates. We also set up a small TILLING population (600 M2 families) that we plan to start screening in 2019 and we project to expand to a higher number of families (see MicroLUP project below). This will give us a possibility to identify alleles of our mutants, in the absence of a stable transformation protocol for white lupin. Although it is worth noting that the last years of the ERC project will keep putting efforts into trying to develop such a protocol that would open the way to genome editing for functional approaches.

-Role of miRNA in rootlet development (conditional sub-axis in the context of a submitted ANR proposal MicroLUP)

In order to better understand the molecular mechanisms controlling cluster root formation, the MicroLUP project will study the role of microRNAs during the development of this organ. The project gathers a consortium of 3 partners including the teams of Benjamin Péret (Scientific coordinator), Martin Crespi (IPS2 unit) and Adnane Boualem (IPS2 unit). This project will combine the development of gene regulatory networks involving miRNAs and their target genes and the generation of genetic resources through TILLING that can be used to improve our understanding of cluster root development as well as for field applications.

- Cluster root determination (P. Doumas/L. Marquès/ B. Péret)

In growing roots, the regular recruitment of meristem cells to form new differentiated root tissues is balanced by cell proliferation within the meristem, sustained by the maintenance of a central root meristem stem cell niche. This steady-state can persist during long periods in most roots that are then said to be with indeterminate growth. Conversely, in some cases, the root meristem is genetically programmed to stop producing new cells at a specific developmental stage. In these cases, the meristem is said to be determinate. Determinate root growth can be found, in particular, in clustered rootlets. Therefore, the activity of genes influencing QC cell or stem cell identity is expected to be at the core of the rootlet meristem maintenance mechanism, while the activity of genes controlling the cell cycle or influencing the switch between the meristematic/differentiating pathways may be crucial for the control of root meristem size. We propose to compare two phenomena, determinate versus indeterminate root growth, in order to develop novel strategies to control root architecture responses.

-New axis of research: quantitative genetics and comparative genomics of lupin (B. Péret/recruitment of CR INRA ?)

Generating a *de novo* sequence for white lupin opened new possibilities that are being exploited by Barbara Hufnagel, current post-doctoral researcher in the team. We believe that exploiting the existing variability in the white lupin species could reveal new regulators of cluster root development. Our collaborators have a 323 collection of *L. albus* varieties with GBS data (Paolo Annicchiarico, CREA, Italy) and a core collection of 40 varieties with full sequence data (Mathew Nelson, Australia) that we plan to use for a GWAS approach. We will take advantage of the PhénoROOT platform for white lupin automated root system phenotyping (in development) and of the 4PMI platform in Dijon (coll. Christophe Salon, UMR Agroécologie, Dijon). We want to extend this approach to other species in the *Lupinus* genus to describe plants with or without the ability to form cluster roots and perform full genome sequencing of a core collection of species. This potential project corresponds to a request for a new position at INRA for a Chargé de Recherche.

Annex 4 – Research products and activities

EVALUATION CAMPAIGN 2019-2020

GROUP A

Name of the team **Development and Plasticity of the Root System**
Acronym: **PLASTICITY**

Theme leader for the current contract: **Benjamin PERET**
Theme leader for the next contract: **Benjamin PERET**

I - PRODUCTION OF KNOWLEDGE AND ACTIVITIES CONTRIBUTING TO THE INFLUENCE AND SCIENTIFIC ATTRACTIVENESS OF THE TEAM

1- Articles

Scientific articles

2016

1. Porco S., Larrieu A, Du Y., Gaudinier A., Goh T., Swarup R., Bishopp A., Keumpers B., Lavenus J., Casimiro I., Hill K., Swarup K., Benkova E., Fukaki H., Brady S.M., Scheres B., Péret B., Bennett M. (2016) Lateral root emergence in Arabidopsis is dependent on transcription factor LBD29 regulation of auxin influx carrier LAX3. Development 143(18): 3340-3349.

2018

2. Bureau C., Lanau N., Ingouff M., Hassan B., Meunier A.C., **Divol F.,** Sevilla R., Mieulet D., Dievart A., Périn C. (2018) A protocol combining multiphoton microscopy and propidium iodide for deep 3D root meristem imaging in rice: application for the screening and identification of tissue-specific enhancer trap lines. Plant Methods. 14 96.

2019

3. **Gallardo C., Hufnagel B., Casset C., Alcon C., Garcia F., Divol F., Marquès L., Doumas P., Péret B. (2019) Anatomical and hormonal description of rootlet primordium development along white lupin cluster root. Physiol. Plantarum. 165: 4-16.**

Review articles

Other articles (professional journals, etc.) (total number)

2- Books

Monographs, critical editions, translations (total number)

Management and coordination of scientific books / Scientific book edition

Book chapters (total number)

Book chapters in English or another foreign language

Edited theses

3- Production in conferences / congresses and research seminars

Articles published in conference proceedings / congress

Other products presented in symposia / congress and research seminars

Invited oral presentations in international symposia/congress

2015

1. Péret B. Unravelling cluster root development in white lupin.(December 3rd 2015) Lateral Root Workshop, Heidelberg (Germany).

2016

2. Péret B. (2016) Unravelling cluster root development in white lupin. *(7-8 april 2016)* UKPPN Root Phenotyping Workshop, University of Nottingham (UK).

2017

3. Péret B. (2017) Lasso mutants link root gravitropic response and salt stress. (June 2017.) 8th International Symposium on Root Development. Umeå (Sweden).
4. Péret B. (2017) Unravelling cluster root development in white lupin. (March 2017) Plant Organ Growth Symposium. Elche, (Spain).

2018

5. Hufnagel B., Marques A., Marande W., Sallet E., Soriano A., Arribat S., Bergès H., Gouzy J., Péret B. (2018) Developing white lupin as a model to study cluster root formation and development Nottingham (17th October 2018) Lateral Root workshop (UK).

Invited oral presentations in national symposia/congress

Selected oral presentations in international symposia/congress

2017

1. Péret B. (2017) Lasso mutants link root gravitropic response and salt stress. (June 2017.) 8th International Symposium on Root Development. Umeå (Sweden).
2. Péret B. (2017) Unravelling cluster root development in white lupin. (March 2017) Plant Organ Growth Symposium. Elche, (Spain).

2018

3. Hufnagel B., Marques A., Marande W., Sallet E., Sorriano A., Arribat S., Bergès H., Gouzy J., Péret B. (2018) Genome sequence of white lupin, a model to study root developmental adaptations. (5-10 august) 12th Congress of the International Plant Molecular Biology. Montpellier (France).

Selected oral presentations in national symposia/congress

2017

1. Gallardo C. (2017) Histological and transcriptomics analysis of cluster root development in white lupin. (27 October) Montpellier Lateral Root workshop. Montpellier (France)

2018

2. Hufnagel B., Marande W., Sallet E., Arribat S., Bergès H., Gouzy J., Péret B. (2018) Genome sequence of white lupin, a model to study root developmental adaptations. Journées Inter Labex Fév 2018. Montpellier (France).
3. Hufnagel B., Marques A., Marande W., Sallet E., Soriano A., Arribat S., Bergès H., Gouzy J., Péret B. (2018) Séquençage du génome du lupin blanc, un modèle pour étudier les adaptations du système racinaire. (17th October 2018) Rencontres Francophones Légumineuses Toulouse (France)

2019

4. Hufnagel B., Soriano A., Lucas M., Guyomarc'h S., Marquès L., Doumas P., Laplaze L., Péret B. Genome Sequence Of White Lupin, A Model To Study Root Developmental Adaptations. PAG Meeting, 12-16th May 2019. San Diego, USA.

Posters in international symposia/congress

2018

1. Divol F., Garcia F., Gallardo C., Hufnagel B., Casset C., Marquès L., Doumas P., Péret B.(2018) Identification of constitutive cluster root mutants in white lupin. (5 -10 août) 12th Congress of the International Plant Molecular Biology. Montpellier (France).

2. Divol F., Garcia F., Gallardo C., Hufnagel B., Casset C., Marquès L., Doumas P., Péret B. (2018) Identification of constitutive cluster root mutants in white lupin (10-14th September) International Plant Systems Biology meeting, Roscoff (France).

3. Divol F., O'connor C., Soriano A., Thompson M., Hrabak E., Péret B. (2018) the Arabidopsis lasso1 mutant links root gravitropic response and salt stress. (25–29 June 2018) 29th International Conference on Arabidopsis Research (ICAR). Turku, (Finland).

4. Hufnagel B., Marques A., Marande W., Sallet E., Soriano A., Arribat S., Bergès H., Gouzy J., Péret B. (2018) Genome sequence of white lupin, a model to study root developmental adaptations (5 -10 août) 12th Congress of the International Plant Molecular Biology. Montpellier (France).

5. Hufnagel B., Soriano A., Lucas M., Guyomarc'h S., Marquès L., Doumas P., Laplaze L., Péret B. (2018) Gene regulatory network controlling cluster root development in white lupin (10-14th September) International Plant Systems Biology meeting, Roscoff (France)

6. Jacquot A., Chaput V., Mauries A., Li Z., Bonillo P., Tillard P., Fizames C., Santoni V., Gojon A., Schulze W., Lejay L. (2018) Post-translational regulation of the root nitrate uptake transporter NRT2.1 in *Arabidopsis thaliana*. (5 -10 août) 12th Congress of the International Plant Molecular Biology. Montpellier (France).

7. Marques A., Hufnagel B., Schubert V., Marande W., Sallet E., Soriano A., Arribat S., Houben A., Bergès H., Gouzy J., Péret B. Genome annotation and chromosome mapping of repetitive DNA sequences as a powerful tool to improve the white lupin (*Lupinus albus*) whole genome assembly (2 - 5 September 2018) XXII International chromosome conference, , Prague (Czech Republic).

8. Marques A., Hufnagel B., Marande W., Sallet E., Soriano A., Arribat S., Bergès H., Gouzy J., Péret B. Estimating the repetitive DNA profile of Lupinus albus and Stylosanthes genomes: a cytogenomic approach. (3 - 6 June 2018) Plant Genome Stability and Change. Gatersleben (Germany).

9. Marques A., Hufnagel B., Marande W., Sallet E., Soriano A., Arribat S., Bergès H., Gouzy J., Péret B. (2018) Estimating the repetitive DNA profile of white lupin (Lupinus albus) genome: a cytogenomic approach (5 -10 août) 12th Congress of the International Plant Molecular Biology. Montpellier (France).

2019

10. Hufnagel B., Soriano A., Lucas M., Guyomarc'h S., Marquès L., Doumas P., Laplaze L., Péret B. (2019) Genome Sequence Of White Lupin, A Model To Study Root Developmental Adaptations. (12-16th May). PAG Meeting, San Diego (USA).

Posters in national symposia/congress

2016

1. Marquès L., Hufnagel B., Marande W., Sallet E., Soriano A., Arribat S., Bergès H., Gouzy J., Péret B. (2016) Unravelling cluster root development in white lupin,(31mai-1 juin 2016) 1ères Rencontres Francophones Légumineuses, Dijon (France).

Invited seminars in foreign institutions

2018

1. Divol F., O'connor C., Soriano A., Thompson M., Hrabak E., Péret B. The *Arabidopsis* lasso1 mutant links root gravitropic response and salt stress. (17th May 2018) Nara Institute of Science and Technology (Japan)

2. Divol F., O'connor C., Soriano A., Thompson M., Hrabak E., Péret B. The *Arabidopsis* lasso1 mutant links root gravitropic response and salt stress. (15th May 2018) Kobe University (Japan)
3. Hufnagel B., Péret B. Unravelling cluster root development in white lupin. (11th April 2018) CREA Lodi (Italy)
4. Péret B. Unravelling cluster root development in white lupin. (22nd November 2018) LMI RICE2 Hanoi (Vietnam)
5. Péret B. Unravelling cluster root development in white lupin. (17th May 2018) Nara Institute of Science and Technology (Japan)
6. Péret B. Unravelling cluster root development in white lupin. (15th May 2018) Kobe University (Japan)

Invited seminars in French institutions

2016

1. Péret B. Unravelling cluster root development in white lupin. (24th November 2016) LIPM Toulouse (France).

2017

2. Péret B. Identifying transcription factor X during lateral root emergence. (20th January 2017) PhD choice Bordeaux (France).

2018

3. Péret B. Unravelling cluster root development in white lupin. (19th march 2018) Agroécologie Dijon (France).
4. Péret B. Unravelling cluster root development in white lupin. (28th march 2018) IPS2 Paris Saclay (France).

4- Electronic tools and products

Softwares

Databases

In 2016-17, we generated several transcriptomics datasets covering cluster root development both from a spatial (8 points) and temporal (12 points) point-of-view as well as an auxin response dataset (8 points). In order to complement this expression analysis, we also invested in a pipetting robot for automated plate production for qPCR analysis (20k€).

5- Instruments and methodology

Prototypes

In 2019, we are developing PhénoROOT, an automated root phenotyping platform for white lupin (capacity 50 plants) that is designed to acquire daily images of up to 3 week-old white lupin root systems (Coll. Laboratoire de Mécanique et de Génie Civil, CNRS/UM, Montpellier).

Platforms and observatories

In 2015, we built scientific equipment for white lupin growth in hydroponic conditions dedicated to screening of 2500 seedlings and developmental/physiological studies. The chambers are environmentally friendly (LED lighting) and semi-automated (osmosed water production and tank filling, medium aeration). They were entirely funded by the ERC LUPIN ROOTS project (400k€).

In 2015, we generated an EMS-mutagenized population for white lupin (AMIGA) that was screened in 2016-17 to search for constitutive cluster root mutant in repressive conditions (high phosphate). It was then used in 2018, to generate a small TILLING population of 600 M2 families to be able to retrieve mutants in reverse genetics approaches. As part of the submitted ANR MicroLUP project, we are planning to extend this population to 8000 M3 families.

6- Other products

Theorised artistic creations, staging, movies

7- Editorial activities

Participation in editorial committees (books, collections, etc.)

Collection and series management

8- Reviewing activities

Reviewing of articles

Journals : **Plant Physiology** - **Planta - Plant, Cell & Environment - Plant and Soil - Philosophical Transactions of the Royal Society B - PloS One - J Exp Bot - Plant Science - Trends Plant Sci (B. Péret)**

Grant evaluation (public or charities)

Projects : **ANR - DFG (ERA-CAPS) - FWO - BARD - Labex SPS - FWF - CSF (B. Péret)**

Reviewing of research institutes

Participation in institutional committees and juries (CNRS, Inserm, etc.)

Jury de concours interne IE (IE710-2017 - F. Divol)

9- Academic research grants

Financial means of the team were provided by the ERC LUPIN ROOT project (2M€), Labex Agro Exploratory ROOT4EVER project (17k€), Labex Agro Soutien ponctuel LATERAL ROOT WORKSHOP project (8k€), Kempe foundation project (2y post-doc to F. JOBERT), mobility grants from MUSE EXPLORE and French Ambassy in Japan. Submitted project: ANR MicroLUP with Martin Crespi and Adnane Boualem (IPS2 - France) Scientific coordinator B. PERET.

European (ERC, H2020, etc.) and international (NSF, JSPS, NIH, World Bank, FAO, etc.) grants – coordination

2015 – 2020 ; **LUPIN ROOTS (ERC, Starting Grants)** ; Unravelling cluster root development in white lupin ; PERET Benjamin

European (ERC, H2020, etc.) and international (NSF, JSPS, NIH, World Bank, FAO, etc.) grants - partnership

Other European grants - coordination

Other European grants - partnership

National public grants (ANR, PHRC, FUI, INCA, etc.) - coordination

National public grants (ANR, PHRC, FUI, INCA, etc.) - partnership

2015 – 2019 ; **ADEME** ; Etude d'un programme de réaménagement et de la faisabilité du phytomanagement ; DOUMAS Patrick

Local grants (collectivités territoriales) - coordination

Local grants (collectivités territoriales) - partnership

PIA (labex, equipex etc.) grants - coordination

2017 – 2017 ; **LATERAL ROOT (Labex AGRO)** ; Montpellier lateral Root workshop 2017 ; PERET Benjamin

2017 – 2018 ; **ROOT FOR EVER (Labex AGRO)** ; Root development: to grow or not to grow ? ; DOUMAS Patrick

2018 – 2019 ; **MUSE EXPLORE (iSITE Montpellier)** ; Programme de soutien à la mobilité internationale Explore 2018 de MUSE ; PERET Benjamin

PIA (labex, equipex etc.) grants - partnership

2018 – 2019 ; **180-PLAT (Labex AGRO)** ; Establishment of a new platform for the analysis of the oxygen isotopic ratio in phosphate extracted form environmental samples : a new opportunity to unravel the role of biological processes in P Cycling ; DOUMAS Patrick

Grants from foundations and charities (ARC, FMR, FRM, etc.) - coordination

Grants from foundations and charities (ARC, FMR, FRM, etc.) - partnership

10- Visiting senior scientists and post-doc

Post-docs (total number)

Depuis 2014: 4

Foreign post-docs

Depuis 2014: 3

Last Name	First Name	Funding/Employer	Nationality	Périod	
Fernández	Virginia	CDD CNRS ERC	ARGENTINA	01/10/2018	30/09/2019
Hufnagel	Bárbara	CDD CNRS ERC	BRAZIL	01/10/2016	30/09/2019
Marques	André	CDD CNRS ERC	BRAZIL	01/02/2018	30/09/2018

Visiting scientists (total number)

Depuis 2014 : 0

Foreign visiting scientists

Depuis 2014 : 0

11- Scientific recognition

Prizes and/or distinctions

IUF members

Chair of learned and scientific societies

Organisations of meetings and symposia (out of France)

Invitations to meetings and symposia (out of France)

II - INTERACTION OF THE TEAM WITH THE NON-ACADEMIC WORLD, IMPACTS ON ECONOMY, SOCIETY, CULTURE OR HEALTH

1- Socio-economic interactions / Patents

Invention disclosures

Filed patents

Accepted patents

Licenced patents

2- Socio-economic interactions

Industrial and R&D contracts

Cifre fellowships

Creation of labs with private-public partnerships

Networks and mixed units

Start-ups

3- Expertise

Consulting

Participation in expert committees (ANSES etc.)

Legal expertise

Expert and standardization reports

4- Public outreach

Radio broadcasts, TV shows, magazines and newspaper

Journal articles, interviews, book edition, videos, other popularization outputs, debates on science and society, etc.

III - INVOLVEMENT OF THE TEAM IN TRAINING THROUGH RESEARCH

1- Educational outputs

Books

E-learning, MOOCs, multimedia lessons, etc.

2- Scientific productions (articles, books, etc.) from theses

Scientific productions (articles, books, etc.) from theses

Scientific articles

Gallardo C., Hufnagel B., Casset C., Alcon C., Garcia F., Divol F., Marquès L., Doumas P., Péret B. (2019) Anatomical and hormonal description of rootlet primordium development along white lupin cluster root. Physiol. Plantarum. 165: 4-16

Mean number of publications per student (Biology & Science and technology only)

3- Training

Habilitated (HDR) scientists

Since 2014 : 3

Last Name	First Name	Status	Employer	Date of HDR
DOUMAS	Patrick	chercheur	INRA MONTPELLIER	2011
MARQUES	Laurence	enseignant-chercheur	UNIVERSITE MONTPELLIER	2015
PERET	Benjamin	chercheur	CNRS	2014

HDR obtained during the period

Since 2014 : 2 HDR

Last Name	First Name	Status	Employer	Date dof HDR
MARQUES	Laurence	enseignant-chercheur	UNIVERSITE MONTPELLIER	2015
PERET	Benjamin	chercheur	CNRS	2014

PhD students (total number)

2 PhD students

PhD students benefiting from a specific doctoral contract

Last Name	First Name	Starting Date	Defense Date	Duration of PhD	Funding
GALLARDO	Cécilia	01/10/2016		36	CDE
LE THANH	Tamara	01/10/2018		36	CDE

Defended PhDs

0 defended PhD

Mean PhD duration

Mean duration:

Internships (M1, M2)

Master 1: 3

Master 2: 4

Last Name	First Name	Level	Period	
Mell	Pauline	Master 1	06/03/2017	30/06/2017
O'connor	Claire	Master 1	06/03/2017	30/06/2017
Laguerre	Malika	Master 1	01/03/2018	31/07/2018
Gallardo	Cécilia	Master 2	04/01/2016	30/06/2016
Bintsi frantzi	Evdoxia	Master 2	01/11/2016	31/01/2017
Le thanh	Tamara	Master 2	08/01/2018	08/07/2018
Thomas	Marion	Master 2	07/01/2019	07/07/2019

People in charge for a mention or a master's degree course (total number)

People in charge for a mention or a master's degree course with international certification (Erasmus mundus)

ELSA team
Self-assessment document

Name of the team concerned by the current contract: Electrophysiology of Plant Mineral Nutrition and Root Symbioses

Acronym of the current contract: ELSA

Team leader for the current contract: Hervé Sentenac

This team will be closed on 31/12/2020. Therefore, no project is presented for the next contract

RESULTS

1 Presentation of the team

Introduction

"*ELSA*" was formed in July 2015, from the former group "*Ion Channels*", a re-organization which also gave rise to two other teams, *TICER* (Ion transpo…in Cereals…) and *KalipHruit* (K^+ transport and fruit acidity in grapevine). At that time, the "*Ion Channels*" group was very large and carried out very distinct research activities. This re-organization was also wished in view of the retirement of the group leader, Hervé Sentenac, in 2021. Within the new organization, *ELSA* gathered the permanent staff working on beneficial interactions between plant roots and soil microbes. Strong connections with research from the former *Ion Channel* team have been maintained, with respect to common interests in electrophysiological analyses of ion channel activities in plants (e.g., Nieves-Cordones ***et al***., 2017 & 2019; Wang ***et al.***, 2019) and new interactions have been developed with *TICER* aiming at implementing a project on beneficial interactions of rhizobia with cereals (durum wheat) roots.

Team's workforce and means

Since July 2015, the permanent staff of the team has comprised Martin Boeglin (TR INRA), Guilhem Desbrosses (Pr., Montpellier University), Lydia Gamet (Technician, Montpellier University, half-time research besides teaching duties), Hervé Sentenac (DR INRA), Bruno Touraine (Pr., Montpellier University) and Sabine Zimmermann (CR CNRS). A newly recruited (June 2017)P) "Assistant-Professor" (MCF, University of Montpellier), Loren Castaing, (previous post-doc in the *TSF* team of BPM, joined the ELSA team at the end of 2017, and rejoined her former research group (TSF) in BPMP in 2019.

Nine PhD students (5 PhD defenses by the end of 2018) have worked or are working in the team *ELSA* (see Annex 4), one of them (T. Rongsawat) in co-supervision with the *TICER* team. *ELSA* has supervised or co-supervised (with TICER) 7 M1 and 6 M2 students (see Annex 4). The group has got funding from 8 National Public grants (including 3 ANR programs) (see Annex 4). ELSA has also welcome 6 foreign visiting scientists (see Annex 4), essentially for training and or experiments on their own research topics (mean duration in the group > 2 months), often in collaboration with the *TICER* team.

Scientific policy

The current programs are based on the following 2 widely reported observations:

(1) In natural conditions, in most ecosystems, plant hydromineral nutrition strongly benefits from root symbiotic interactions with fungal and bacterial species, especially mycorrhizal fungi, N_2 fixing rhizobia and bacteria generically named PGPR for Plant Growth Promoting Rhizobacteria. Deep understanding of the functioning of root systems and their interactions with soil microorganisms, especially in term of hydromineral nutrition, is required to better exploit the genetic variation in the productivity of crops in poorly fertile soils (or in absence of high levels of fertilization/irrigation) and to develop new crop cultivars with enhanced capacity for soil resource acquisition (Brown et al., 2013).

(2) The beneficial microbial partners, which can act by solubilizing, mobilizing and taking up nutrients that they render available for/secrete towards the roots of the plant partner, also affect the architecture of the root system and the development of root hairs. For instance, interactions with PGPR can result in strong development of root hairs, a process that is likely to improve plant nutrition by increasing the volume of exploited soil.

ELSA is especially interested in (1) ectomycorrhizal symbiosis and the fungal membrane transporters and channels that mediate the fluxes of nutrients from the soil to the roots (the theme that has initiated the work on root symbioses in the former *Ion Channel* team at the end of the 1990's), (2) transporters and ion channels active at the root hair cell membrane and likely to contribute to nutrient acquisition and, in legume, to the electrical signaling events triggered by perception of bacterial Nod factors, (3) root responses to rhizobia, in terms of root architecture and root hair development, in the model plant Arabidopsis and in cereals (in collaboration with the *TICER* team).

Common key words describing these research activities: plant mineral nutrition, electrophysiological analyses, ion transporters and channels, root system development and functioning, soil exploration/exploitation by root hairs and fungal hyphae.

2- Research products and activities for the team

1. Identification and characterization of membrane transport systems involved in nutrient exchange in ectomycorrhizal symbiosis

S. Zimmermann / PhD students: K. Garcia, C. Guerrero-Galán, G Houdinet and M. Usman

The vast majority of land plants associates with mycorrhizal fungi and rely on them for their nutrient and water supply (Smith & Read, 2008; Brundrett & Tedersoo, 2018). The most widespread and studied types are arbuscular mycorrhizal and ectomycorrhizal (ECM) symbioses. These beneficial interactions involve controlled expression and activity of specialized membrane transport systems in fungi and plants (Garcia **et al.**, 2016; Courty **et al.**, 2017; Guerrero-Galán **et al.**, 2018a). We have been working at characterizing fungal transport systems and at deciphering their roles in the symbiotic interaction using an ECM model that allows molecular and functional analyses (Becquer **et al.**, 2019), ***Hebeloma cylindrosporum***, symbiotic partner of ***Pinus pinaster***. This fungus can be transformed (Ngari **et al.**, 2009), its genome has been sequenced and annotated in 2011 (JGI; Kohler **et al.**, 2015), and transcriptomic data are available (Doré **et al.**, 2015, 2017). Objective of our program on ECM symbiosis is to investigate the fungal membrane transport systems and to analyze the specialization between the sites of nutrient ion uptake from the soil and the sites of secretion towards root cells. As the fungal mechanisms responsible for nutrient secretion towards the host root cells at the symbiotic interface, the Hartig net, are still poorly known, a challenging work during the last period has been aimed at identifying and characterizing potassium (K^+) and phosphate (Pi) membrane transport systems contributing to such secretion.

Progress towards this objective has required significant methodological investments from our group. Transformation of the ECM fungus ***H. cylindrosporum*** by ***Agrobacterium*** was established (ANR "Transmut", 2010-2014), dedicated transforming vectors for localization and overexpression or RNAi have been constructed (PhD K. Garcia 2013), and *in situ* hybridization was developed. These tools have been used first to investigate the Pi-dependent expression and spatial localization of the fungal Pi transporter ***HcPT1.1*** (Garcia **et al.**, 2013). In continuity, in collaboration with C. Plassard (UMR Eco&Sols, Montpellier), the Pi transporter HcPT2 has been shown to be involved in both Pi uptake and Pi transfer towards the plant host (Becquer **et al.**, 2018). Regarding our main project on K^+ transport (Garcia & Zimmermann, 2014), 2 K^+ transporters from the Trk family, 1 K^+ transporter from the HAK family, 3 K^+ channels from the TOK family and 1 K^+ channel from the Shaker family have been identified in the genome of ***H. cylindrosporum*** (http://genome.jgi-psf.org/Hebcy2/Hebcy2.home.html). The Shaker channel, HcSKC (Garcia **et al.**, in revision), and one of the two Trk transporters, HcTrK1 (Corratgé **et al.**, 2007, 2010), were already identified and in part characterized using a cDNA library and EST collection produced in the team (Lambilliotte **et al.**, 2004). All these K^+ transport systems are under analyses regarding (i) functional characterization (ion selectivity, direction of transport) by heterologous expression in ***Xenopus laevis*** oocytes and two electrode voltage-clamp analyses or by yeast complementation experiments, and (ii) expression and localization (Q-RT-PCR, *in situ* hybridization and/or transgenic lines expressing reporter constructs). (iii) Moreover, transgenic fungal lines with modified expression levels (RNAi or overexpression) to study the putative roles of these transport systems are produced and analyzed. In particular, fungal lines with reporter markers and with modified transcript levels of the ***HcTrk1*** K^+ transporter have shown that ***HcTrk1*** is mainly expressed in extraradical hyphae and in the hyphal mantle, and that alteration of its expression levels affects the plant K^+ nutrition (K^+ shoot contents) and Pi homeostasis in ECM plants (Garcia **et al.**, 2014). Interestingly, in addition to the first candidates implicated in K^+ uptake (HcTrk1) and putatively in K^+ secretion (HcSKC), we have identified in the genome three members of the two-pore K^+ (TOK) channel family (HcTOK1, 2.1, 2.2) raising the question of their specificities. Combination of functional approaches and localization analyses gave interesting results suggesting a role of HcTOK2.2 for K^+ transfer at the symbiotic interface and for HcTOK1, that is functionally similar to the yeast TOK (Ketchum **et al.**, 1995), a more general role in the fungal K^+ homeostasis (PhD C. Guerrero-Galán 2017; Guerrero-Galán **et al.**, 2018b,c). However, characterization of HcTOK2.2 could not yet be completed and requires attempts of functional characterization in yeast as well as of establishing CRISPR/Cas tools for the ECM fungus (PhD G. Houdinet, 2016-2019). Characterization of HcTrk2 and HcHAK will complete the analyses of K^+ transport within the ECM fungus (PhD M. Usman, 2018-2021). In addition to the significant improvement of K^+ nutrition in ECM pine seedlings under K^+ starvation conditions (Garcia **et al.**, 2014), ECM symbiosis improves plant performance also under abiotic stress as salinity (Guerrero-Galán **et al.**, 2019a, in press). RNAseq data (Doré **et al.**, 2015, 2017) have identified ECM-regulated ***H. cylindrosporum*** membrane transporters that might be involved in symbiosis (Guerrero-Galán **et al.**, 2019b, in prep.). Among these transporters, we found macronutrient transporters for Pi and K^+ (HcPT2, HcTOK2.2,), but interestingly, also a CDF member (Cation Diffusion Facilitator) annotated as Zn transporter (Ryutinx **et al.**, 2019, submitted). Dissection of the role of this candidate will be challenging to understand the role of such micronutrients in ECM symbiosis functioning and/or establishment (ANR pre-project "MYCOTRANS" preselected 2019; PhD M. Usman).

2. Interaction between Arabidopsis and plant growth promoting Rhizobiaceae

G. Desbrosses & B. Touraine. L. Gamet; PhD student: S Mashkoor

To understand the impact of beneficial bacteria (PGPR) on plants we created a model of beneficial interaction composed of *Arabidopsis thaliana* inoculated with the model nitrogen fixing rhizobacteria *Mesorhizobium loti*. *In vitro*, several phenotypes are successively visible in inoculated Arabidopsis plantlets: (*i*) significant root hair elongation within a few hours after inoculation, (*ii*) significant promotion of lateral root length within 3 to 5 days, (*iii*) significant increase in shoot biomass within a couple of weeks. The latter, the most interesting one in terms of potential of green technologies, is likely to be a consequence of the activation of a signaling pathway (how a beneficial bacterium is detected?) resulting in the adjustment of root development (how plant development/growth is affected), and eventually the plant nutrition (how a beneficial bacteria would affect photosynthesis and nutrient uptake?). Over the 2015-now period, we have mainly addressed the development and nutrition questions.

How can microbes affect root development during their interaction with the plant? To understand how a beneficial microbe affects root development, we carefully characterized the Arabidopsis root system responses to inoculation. Using the Arabidopsis/Mesorhizobium biological model, we established that *M. loti* can repress or not primary root growth depending on the mode of preparation of the inoculum and that the plant auxin pathway plays a central role in the modification of root development in presence of a PGPR (Poitout *et al.*, 2017). We also demonstrated that the effects on root development are local (Poitout *et al.*, 2017). One possibility is that *M. loti* secretes some compound/molecule affecting the root development. We got confirmation of that when we observed that an *M. loti* colony grown on a filter paper laid on the media but at distance from the root tip, had similar effect on root development as a classical inoculation. Using a proteomic approach, we try to identify some of the diffusible compounds involved (Proteosphere project; funded by INRA BAP). The first results are suggesting that some of these compounds are typical bacterial peptidic effector (Ef-tu; Mashkhoor, unpublished).

Primary root growth inhibition like that triggered by *M. loti* inoculation in Arabidopsis is a phenotype also observed during abiotic stresses such as Pi deficiency. It has been tempting to assume that the response to Pi deficiency and to *M. loti* shares some common nodes in Arabidopsis. We tested this hypothesis by growing Arabidopsis plants in 4 different conditions (± Pi x ± *M. loti*) and compared the different observed root phenotypes Our results show that the effects of *M. loti* on root development are independent from those of Pi deficiency (Mashkoor et al. In prep).

How can microbe affect plant mineral nutrition? PGPR affect the development of the root system and especially the elongation of root hairs while, at the same time, it promotes overall growth. It is thus reasonable to speculate that the latter effect is a direct consequence of the former. In such a model, the increased root surface in response to a beneficial bacterium would directly improve mineral nutrient uptake. To test that hypothesis, we measured plant growth in presence or in absence of PGPR, for different plant genotypes affected in either root hair elongation or lateral root development and grown at different concentration of Pi. We observed that, *in vitro*, Arabidopsis growth was independent of the presence of root hairs (length and density), but dependent on the number and total length of lateral roots (Mashkoor et al. In prep). This would suggest that plant growth promotion by *M. loti* might involved the positive effect that the bacteria had on the total length of the lateral root system.

Cross-talk between plant response to PGPR and plant NO_3^- nutrition. We previously showed that *NRT2.5* and *NRT2.6* gene products are required for Arabidopsis growth promotion by the *Phyllobacterium brassicacearum* STM196 PGPR strain (Mantelin *et al.*, 2006). Yet, their precise roles remain unclear. We investigated a possible link with N nutrition using nitrate reductase Arabidopsis mutants. Altogether, our results show that the root architecture and plant growth promotion responses were not affected by the resulting alterations of NO_3^- transport, accumulation and metabolism (Kechid et al., 2013). Interestingly, we identified the *NR1* gene coding for NO-producing NITRATE REDUCTASE 1 as a possible target in the plant response to PGPR. A *nia1*, *nr1* knock-out, mutant recently provided by Dr. Nigel Crawford will be a powerful tool to address the role of NO signalling in plant responses to PGPR.

3. Early electrical and calcium signals triggered by Nod Factor perception in legume root hairs

H. Sentenac. M. Boeglin; PhD students; Alice Drain & Julien Thouin. Collaboration with Ticer (AA Véry; PhD student; L Wang & MY Guo)

Initiation of symbiotic interactions with N_2-fixing rhizobia is often triggered at the root hair cell membrane in response to nodulation factors (NF) secreted by rhizobia, and involves complex signaling events. Amongst these events, the earliest ones, together with ROS production, are changes in H⁺, Ca²⁺, K⁺ and anion (Cl⁻) fluxes, which induce a transient depolarization of the cell membrane. *ELSA* (in collaboration with TICER) is interested in deciphering the molecular mechanisms underlying these signaling events in the model legume *Medicago truncatula* (ANR "CAROLS" coordinated by *ELSA*, involving scientists from Sophia-Antipolis and Gif/Yvette). In a first step, a *M. truncatula* root hair transcriptome has been obtained (RNA-Seq) and analyzed, *ELSA* and *TICER* especially aiming at getting a "molecular" repertoire of the candidate ion channels likely to play a role in the early electrical and calcium signaling events triggered by NF perception (Damiani *et al.*, 2016). Then, *ELSA* and *TICER* have been working at obtaining the "functional" counterpart of this molecular repertoire by identifying the major ion channel activities (ionic conductances) active of the root hair cell membrane (Wang *et al.*, 2019). This required developing an experimental setup allowing laser-assisted production of root hair spheroplasts for patch-clamp recordings (Wang *et al.*, 2019). The results of this work and further analyses of the effects of NF treatments on ion channel activities at the root hair cell membrane have led us (1) to characterize a K⁺ channel shown to be involved in root hair membrane repolarization following the initial NF induced depolarization and to analyze the physiological roles of this channel using mutant plants (PhD students: A Drain and J Thouin; manuscript in preparation), and (2) to identify a NF-induced Ca²⁺ conductance and to select candidate genes from the CNGC and GLR families in order to determine the molecular structure corresponding to this conductance (L Wang, J Thouin, M-Y Guo; recent and present work), in collaboration with Toulouse and Sophia-Antipolis scientists: J Cullimore, C Gough and N Pauly).

4. Effects of rhizobia on root architecture development and root hair elongation promotion in durum wheat

H. Sentenac (in collaboration with J-B Peltier from TICER; co-supervision of the PhD Thesis of T Rongsawat)

The work carried out by G Desbrosses (*Cf.* above) shows that a *bona fide* rhizobial species can engage PGPR-like interactions with the non-host plant Arabidopsis, including changes in root architecture development and promotion of root hair elongation, besides plant growth promotion. Evidence of such PGPR-behavior of rhizobia on various non-host plant species is also available in the literature (Mehboob *et al.*, 2009; Gopalakrishnan *et al.*, 2015). Interested in such positive interactions in cereals, we (*ELSA* and *TICER*) have shown that durum wheat was able to interact with different rhizobial strains, at least in terms of promotion of root hair elongation. Furthermore, in plants grown for few days on agar plates in "absence" of Pi, the magnitude of the stimulating effects on root hair elongation was found to be strongly dependent on the rhizobial strain (4 strains having been tested). We have considered that these observations were sufficiently promising to engage strong methodological efforts in order to become able to characterize the effects of rhizobial strains with "quantitative" parameters in older plants grown in various conditions (hydroponics/aeroponics conditions in culture devices obtained by 3-D printing, and "rhizotron-like" devices). Images of root systems and root hair zones obtained with the present device prototypes are available and can be zoomed at https://www1.montpellier.inra.fr/wp-inra/bpmp/recherche/les-equipes/elsa/. A pilot version of a software allowing to quantify the development of the root hair zones has been developed (in collaboration with an informatician: Volker Bäcker, RIO-Imaging, CRBM Montpellier). Most of this work is carried out within the framework of the PhD Thesis of T Rongsawat.

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Annex 4 – Research products and activities

EVALUATION CAMPAIGN 2019-2020

GROUP A

Name of the team: **Electrophysiology of plant mineral nutrition and root symbioses**
Acronym: **ELSA**

Theme leader for the current contract: **Hervé Sentenac**

I - PRODUCTION OF KNOWLEDGE AND ACTIVITIES CONTRIBUTING TO THE INFLUENCE AND SCIENTIFIC ATTRACTIVENESS OF THE TEAM

1- Articles

Scientific articles

Twenty % most significant articles:

1. **Garcia K., Delteil A., Conéjéro G., Becquer A., Plassard C., Sentenac H. , Zimmermann S. (2014) Potassium nutrition of ectomycorrhizal *Pinus pinaster*: overexpression of the *Hebeloma cylindrosporum HcTRK1* transporter affects the translocation of of both K$^+$ and phosphorus in the host plant. New Phytol. 201(3): 951-960.**

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Other articles (professional journals, etc.) (total number)

2017

1. Bouain N., Satbhai S.B., Saenchai C., Desbrosses G., Berthomieu P., Busch W., (2017) Zinc availability modulates plant growth and immune responses via AZI1. bioRxiv.

2- Books

Monographs, critical editions, translations (total number)

Plant physiology Textbooks in French:

2016

1. Coupé M., Touraine B. (2016) Physiologie Végétale ; Collection LMD SVT ; Ellipses, Paris, 354 p

2017

2. Morot-Gaudry JF, Moreau F, Prat R, Maurel C, Sentenac H (2017) Biologie Végétale – Nutrition et Métabolisme ; Collection « Sciences Sup », Dunod 3$^{\text{ème}}$ édition, Paris, pp 1-242.

Management and coordination of scientific books / Scientific book edition

Book chapters (total number)

Book chapters in English or another foreign language

2016

1. Nieves-Cordones M., Al Shiblawi F. R., Sentenac H. (2016) Roles and Transport of Sodium and Potassium in Plants. Met. Ions Life Sci. 16 291-324.

2017

2. Courty P.E., Doidy J., Garcia K., Wipf D., Zimmermann S.D. (2017) The transportome of mycorrhizal systems. Chapter 14 *In Molecular Mycorrhizal Symbiosis* (F. Martin eds) *Wiley-Blackwell* pp 239-256.

2018

3. Guerrero-Galán C., Houdinet G., Calvo-Polanco M., Bonaldi K.E., Garcia K., Zimmermann S.D. (2018) The role of plant transporters in mycorrhizal symbioses. Chapter 10 in “Membrane Transport in Plants”, Adv. Bot. Res. 87: 303-342.

2019

4. Becquer A., Guerrero-Galán C., Eibensteiner J.L., Houdinet G., Bücking H., Zimmermann S.D., Garcia K. (2019) The ectomycorrhizal contribution to tree nutrition. Chapter 5 in "Molecular Physiology and Biotechnology of Trees"; Adv. Bot. Res. 89: 77-126.

Edited theses

3- Production in conferences / congresses and research seminars

Articles published in conference proceedings / congress

2015

1. Becquer A., Garcia K., Amenc L., Russet S., Baeza Y., Zimmermann S., Plassard C. (2015) The *Hebeloma cylindrosporum* phosphate transporter HcPT2 was involved in phosphate efflux at the fungus-plant interface. 8. International Conference on Mycorrhiza (ICOM8). Flagstaff (USA).

Other products presented in symposia / congress and research seminars

Invited oral presentations in international symposia/congress

2018

1. Sentenac H., Peltier J.-B. (2018) Interactions between rhizobia and root hairs: from *Medicago truncatula* to wheat via *Arabidopsis thaliana.* The 3rd *Molecular Plant* International Symposium. June 12-15, 2018·Xi'an, China

Invited oral presentations in national symposia/congress

Selected oral presentations in international symposia/congress

2014

1. Zimmermann S., Garcia K., Gay G., Doré J., Perraud M. (2014) Genome descriptions by species: *Hebeloma cylindrosporum*. 4th Mycorrhizal Genomics Workshop 2014, Seville (Spain).
2. Becquer A., Amenc L., Garcia K., Ruset S., Baeza Y., Zimmermann S., Plassard C. (2014) The phosphate transporter HcPT2, first candidate for phosphate efflux in ectomycorhizal symbiosis? 5th Phosphorus in Soils and Plants Symposium PSP5 Montpellier, August 2014, Montpellier, France.

2015

3. Becquer A., Garcia K., Amenc L., Ruset S., Baeza Y., Zimmermann S., Plassard C. (2015) The *Hebeloma cylindrosporum* phosphate transporter HcPT2 was involved in phosphate efflux at the fungus-plant interface. ICOM8, 8th International Conference on Mycorrhiza, August 2015, Northern Arizona University, Flagstaff, Arizona, USA.

2016

4. Ben-Amar A, El Bouhmadi K, Sentenac H, Véry A-A, Mahboub S. Study of leaf rolling upon abiotic stress in wheat and rice. (18.12.2016-22.12.2016) Journées Internationales de Biotechnologie (JIB 2016) de l'Association Tunisienne de Biotechnologie. Sousse (Tunisia).
5. Nguyen T-H, Meynard D, Guiderdoni E, Sentenac H, Véry A-A. Control of leaf transpiration in rice under salt stress : functional characterization and roles of potassium channels expressed in rice stomata. (26.09.2016-28.09.2016) 14th International Symposium on Rice Functional Genomics. Montpellier (France).
6. Nieves-Cordones M, Mohamed S, Meynard D, Périn C, Sentenac H, Guiderdoni E, Véry A-A. Functional and molecular analysis of Cs^+ uptake in rice, and its interaction with K^+ uptake. (26.09.2016-28.09.2016) 14th. International Symposium on Rice Functional Genomics. Montpellier (France).

2017

7. Guerrero-Galán C., Delteil A., Garcia K., Houdinet G., Conéjéro G., Sentenac H., Zimmermann S.D. (2017) Novel functions of fungal TOK channels: putative roles in symbiotic potassium transfer. ICOM9, 9th International Conference on Mycorrhiza, Aout 2017, Prague (Czech Republic).

8. Plassard C., Becquer A., Garcia K., Amenc L., Doré J., Gay G., Zimmermann S. (2017) Phosphate unloading in ectomycorrhizae: why is the HcPT2 transporter of *Hebeloma cylindrosporum* a good candidate? ICOM9, 9th International Conference on Mycorrhiza, Aout 2017, Prague (Czech Republic).

9. Zimmermann S.D. (2017) Unravelling nutrient exchange in ectomycorrhizal symbiosis. 3rd international Molecular Mycorrhiza Meeting iMMM, July 2017, Toulouse (France).

2018

10. Guerrero-Galan C., Houdinet G., Delteil A., Garcia K., Zimmermann S.D. (2018) Unravelling nutrient exchange in ectomycorrhizal symbiosis contributing to plant potassium nutrition. (4-6 July 2018) International Conference Saclay Plant Sciences (SPS) 2018. Campus Paris-Saclay (France)

11. Guo M.Y., Wang L., Sentenac H., Véry A.A. (2018) Building a functional repertoire of ion channels active at the plasma membrane of apical regions of medicago truncatula legume root hairs. (5 -10 août) 12th Congress of the International Plant Molecular Biology. Montpellier (France).

12. Paris N., Martinière A., Gibrat R., Sentenac H., Dumont X., Gaillard I. (2018) Non invasive imaging use to shed light on pH at both sides of the plasma membrane interface in living root. (5 -10 août) 12th Congress of the International Plant Molecular Biology. Montpellier (France).

Selected oral presentations in national symposia/congress

2014

1. Wang L. Characterisation of legume root hair ion channels signalling the presence of symbiotic bacteria. Patch-Club Meeting, Colloque annuel du Patch Club de Montpellier. (06.06.2014) Castelnau le Lez (France).

2015

2. Guerrero Galán C., Zimmermann S.D. (2015) Identification and characterization of three two-pore K^+ channels from the ectomycorrhizal fungus *Hebeloma cylindrosporum*. Patch-Club Meeting, Colloque annuel du Patch Club de Montpellier. June 2015, Montpellier (France).

2017

3. Mohamed S., Nieves-Cordones M., Sentenac H., Véry A.-A., Guiderdoni E. (2017) Contrôle de l'accumulation de cations toxiques chez le riz par modification des transporteurs, de l'architecture et de l'anatomie racinaire : application à une culture sur des sols contaminés par le césium 137. "Rencontres Céréales" (03.03.2017) Montpellier (France).

4. Peltier J.-B., Sentenac H. (2017) Réponses des poils absorbants à l'environnement abiotique et biotique, et développement d'un programme de phénotypage automatique. 5th Meeting on Nitrogen-Fixing Root Endosymbioses. (12.10.2017-13.10.2017) Toulouse (France).

5. Peltier J.-B., Sentenac H. (2017) Phénotipage des réponses racinaires du blé dur à l'inoculation par des bactéries diazotrophes. Workshop "*Interactions céréales micro-organismes bénéfiques du sol*" (Organis : Benoît Lefebvre, LIPM). (16.11.2017) Paris (France)..

6. Rongsawat T., Peltier J.-B., Sentenac H. (2017) Phénotypage du développement de la zone pilifère en réponse à des bactéries rhizosphériques chez le blé : dispositif expérimental et analyse d'image automatisée. Journées du Réseau PhytoMic 2017 "*Diversité et rôle du microbiote dans la performance et l'adaptation de la plante",* Round Table "*Génétique et amélioration des plantes au sein du réseau PhytoMic*". (9.11.2017-10.11.2017) Rennes (France).

7. Rosales M.A. (2017) Abscisic Acid Participates in Root Developmental and Hydraulic Responses to Water Deficit in Arabidopsis. Lateral root Workshop, Montpellier (France).

8. Zimmermann S.D. (2017) Unravelling nutrient exchange in ectomycorrhizal symbiosis. 3rd international Molecular Mycorrhiza Meeting iMMM, Juillet 2017, Toulouse, France.

Posters in international symposia/congress

2014

1. Drain A, Wang L., Véry A.-A., Fizames C., Pauly N., Gaillard I., Sentenac H. (2014) Early steps in nodulation signaling: role of ion channels in *Medicago* root hair (July 6-10) XVI International congress on Molecular Plant-Microbe Interactions., Rhodes Island (Greece).

2. Leung J., Leonhardt N., Tran D., Véry A.A., Vavasseur A., Renou J.P., Kwak J., Sentenac H., Bouteau F., Jammes F. (2014). Metabolite switch with anti abscisic acid actvity (28 july-1 august 2014). 25th International Conference on Arabidopsis Research (ICAR), Vancouver (Canada).

3. Martinière A., Gibrat G., Sentenac H., Paris N. (2014) Media acidification and proton gradient in *Arabidopsis thaliana* roots. (8-11 September 2014) XVII meeting of European Network for Plant Endomembrane Research. Lecce (Italie).

4. Tounsi S, Véry A-A, Sentenac H, Masmoudi K, Brini F. Caractérisation fonctionnelle par électrophysiologie d'un transporteur HKT1;4-A1 de *Triticum monococcum*. (20-24 dDecember 2014) Journées Internationales de Biotechnologie 2014 (Association Tunisienne de Biotechnologie). Yasmine Hammamet (Tunisie)

2015

5. Ben Amar S, Masmoudi K, Véry A-A, Sentenac H, Brini F. Isolement et étude de l'activité des promoteurs des gènes *TdHKT1;4-1* et *TdHKT1;4-2* chez des plantes transgéniques de blé. (20.12.2015-24.12.2015) Journées Internationales de Biotechnologie 2015 (Association Tunisienne de Biotechnologie). Djerba (Tunisie).

6. Guerrero-Galán C., Delteil A., Garcia K., Sentenac H., Zimmermann S.D. (2015) Fungal molecular players involved in plant potassium nutrition via ectomycorrhizal associations. (2-4 September 2015) 2nd International Molecular Mycorrhiza Meeting IMMM 2015. Cambridge (Royaume-Uni).

7. Hmidi D., Messedi D, Sentenac H, Véry A-A, Abdelly C. Clonage et caractérisation électrophysiologique d'un transporteur HKT chez l'orge sauvage : *Hordeum maritimum.* (20-24 December 2015) Journées Internationales de Biotechnologie de l'Association Tunisienne de Biotechnologie. Djerba (Tunisie).

8. Nguyen T-H, Meynard D, Guiderdoni E, Sentenac H, Véry A-A. Identification and functional characterization of inward Shaker channels expressed in rice stomata. (13-16 September 2015) 26ème colloque "Canaux Ioniques", Sète, France.

9. Tounsi S, Véry A-A, Sentenac H, Masmoudi K, Brini F. Etude fonctionnelle de deux transporteurs HKT1;4 de *Triticum monococcum*. (20.12.205-24.12.2015) Journées Internationales de Biotechnologie 2015 (Association Tunisienne de Biotechnologie), Djerba, Tunisie.

10. Wang L, Sentenac H, Véry A-A. Electrophysiological characterization of *Medicago truncatula* root hair conductances potentially involved in the early Nod factor signaling in rhizobial symbiosis. (13.09.2015-16.09.2015) 26ème colloque "Canaux Ioniques". Sète (France).

2016

11. Al Shiblawi FR, Meynard D, Périn C, Guiderdoni E, Sentenac H, Véry A-A. Production of loss-of-function rice mutant lines in Na^+ transporter genes by using the CRISPR-Cas9 biotechnology. (26.09.2016-28.09.2016) 14th International Symposium on Rice Functional Genomics. Montpellier (France).

12. Ben-Amar A, El Bouhmadi K, Sentenac H, Véry A-A, Mahboub S. Enroulement foliaire chez le blé dur en conditions de stress hydrique. (18-19 October 2016) International workshop "L'Afrique face aux changements climatiques" ("Afrika under drought stress"), labellisé "COP22" (COP22 Label). Faculté de Médecine et de Pharmacie, Casablanca (Maroc).

2017

13. Guerrero-Galan C., Houdinet G., Delteil A., Garcia K., Conéjéro G., Sentenac H., Zimmermann S.D. (2017) Unravelling nutrient exchange in ectomycorrhizal symbiosis. (26-27 July 2017) 3rd international Molecular Mycorrhiza Meeting iMMM. Toulouse (France).

14. Guerrero-Galan C., Delteil A., Garcia K., Houdinet G., Conéjéro G., Sentenac H., Zimmermann S.D. (2017) Novel functions of fungal TOK channels: putative roles in symbiotic potassium transfer. (30 July.-04 August 2017) 9th International Conference on Mycorrhiza, ICOM9. Prague (Czech Republic).

2018

15. Houdinet G., Guerrero-Galán C., Garcia K., Delteil A., Corratgé-Faillie C., Conéjéro G., Gaillard I., Touraine B., Sentenac H., Zimmermann S.D. (2018) Unravelling nutrient exchange in ectomycorrhizal symbiosis contributing to plant potassium nutrition. XIIth Congress of the International Plant Molecular Biology, 5-10 August 2018, Montpellier (France).

2019

16. Houdinet G., Guerrero-Galán C., Garcia K., Delteil A., Conéjéro G., Gaillard I., Sentenac H., Touraine B., Zimmermann S.D. (2019) Role(s) of fungal proteins involved in plant potassium nutrition during ectomycorrhizal symbiosis. 4th international Molecular Mycorrhiza Meeting iMMM, 6 - 8 February 2019, Torino (Italy).

Posters in national symposia/congress

2017

1. Mohamed S, Nieves-Cordones M, Meunier A-C, Vernet A, Périn C, Guiderdoni E, Sentenac H, Véry A-A. Production of low-Cs^+ rice plants by inactivation of the K^+ transporter OsHAK1 with the CRISPR-Cas9 system. Colloque Inter-LabEx CRISPR-Cas9. (17.05.2017) Montpellier (France).

2. Al Shiblawi FR, Meynard D, Périn C, Guiderdoni E, Sentenac H, Véry A-A. Production of loss-of-function rice mutant lines in Na^+ transporter genes by using the CRISPR-Cas9 biotechnology. Colloque Inter-LabEx CRISPR-Cas9. (17.05.2017) Montpellier (France).

2018

3. Mashkoor S., Rodrigues O., Gamet L., Touraine B., Desbrosses G. Comment une bactérie bénéfique inhibe *in vitro* la croissance de la racine d'*Arabidopsis thaliana* ? 13ième Rencontres Plantes – Microorganismes, 21 janvier – 2 février 2018, Aussois (France)

Invited seminars in foreign institutions

2015

1. Desbrosses G. What can we learn from a non host interaction between Arabidopsis thaliana and a regular nitrogen fixing rhizobium. Department of Molecular Biology and Genetics, University of Aarhus (DK); August the 27th 2015

2017

2. Desbrosses G. From root developement to mineral nutrition, the impact of a beneficial microbe on Arabidopsis. Department of Molecular Biology and Genetics, University of Aarhus (DK); May the 18th 2017

Invited seminars in French institutions

2016

1. Sentenac H. (2016) Early electrical and calcium signaling in rhizobial symbiosis in *Medicago truncatula*: electrophysiological and molecular approaches. February 29, 2016. UMR Agroécologie, INRA, Dijon.

2018

2. Sentenac H. (2018) Interactions entre rhizobia et poils absorbants chez *Medicago truncatula* et le blé. May 22, 2018. UMR Ecologie Microbienne, Univ. Lyon 1

4- Electronic tools and products

Softwares

Databases

5- Instruments and methodology

Prototypes

Platforms and observatories

6- Other products

Theorised artistic creations, staging, movies

7- Editorial activities

Participation in editorial committees (books, collections, etc.)

Sentenac H. Member of the Editorial Board of the Journal "Molecular Plant".

Zimmermann S.D. Member of the Reviewer Panel “Plant-Microbe Interactions” of “Frontiers”; Member of the Editorial Board of the Journal “Plants” and of the Journal “Plant Signaling & Behaviour”.

Collection and series management

Zimmermann SD. Topic Editor of a Research Topic in “Frontiers”: “*Importance of Root Symbiomes for Plant Nutrition: New Insights, Perspectives and Future Challenges*

8- Reviewing activities

Reviewing of articles

Zimmermann S.D. Reviewing of research articles in “Frontiers”, “New Physiologist”, “Plant Physiology”, “Plant Journal”, “Environmental Microbiology”, etc.

Desbrosses G. Reviewing of research articles in “Plant Science”, “Molecular Plant-Microbe Interactions”, “The journal of experimental Botany” …

Touraine B. Reviewing of research articles in “Journal of Experimental Botany”, “New Phytologist”, “Journal of Plant Physiology”, “PLOS-One”, “Planta”, “Plant, Cell & Environment”, etc.

Grant evaluation (public or charities)

Zimmermann S.D. Reviewing of research projects for funding organzations in France, Switzerland, Austria, etc.

Reviewing of research institutes

Desbrosses G. member of the Hcéres committee for the evaluation of BIAM (UMR7265, Aix-Marseille) as a representative of CNU 66 ; January 2017

Touraine B. member of the Hcéres committees for the evaluation of LRSV (Toulouse, 2014), RDP (Lyon, 2015), EVA (Caen, 2015), BIOPI (Amiens, 2016), IRHS (Angers, 2016), BBV (Tours, 2017).

Participation in institutional committees and juries (CNRS, Inserm, etc.)

Desbrosses G. involved in the recruitment of an Assistant Professor: University of Avignon (France) May 2014

Touraine B. Vice-President (2011-2015) and President (2015-2019) of the CNU-66 (National Council of Universities, Section Physiology); as such, has vice-chaired and then chaired three 3-day meetings of the Section each year.

9- Academic research grants

European (ERC, H2020, etc.) and international (NSF, JSPS, NIH, World Bank, FAO, etc.) grants - coordination

European (ERC, H2020, etc.) and international (NSF, JSPS, NIH, World Bank, FAO, etc.) grants - partnership

Other European grants - coordination

Other European grants - partnership

National public grants (ANR, PHRC, FUI, INCA, etc.) - coordination

2012 – 2016 ; **CAROLS (ANR PRC)** ; Canaux ioniques et espèces actives de l'oxygène dans le poil absorbant de légumineuse : rôle dans la symbiose avec Rhizobium ; SENTENAC Hervé

2017 – 2018 ; **ProteoSphère (Department BAP INRA)** ; Analyse protéomique de la rhizosphère au cours d’une symbiose associative entre plantes et microorganismes bénéfiques ; DESBROSSES Guilhem

2019 – 2020 ; **PHENOPILI (Department BAP INRA)** ; Mise au point d'une méthodologie de phénotypage automatisé de la croissance du poil absorbant et du développement de la zone pilifère, et application à l'analyse des réponses racinaires de cultivars de blé à des rhizobia ; SENTENAC Hervé

National public grants (ANR, PHRC, FUI, INCA, etc.) - partnership

2011 – 2014 ; **TRANSMUT (ANR PRC)** ; Rôle du transportome aux interfaces biotrophes des interactions mutualistes plantes-champignons ; ZIMMERMANN Sabine

2018 – 2020 ; **CROSYMED (ANR ERA-Net ARIMNet)** ; Enhancing nutrient use efficiency through legumes in agro-ecosystems of the Mediterranean basin ; CASTAINGS Loren

Local grants (collectivités territoriales) - coordination

Local grants (collectivités territoriales) - partnership

PIA (labex, equipex etc.) grants - coordination

2011 – 2015 ; **RHIZOPOLIS (Labex AGRO)** ; A federative project for plant root research ; SENTENAC Hervé

PIA (labex, equipex etc.) grants - partnership

2013 – 2019 ; **DEMETERRES (ANR RNSR)** ; Développement de méthodes bio-et Ecotechnologies pour la remédiation raisonnée des effluents et des sols en appui à une stratégie de réhabilitation post-accidentelle ; SENTENAC Hervé, VERY Anne-Aliénor

2016 – 2019 ; **APLIM (Labex AGRO)** ; Development of magnetic resonance technologies (NMR,MRI and nanoprobes) to support integrative biology of plant response to abiotic and biotic constraints - Advanced Plant Life Imaging and Metrology ; (Coord. JL Verdeil, CIRAD, and C. Goze-Bac, CNRS), MAUREL Christophe, SENTENAC Hervé, GAILLARD Isabelle

Grants from foundations and charities (ARC, FMR, FRM, etc.) - coordination

Grants from foundations and charities (ARC, FMR, FRM, etc.) - partnership

10- Visiting senior scientists and post-doc

Post-docs (total number)

Since 2014: 0

Foreign post-docs

Since 2014: 0

Visiting scientists (total number)

Since 2014 : 7

Foreign visiting scientists

Since 2014 : 7

Family Name	First name	Financing source	Nationality	Date of arrival	Date of departure)	Status	University of origin
Amrani	Nassima	Salaire	ALGERIAN	01/05/2015	30/06/2015	Maitre-assistant invité	Université de Batna Algerie
Ellijimi	Chedly	Bourse	Tunisien	01/03/2016	30/04/2016	Doctorant invité	Institut pasteur de Tunis
Ellijimi	Chedly	Bourse	Tunisien	06/11/2017	22/12/2017	Doctorant invité	Institut pasteur de Tunis
Lahrizi	Yahya	Bourse campus France	Marocain	01/09/2018	31/12/2018	Doctorant invité	Université Cadi AYYAD
Mashkoor	Sulaiman	Salaire	Iraquien	07/01/2019	08/07/2021	Maitre-assistant invité	Université de Kufa Iraq
Salgado Guedes	Marco	Bourse EMBO	Suedois	17/08/2015	04/09/2015	Doctorant invité	Departement d'ecologie environnement et science des plantes Stockholm University
Yakoubi	Fatima	Bourse	ALGERIAN	18/06/2018	03/08/2018	Doctorant invité	Laboratoire de physiologie végétale Université d'Oran 1 Ahmed Ben Bella Algérie

11- Scientific recognition

Prizes and/or distinctions

Hervé Sentenac : Médaille d'Or (2014) de l'Académie d'Agriculture de France

IUF members

Chair of learned and scientific societies

Organisations of meetings and symposia (out of France)

Invitations to meetings and symposia (out of France)

II - INTERACTION OF THE TEAM WITH THE NON-ACADEMIC WORLD, IMPACTS ON ECONOMY, SOCIETY, CULTURE OR HEALTH

1- Socio-economic interactions / Patents

Invention disclosures

Filed patents

Accepted patents

Licenced patents

2- Socio-economic interactions

Industrial and R&D contracts

Cifre fellowships

Creation of labs with private-public partnerships

Networks and mixed units

Start-ups

3- Expertise

Consulting

Participation in expert committees (ANSES etc.)

Legal expertise

Expert and standardization reports

4- Public outreach

Radio broadcasts, TV shows, magazines and newspaper

Touraine B., Biologie-agrosciences, un domaine d’excellence scientifique de niveau international au service des grands défis, Nouvel Observateur, May 2014

Journal articles, interviews, book edition, videos, other popularization outputs, debates on science and society, etc.

Desbrosses G. and Touraine B., regular participation at various events to inform high school student about university studies.

Touraine B., chairman of a round table about university studies and animal and plant physiology in university curricula (Ajaccio, May 2019).

III - INVOLVEMENT OF THE TEAM IN TRAINING THROUGH RESEARCH

1- Educational outputs

Books

2016

Coupé M., Touraine B. (2016) Physiologie Végétale ; Collection LMD SVT ; Ellipses, Paris, 354 p

2017

Morot-Gaudry JF, Moreau F, Prat R, Maurel C, Sentenac H (2017) Biologie Végétale – Nutrition et Métabolisme ; Collection « Sciences Sup », Dunod $3^{ème}$ édition, Paris, pp 1-242.

E-learning, MOOCs, multimedia lessons, etc.

2- Scientific productions (articles, books, etc.) from theses

Scientific productions (articles, books, etc.) from theses

Scientific articles

Ben Amar S., Brini F., Sentenac H., Masmoudi K. ,Véry A.A. (2014) Functional characterisation in Xenopus oocytes of Na+ transport systems from durum wheat reveals diversity among two HKT1;4 transporters. J. Exp. Bot. 65: 213-222

Garcia K., Delteil A., Conéjéro G., Becquer A., Plassard C., Sentenac H. , Zimmermann S. (2014) Potassium nutrition of ectomycorrhizal *Pinus pinaster*: overexpression of the *Hebeloma cylindrosporum HcTRK1* transporter affects the translocation of of both K^+ and phosphorus in the host plant. New Phytol. 201(3): 951-960.

Taochy C., Gaillard I., Ipotesi E., Oomen R., Leonhardt N., Zimmermann S., Peltier J.B., Szponarski W., Simonneau T., Sentenac H., Gibrat R., Boyer J.C. (2015) The Arabidopsis root stele transporter NPF2.3 contributes to nitrate translocation to shoots under salt stress. Plant J. 83(3): 466-479

Damiani I., Drain A. (co-premier auteure), Guichard M., Balzergue S., Boscari A., Boyer J.C., Brunaud V., Cottaz S., Rancurel C., Da Rocha M., Fizames C., Fort S., Gaillard I., Maillol V., Danchin E. G. J., Rouached H., Samain E., Su Y.H., Thouin J., Touraine B., Puppo A., Frachisse J.M., Pauly N., Sentenac H. (2016) Nod factor effects on root hair-specific transcriptome of *Medicago truncatula*: Focus on plasma membrane transport systems and reactive oxygen species networks. Front Plant Sci. 7: 794.

Charpentier M., Sun J., Vaz Martins T., Radhakrishnan G.V., Findlay K., Soumpourou E., Thouin J., Véry A.A., Sander D., Morri R.J., Oldroyd G.E.D. (2016) Symbiotic calcium oscillations require nuclear-localised cyclic nucleotide gated channels. Science 352(6289): 1102-1105

Damiani I., Drain A., Guichard M., Balzergue S., Boscari A., Boyer J.C., Brunaud V., Cottaz S., Rancurel C., Da Rocha M., Fizames C., Fort S., Gaillard I., Maillol V., Danchin E. G. J., Rouached H., Samain E., Su Y.H., Thouin J., Touraine B., Puppo A., Frachisse J.M., Pauly N., Sentenac H. (2016) Nod Factor Effects on Root Hair-Specific Transcriptome of Medicago truncatula: Focus on Plasma Membrane Transport Systems and Reactive Oxygen Species Networks. Front Plant Sci. 7 : 794

Lefoulon C., Boeglin M., Moreau B., Véry A.A., Szponarski W., Dauzat M., Michard E., Gaillard I., Chérel I. (2016) The Arabidopsis AtPP2CA protein phosphatase inhibits the GORK K+ efflux channel and exerts a dominant suppressive effect on phosphomimetic activating mutations. *J. Biol. Chem.* **291(12) : 6521-6533**

Nguyen T.H., Huang S., Meynard D., Chaine C., Michel R., Roelfsema R., Guiderdoni E., Sentenac H., Véry A.A. (2017) A dual role for the OsK5.2 ion channel in stomatal movements and K+ loading into xylem sap. Plant Physiol. 174(4): 2409-2418. (doct, fr, eu)

Nieves-Cordones M., Mohamed S., Tanoi K, Kobayashi N., Takagi K., Vernet A., Guiderdoni E., Perin C., Sentenac H., Véry A. A. (2017) Production of low-Cs+ rice plants by inactivation of the K+ transporter OsHAK1 with the CRISPR-Cas system. Plant J. 92(1): 43-56.

Tounsi S., Ben Amar S., Masmoudi K., Sentenac H., Brini F., Véry A. A. (2016) Characterisation of two HKT1;4 Transporters from Triticum monococcum to elucidate the Determinants of the Wheat Salt Tolerance Nax1 QTL. Plant Cell Physiol. 57(10): 2047-2057.

Poitout A., Martinière A., Kucharczyk B., Queruel N., Silva-Andia J., Mashkoor S., Gamet L., Varoquaux F., Paris N., Sentenac H., Touraine B., Desbrosses G. (2017) Local signalling pathways regulate the Arabidopsis root developmental response to Mesorhizobium loti inoculation. J. Exp. Bot. 68 (5): 1199-1211

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Review articles

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Garcia K., Zimmermann S.D. (2014) The role of mycorrhizal associations in plant potassium nutrition. Front Plant Sci. (5): 337.

Véry A.A., Nieves-Cordones M., Daly M., Khan I., Fizames C., Sentenac H. (2014) Molecular biology of K$^+$ transport across the plant cell membrane: What do we learn from comparison between plant species? J Plant Physiol. 171(9):748-769.

Book chapters in English or another foreign language

Guerrero-Galán C., Houdinet G., Calvo-Polanco M., Bonaldi K.E., Garcia K., Zimmermann S.D. (2018) The role of plant transporters in mycorrhizal symbioses. Chapter 10 in “Membrane Transport in Plants”, Adv. Bot. Res. 87: 303-342.

Becquer A., <u>Guerrero-Galán C.</u>, Eibensteiner J.L., <u>Houdinet G.</u>, Bücking H., Zimmermann S.D., Garcia K. (2019) The ectomycorrhizal contribution to tree nutrition. Chapter 5 in "Molecular Physiology and Biotechnology of Trees"; Adv. Bot. Res. 89: 77-126.

Mean number of publications per student (Biology & Science and technology only)

3- Training

Habilitated (HDR) scientists

Since 2014: 4

Family Name	First name	Position	Employer	Defense Date
DESBROSSES	Guilhem	enseignant-chercheur	UNIVERSITE MONTPELLIER	2006
SENTENAC	Hervé	chercheur	INRA MONTPELLIER	1988
TOURAINE	Bruno	enseignant-chercheur	UNIVERSITE MONTPELLIER	1992
ZIMMERMANN	Sabine	Chercheur	CNRS	2010

HDR obtained during the period

Since 2014 : 0 HDR

PhD students (total number)

8 PhD students

PhD students benefiting from a specific doctoral contract

Last Name	First Name	Starting date	Defense date	Duration (months)	Funding
DRAIN	Alice	06/02/2012	12/06/2015	34	ETR
GUERRERO	Maria Carmen	01/10/2014	24/11/2017	36	CDE
GUO	Man-Yuan	01/11/2016		36	ETR
HOUDINET	Gabriella	01/10/2016		36	CDE
MASHKOOR	Soulaiman	01/10/2014	18/10/2018	48	ETR
RONGSAWAT	Thanyakorn	01/09/2015		36	ETR
THOUIN	Julien	01/10/2015	05/12/2018	38	CDE
USMAN	Muhammad	01/10/2018		36	ETR

PhD students benefiting from a specific doctoral contract

Defended PhDs

4 defended PhDs

Last name	First Name	Defense Date
DRAIN	Alice	2015
GUERRERO	Maria Carmen	2017
MASHKOOR	Soulaiman	2018
THOUIN	Julien	2018

Mean PhD duration

Mean duration: 39 months

Internships (M1, M2)

Master 1: 4

Master 2: 7

Last name	First Name	Status	Period	
Flandin	Leo	Master 1	01/03/2014	30/06/2014
Alali	Mellis	Master 1	01/03/2015	31/07/2015
Carrié	Emma	Master 1	06/03/2017	30/06/2017
Lee Chai Shit	Terence	Master 1	02/05/2017	09/06/2017
Guerrero	Maria	Master 2	15/01/2014	15/07/2014
Thouin	Julien	Master 2	05/01/2015	05/07/2015
Flandin	Leo	Master 2	01/03/2015	28/08/2015
Houdinet	Gabriella	Master 2	15/01/2016	15/07/2016
Prou	Jérémie	Master 2	04/01/2016	01/07/2016
Zhour	Houssein	Master 2	01/03/2018	31/08/2018
ZRIBI	Ikram	Master 2	01/09/2018	28/02/2019

People in charge for a mention or a master's degree course (total number)

Desbrosses G: in charge of a 6 different MSc courses (M1 and M2);

Touraine B: in charge of the Master mention “Biology, Agrosciences” (includes 5 “parcours” (cursus?)/degree cursus, ca. 80 students graduated each year), in charge of 3 MSc courses (teaching units)

People in charge for a mention or a master's degree course with international certification (Erasmus mundus)

HoNuDe team
Self-assessment document

Name of the team concerned by the current contract: Hormones, Nutrients & Development
Name of the team concerned by the next contract (if different):
Acronym of the current contract: HoNuDe
Acronym of the next contract (if different):
Team leader for the current contract: Benoît Lacombe
Team leader for the next contract: Benoît Lacombe

RESULTS

1- Presentation of the team

Introduction

History

HONUDE (Hormones, Nutrients & Development) was created in July 2015. The team emerged from the Integration group (Alain Gojon) and consisted of: Benoit Lacombe (CR CNRS, now DR), Gabriel Krouk (CR CNRS), Sandrine Ruffel (CR INRA), Anna Medici (MC SupAgro, recruited in September 2015) and Chantal Brachet (TR INRA). Then Wojciech Szponarski (IR1, now IRHC, INRA, 2015), Hatem Rouached (CR INRA, 2016) and Virginie Thiry (AJT SupAgro, 2019) joined the team.

Noteworthy that some of the results presented in this document has been obtained in the team "Intégration des Fonctions Nutritives dans la Plante Entière" before July 2015 by the actual members of the Honude Team.

Structure of the team

The group leader is Benoit Lacombe. Each researcher has its own project(s) to have the opportunity to express its creativity in a complementary manner. This(ese) project(s) should be related to nitrogen transport and sensing and each researcher is actively trying to seek financial support. The role of the team members is to share their knowledge to the rest of the group to reach common successes.

Team's workforce and means

The number of permanent positions has strongly increased (x2) since the beginning. Whereas, at the same time, the non-permanent position has been drastically reduced.

This is related to the financial incomes that will finish by the end of 2019 (see details in the annexes). We have submitted 6 ANR second round proposals this year. The outcome will strongly influence our project for the next period.

Scientific policy

Our scientific objective is to identify the mechanisms through which plants perceive their mineral environment and modify their development to adapt to nutritional constraints by using complementary approaches: targeted approaches on different nitrogen, auxin, ABA and cytokinins transporters, and approaches without preconceptions using systems biology and GWAS.

2- Research products and activities for the team

Scientific track record

The mineral nutrition of most terrestrial organisms (bacteria, fungi, plants) relies on the uptake of inorganic ions from the soil. However, the availability of these ions dramatically fluctuates in both time and space. To face this constraint, all organisms are able to develop a wide range of adaptive responses triggered by sensing systems that perceive the external nutrient availability. The identification of these sensing systems has a strategic importance at both fundamental and applied levels to unravel and manipulate the molecular mechanisms involved in tolerance to nutrient stress. Flexible modulation of the postembryonic organogenesis in response to nutrient availability is one of the most prominent adaptation strategies, and a major determinant of the plant body architecture. This plant ability to respond to environmental conditions is mediated through signalling molecules called plant hormones. Thus, the current insights into hormone and nutrition interplay indicate existence of complex network in which hormones act as endogenous mediators in response to nutrient fluctuation and at the same time fine tune plant capacity for nutrient acquisition. The current data support the concept of nutrient – hormonal regulatory network control plant behavior in response to fluctuating nitrogen environments and feedback on the nutrition acquisition capacity. Together with the influence of nitrogen on hormonal status of the plant this builds the conceptual cycle where nutrients control growth, which in turn control nutrient use and perception. However, molecular mechanisms underlying this nutrient – hormones regulated plant development are still scarcely understood. We develop complementary approaches to understand the molecular basis of development regulated by the hormone-nutrient interactions (Nitrogen) and nutrient-nutrient interactions. We focus on interactions during root development with different approaches taking advantage of our skills in biophysics, molecular and cellular biology, physiology and systems biology. The project uses an approach at the interface between biology and modeling to reveal the molecular mechanisms involved in the interaction between nutritional and hormonal signals in plants. In the following paragraphs, we present the main data obtained during the evaluated period.

- **Long Distance Signaling (Sandrine Ruffel, Benoit Lacombe)**

We performed split-root experiments and used a combination of CK-related mutant analyses, hormone profiling, transcriptomic analysis, NO_3^- uptake assays, and root growth measurements to gain insight into systemic N signaling in Arabidopsis thaliana. By comparing wild-type plants and mutants affected in CK biosynthesis and ABCG14-dependent root-to-shoot translocation of CK, we revealed an important role for active trans-zeatin (tZ) in systemic N signaling. Both rapid sentinel gene regulation and long term functional acclimation to heterogeneous NO_3^- supply, including NO_3^- transport and root growth regulation, are likely mediated by the integration of tZ content in shoots. Furthermore, shoot transcriptome profiling revealed that glutamate/ glutamine metabolism is likely a target of tZ root-to-shoot translocation, prompting an interesting hypothesis regarding shoot-to-root communication. Finally, this study highlights tZ-independent pathways regulating gene expression in shoots as well as NO_3^- uptake activity in response to total N deprivation (Poitout et al., 2018).

Related publications: Ruffel et al., 2015; Lacombe and Achard, 2016; Poitout et al., 2018; Ruffel, 2018

- **Nitrate-Phosphate Interaction (Gabriel Krouk, Anna Medici, Wojciech Szponarski, Hatem Rouached)**

In our research on HRS1-mediated N and P interactions (Medici et al., 2015), we treated Arabidopsis plants with a combination of different N and P to observe any change in their root development and gene expression. We observed the response of PSR marker genes systematically to verify that our treatments were felt by the plant. In these experiments, we found in wild-type plants (WT) that the responses of the PSR marker genes (IPS1, SPX1, miR399D, PHT1-1) were dependent on N provision. Indeed, the transcripts of these genes accumulate under P-depleted conditions only in the presence of at least 0.05 mM NO_3^-, and these transcripts are almost undetectable at 0 mM nitrate. Based on this original observation, we decided to study further the molecular mechanisms that could be at the heart of this important cross-dialog. We have shown that this control of the PSR response by N is under the influence of several genes already known in the PNR (NRT1.1) and PSR (PHR1 and PHO2) pathways. Indeed, mutations of NRT1.1 and PHR1 affect the response of plants to P deficiency in the presence of N. To understand the underlying mechanism, we studied the accumulation of the protein PHR1 protein and could show that the latter is strongly influenced by N. This could explain the mechanism: in the absence of N, PHR1 the "P-co-receptor" is degraded so as to repress the PSR. Moreover, we have discovered that *PHO2*, being known downstream of the PSR pathway, was itself transcriptionally activated by N deficiency and that its mutation resulted in a de-repression of the marker genes of PSR in the absence of N. This demonstrates genetically that repression of PSR by N is an active phenomenon and that PHO2 is induced to repress the pathway in a N-dependent manner. This places PHO2 both downstream and upstream of the PSR. Finally, by establishing a collaboration with Milos Tanurdzic (University of Queensland, Australia), we were able to show that this phenomenon is conserved in two cultivated species: rice and wheat (Medici et al., 2019).

Related publications: Medici et al., 2019

- **NPF proteins at the crossroad of nutrient and hormone signalings (Benoit Lacombe, Chantal Brachet)**

Whilst the data about functional properties of NPF (Corratgé-Faillie and Lacombe, 2017), should give us a clearer view of the NPF substrates, what becomes evident is the multi-substrate nature of most of the NPF members. Competitive interactions between different substrates, such as nutrients and hormones, should now be studied in detail to strengthen the hypothesis that NPF members are the basis of the integration of environmental and physiological information linked to the relative availability of the different nutrients.

The functional characterization of NPF6.3/NRT1.1 has revealed interesting transport properties: the transport of nitrate and auxin, a dual-affinity transport behavior for nitrate depending on external nitrate concentration and the eletrogenicity of the nitrate transport. However, some of these properties remained controversial and were challenged here. We used a combination of uptake experiments using 15N-labelled nitrate and two-electrode voltage-clamp on xenopus oocytes expressing WT and mutant proteins. In our experimental conditions in xenopus oocytes, we could not show NPF6.3/NRT1.1 electrogenicity and only low-affinity transport properties were recorded. Moreover, further functional characterization of a NPF6.3/NRT1.1 point mutant, P492L, allowed us to hypothesize that NPF6.3/NRT1.1 is regulated by internal nitrate concentration and that the internal perception site involves the P492 residue (Noguero et al., 2018).

Unraveling the molecular basis of nitrate sensing and regulation of nitrate uptake should enable the development of strategies to increase the efficiency of nitrogen use and maximize nitrate uptake by plants, which would aid in reducing nitrate pollution. NPF6.3 (also known as NRT1.1), which functions a nitrate sensor and transporter, the kinase CIPK23, and the calcium sensor CBL9 form a complex that is crucial for nitrate sensing in Arabidopsis thaliana. We have identified two additional components of that regulate nitrate transport, sensing, and signaling: the calcium sensor CBL1 and protein phosphatase 2C family member ABI2 (Léran et al., 2015), which is inhibited by the stress-response hormone abscisic acid. Bimolecular fluorescence complementation assays and in vitro kinase assays revealed that ABI2 interacted with and dephosphorylated CIPK23 and CBL1. Coexpression studies in Xenopus oocytes and analysis of plants deficient in ABI2 indicated that ABI2 enhanced NPF6.3-dependent nitrate transport, nitrate sensing, and nitrate signaling. These findings suggest that ABI2 may functionally link stress-regulated control of growth and nitrate uptake and utilization, which are energy-expensive processes.

Related publications : Léran et al., 2015 ; Corratgé-Faillie and Lacombe, 2017 ; Noguero et al., 2018 ;

Key events

During this period, we would like to highlight two key events. Our team has organized two important meetings in 2018:

- **IPMB2018** (Montpellier August 2018, headed by Benoit Lacombe):

The IPMB congress is a triennial meeting gathering plant molecular biologists from all over the world since 1984. The IPMB2015 was held in Brazil (Iguazu) and was preceded by IPMB2012 in Korea (Jeju Island) and IPMB2009 in USA (St Louis). Each of the previous meetings demonstrates the importance to develop multidisciplinary approaches to face climate changes and population increase. IPMB2018 offered an attractive scientific program highlighting major impact recent discoveries from basic molecular biology to crop biotechnology. Combination of plenary and parallel sessions demonstrate the importance of plant molecular biology to fulfill the need for the development of an ecologically intensive farming.

The congress has taken place in a conference center offering all facilities in old downtown Montpellier. Nestled between the countryside, the sea and the mountains, Montpellier is the capital of the biggest wine area in the world. After two keynote talks by Detlef Weigel (MPI Tübingen, Germany) and Pamela Ronald (University of California), the scientific program (https://www.ipmb2018.org/program/) has been built with a mix of 15 plenary and 50 concurrent sessions and two poster sessions.

- **IPSB2018** (Roscoff September 2018, Jacques Monod Conference, Headed by Gabriel Krouk):

System Biology aim is to use mathematical modeling procedures together with computer science to study biological objects as a whole trying to explore their emerging properties. Many plant biology labs around the world are taking these new avenues of research and are even developing landmark datasets and analysis, bridging biology, modeling and computer science. The Jacques Monod conference (1st international Plant Systems Biology [iPSB] meeting) is meant to consolidate the plant biology community around this emerging field that represents an important shift in plant biology studies (https://sites.google.com/site/iplantsystemsbiol/home).

FIVE-YEAR PROJECT AND STRATEGY

1- SWOT analysis

- its strong points;

- All permanent researchers have published a first and/or last author paper during the evaluated periods
- the team has an international recognition (invitation for seminar, meeting, reviews)
- we are involved in teaching at Montpellier SupAgro (Biology & Ecology Department) and the UM
- we welcomed two colleagues for their sabbatical (Milos Tanurdzic, University of Queensland and Geert de Jaeger, VIB Ghent)
- each researcher has local, national and/or international collaborations (Sue Rhee, Gloria Coruzzi (ongoing LIA CNRS), Jörg Kudla, Wolfgang Busch, Dirk Becker, Eva Benkova, Ondrej Novak…)

- needs for improvements;

- we should be more focused
- we should take care about our involvement in 'technical' subcontracting (oocyte expression, transcriptomic analysis, in the future stable isotope analysis).

- possibilities brought in by the context / surrounding environment;

- we were very successful in our previous grant application
- we had financial support from diverse funding agencies (ANR, NSF, Région LR, Agropolis Fondation, INRA, CNRS, SupAgro)

- risks linked to the context / environment

- we will see after the results of the ANR calls
- we should not stop our discussion about the best model for basic research (our answer today is to do our best with Arabidopsis)
- Limited funding will reduce the number of non-permanent

2- Structure, workforce and scientific orientations

The structure of the team is planned to be conserved (but everybody has the freedom to discover new research environment – and it would be very positive). We expect to have two retirements during the next period (Wojciech Szponarski and Chantal Brachet). We will have the responsibility of the AIS (Stable Isotopes Platform) and for that we need a specific technical support. We have asked INRA for an AI position to run the two mass spectrometers. The use of ^{15}N and ^{13}C is very important for us and we will introduce the use of ^{2}H (instead of ^{3}H) especially for use of labelled hormone.

While we mainly focused on the nutrient sensing of one specific nutrient (mainly nitrate), one of our main objective is to understand the molecular basis of combinatorial effects of multi-nutrients (Multi-N, N/P, N/Zn/P, K/N) and nutrient with hormones.

Concerning the approaches, even if we still have our favorite targets: transporters and their regulators (NPF, CIPK, CBL, PP2C) and transcription factors (HRS1/HHO, TGA1), we will strengthen our "unbiased" approaches using system biology and GWAS. The predictive modeling approaches are supported by our collaborations with mathematician (Pr André Mas, University of Montpellier) and will be developed in combination with GWAS approaches started on root development.

We have dedicated a lot of effort on system biology approaches and our relative success is challenging because we are often asked by colleagues to analyze their transcriptomic data. Whereas we are very glad to collaborate we believe that this kind of work should be done by a specific platform which should be developed in-house. On our side, we continue our effort by developing different strategies that should provide data and hypothesis for our biological approaches.

We often discuss the move to crops. With the arrival of Hatem Rouached in the team, we have decided to focus on Durum wheat. We will see in the future if this topic should be developed in a specific team headed by Hatem Rouached.

This is very important for us to stay at the top of: transport studies (with the specific use of stable isotope labelling), long distance signaling, combinatorial studies and system biology and we would like to be identified in foliar nutrient sensing.

Long distance signaling, TIP/TOP as a new set up to identify the signaling molecules (Sandrine Ruffel)

Overall, the complexity of the N-systemic signaling network (i.e., multiplicity of signals not originating from the same place in the plant) prompted us to question the relationship of the different parts of the root system with systemic signaling. To do so, we characterized the responses of Arabidopsis in a vertical split-root system. This system mimics a heterogeneous NO_3^- environment between the upper and the lower part of the root system. In brief, plants, beforehand grown in a homogeneous medium for 11 days, were transferred on the different combinations of N-provision: Control+N, N-Top, N-Tip, Control-N. In these preliminary experiments, what we designed as the "root-top" and "root-tip" were the portion of the primary root with and without any visible lateral roots, respectively. First, the level of plant NO_3^- acquisition in these different conditions were assessed after 8 hours following the transfer in order to evaluate the responsiveness of the roots in these conditions. It was interesting to observe that the level of acquisition between N-Top and N-Tip plants is only slightly different. This indicates a strong activity and responsiveness of this bottom and small part of the root system regarding NO_3^- provision. Moreover, after few days of treatment, the root system of N-Tip or N-Top plants displayed the characteristic root developmental response to NO_3^- heterogeneity. It corresponds to a higher lateral root growth in the NO_3^--rich patch compared to the respective Control+N root portion. This developmental response indicated that long-distance signaling operated to integrate NO_3^- heterogeneity at the whole plant level and to direct a compensatory response where NO_3^- is available. Therefore, the next question was to evaluate if shoots were rapidly able to discriminate between a NO_3^- related signaling originating from the top versus the tip of the primary root after few hours of treatment. Indeed, using a transcriptomic approach, we observed signature responses (i.e., specific gene regulation) in shoots depending on the NO_3^- supplied root portion. We identify 161 genes responding specifically if NO_3^- is provided to the "root-top" and interestingly, 28 genes responding specifically if NO_3^- is provided to the "root-tip". Altogether, these preliminary results suggest that the younger part of the primary root actively perceives and transduce the availability of NO_3^- directly to the shoots that are in turn able to process this information and trigger an active response.

By bringing together the questions of N-signaling and genotype grafting, the objective of the project is to provide important insights into the molecular mechanisms of long-distance communication triggered by root NO_3^- provision and targeting shoot activity, in plants. Basic mechanisms associated with root-shoot signaling will be elucidated in Arabidopsis thaliana, exploiting the vast genetic and genomic tools available in this model plant, the previous knowledge obtained on NO_3^- related long distance signaling and the rapidity with which different hypothesis can be tested. We will test the hypothesis that the most active parts of the plant for growth, *i.e*., the root and shoot apexes, directly communicate. In other words, do the root tips themselves perceive NO_3^- and trigger a specific systemic signaling that is essential to control the shoot and root development.

Foliar nutrient sensing (Anna Medici, Virginie Thiry)

Foliar fertilization is a widely-used tool in agriculture. Despite the recognized benefits of this practice on crops cultures, the molecular mechanisms driving the sensing of mineral and organic nutrients at the leaf level and their absorption are largely unknown. Nitrogen (N) is the most important macronutrient affecting crop growth and yield. Urea and nitrate are the two main organic and inorganic forms of nitrogen used in foliar fertilization. At present, the molecular basis of the roots sensing and transport of different N forms and the response to root supply have been largely studied. At the contrary, there are no evidence for i) the involvement of specific transporters for urea and nitrate leaf uptake, and ii) the involvement of specific genes in the perception and regulation of the foliar uptake. The project is an exploratory program, which aim is to discover new molecular elements driving the foliar sensing and acquisition of one main mineral and one main organic form of nitrogen. For that, we will combine molecular physiology, transcriptomic, and genetic approaches. First, we will characterize the kinetic and dynamic of urea and nitrate leaf absorption and translocation in planta. We will assess influx and uptake experiments from leaves, treated with isotope-labelled urea and nitrate solutions. Secondly, we will perform a genome-wide analysis of the leaf transcriptional response specific to urea or nitrate supply, and resulting from the combination of both. We will generate and analyze the gene regulatory networks driving the responses to leaf nitrogen supply, to identify master regulator genes. Thirdly, we will develop a second genome-wide approach, by studying the genetic variability associated to urea and nitrate acquisition from leaves in 223 Arabidopsis accessions. The aim of this GWAS approach is to find loci linked to the mechanism of acquisition of nitrogen from leaves. Finally, we will develop a more targeted approach, to verify the involvement of stomata, cuticle and the NIGT1 transcription factors in the regulation of nitrogen acquisition from leaves. In fact, the literature suggests their role in this process. Using a reverse genetic approach, we will study urea and nitrate leaf transport properties, in two genotypes showing modified stomata opening behavior (*ost2-2* and OX-NCED8) and cuticle composition (*shn1*). In this way, we will validate or not the potential role of this two leaf components in nitrogen absorption. Then we will investigate the role of NIGT1 transcription factors in regulating the transcription of the urea transporter DUR3 and other identified urea-responsive genes in leaves, using nigt1 mutants. At the end of this program that we named Nutri-leaf, we should obtain a list of genes, validated for their role in urea and nitrate absorption from Arabidopsis leaves, and a model explaining the mechanism of transport and allocation of these molecules from the leaf surface to the rest of the plant. These results on nitrogen-based molecules will have a real potential for the transfer i) to other species of agronomic interest and ii) to other macro or micro-elements important in the foliar feeding. We think that Nutri-leaf results could have a strong impact on the research community, driving a big shift from roots to shoots studies in the plant nutrition domain. At long term, the knowledge generated through this plant nutrition research could be used for establishment of sustainable nutrient management systems but also to develop more nutrient-efficient crop plants.

Nitrate/phosphate interactions (Gabriel Krouk, Wojciech Szponarski, Anna Medici)

Nitrogen (N) and phosphorus (P) are the most important macroelements affecting plant growth. The points of interaction between nitrate and phosphate signaling pathways are critical for plant development, but have received scarce attention. Our previous findings demonstrated the centrality of some molecular actors (NRT1.1 and PHO2 for instance) in the nitrate-phosphate signals interaction. We will continue this research topic, searching for the relationships between these molecular actors to define the molecular network neighboring these two central integrators of nutritional signals. Since PHO2 is a post-translational regulator, we decided to develop a combinatorial study (N/P) at the protein level. For building a mechanistic model in Arabidopsis we will 1) search for new candidate proteins of the network via proteomic associated to transcriptomic approaches and 2) characterize the candidates via a molecular physiology approach in Arabidopsis.

Nitrate/ABA and its link to other macronutrient (Benoit Lacombe, Chantal Brachet)

To address the molecular principles underlying combinatorial signals in hormone – nutrient regulated development we will focus on nitrate – potassium interplay and its link to ABA signaling. Nitrate (NO_3^-) and Potassium (K+) are not only major nutrients (known as Macronutrients) sources but in addition serve as signaling molecules that modifies root growth and development. The sensing mechanism is, at least partly, mediated by a member of the CBL-INTERACTING PROTEIN KINASE (CIPK), CIPK23.

Our recent work on this kinase demonstrates that it belongs to a protein regulatory network involving a transceptor (NPF6.3), calcium sensors (CBL1/9) and a PP2C (ABI2) (Léran et al., 2015). In parallel, published results (Kanno et al., 2012; Huang et al., 1999) and preliminary data obtained in our laboratories indicate that another member of this NPF family, NPF4.6, is a nitrate and an ABA transporter and that it is regulated by a similar regulatory network involving CIPK23 and CBL1. But it was also demonstrated previously that CIPK23 targets several potassium transporters involved in potassium uptake, namely, AKT1, AtKC1 and HAK5). The role of CIPK23 in the low K+ response has now firmly be established by different research groups (Xu et al., 2006, Cheong et al., 2007, Geiger et al. 2011). Own preliminary work performed by the German partner further shows that the CBL1/9, CIPK23, ABI2 module regulates potassium homeostasis via K+ efflux channels. Interestingly, and beside its interaction with plasma membrane nutrient transporters, it has been recently demonstrated that CIPK23 interacts with the bZIP transcription factor (TF) TGA1 (AtbZIP47) (Yazaki et al., 2016) known to be involved in nitrate signalling (Alvares et al., 2014). Furthermore, other CIPK and CBL are known or supposed to be important in either potassium, nitrate and/or ABA signaling. For example, CIPK14 and CIPK15 have been established to be central hubs in ABA signaling (Lumba, et al., 2014), CBL7 is involved in nitrogen starvation response (Ma, et al., 2015) and CIPK8 is involved in the primary nitrate response (Hu et al., 2009).

Hence, CIPK23 might act as an important convergence point at which nitrate, potassium and ABA mediated signaling meet. However, how the activity of CIPK23 towards its different targets is integrated into fast (nutrient sensing and transport) and long-term (transcriptional regulation) remains to be elucidated.

System biology and predictive modeling (Gabriel Krouk)

Determining the fundamental structure of Transcriptional Gene Regulatory Networks (GRNs) is a major challenge of biology. Indeed, being able to learn the GRN will enable to manipulate the cell as a system and potentially control many physiological events that are related to GRN activity. The actual scientific question is to determine GRN structure using machine-learning algorithms applied on transcriptomic datasets [being the most exhaustively measures level of the system (commonly assayed by micro-arrays or next generation sequencing)]. One striking observation concerning the Gene Regulatory Networks, identified so far, is that they are really strongly branched in order to adapt to mutation and to fluctuating environments. This is true from bacteria to more complex organisms such as plants and humans. For most of the studied eukaryotic organisms, 5 to 10% of the genes are involved in transcriptional control (are transcription factors [TF]). Thus, this biological complexity represents an exciting challenge for Math and Computer science.

Several algorithms have been developed to model as well as to solve GRNs. Interestingly, most if not all of the GRN simulating algorithms are based on solving Ordinary Differential Equations (ODEs) that make the algorithms restricted to several hundred of genes due to computational restrictions. Here we have the ambition to propose a tool that will in fine be able to simulate a whole genome GRN. The philosophy of this project is also definitively oriented to reverse engineering. You will see below that we plan to develop an algorithm that is able to simulate gene expression per se trying to solve some questions about how to build the first steps towards a in silico living cell.

Thus, we will: (i) develop large GRN simulation algorithm aimed at providing an “almost infinite” *in silico* expression of a simulated stable network containing several thousands of genes imitating, as close as possible, real transcriptomic data; (ii) develop new kinds of GRN machine learning processes in order to learn an unprecedented large GRNs using prior knowledge about GRN structure; (iii) the learning of real eukaryotic GRNs using real transcriptomic datasets in order to provide biologists reliable GRNs predictions.

We wish to highlight here that, an important aspect of the project is that we aim at using experimentally probed and active TF <-> Target relationships and ask Math to learn which features/rules in genome scale datasets could have been used to predict such connections. Indeed, usually GRN predictions are meant to be validated by experimental investigations. Here we flip the necessity of experimental validations into a mean to improve GRN learning. To achieve this goal, we undertook a reverse engineering approach in order to simulate GRNs as close as possible to reality, in order to be able to fully validate the machine learning algorithms trained at the same time on in silico expression data and prior knowledge of pieces of the network.

Systems genetics of phosphate homeostasis in crops (Hatem Rouached)

An intriguing observation has been made in the 70s, showing that plants overaccumulate P in the shoots when they grow under zinc (Zn) deficiency. Nevertheless, despite its fundamental importance, this P-Zn interaction in plants remains unexplained at the molecular level. Deciphering the molecular pathways that lead to overaccumulates Pi under Zn deficiency will unravel a new route for the accumulation of Pi in plants, and will help to design an innovative strategy to improve P-use efficiency in plants. Recently, we have revealed, using Genome-Wide Association Study (GWAS) in the model plant *Arabidopsis thaliana*, the existence of genetic factors that control Pi accumulation in Zn dependent manner (Kisko et al, 2018). These results are very encouraging to discover genes and molecular pathways regulating Pi accumulation in an economically important crop(s). Therefore, we will identify genes controlling Pi accumulation in shoots of durum wheat grown under different Zn regimes. Durum wheat was chosen because of its high strategic, economic and social importance in France and considered as a major staple food source comprising about one-third of the global cereal production. Using a unique panel of Evolutionary Pre-breeding population (EPO) consisting in 211 varieties of durum wheat, we obtained preliminary data showing that there is widespread natural variation in shoot Pi concentration in durum wheat. This supports the existence of genetic factors that affect this trait in wheat. The genetic diversity of EPO makes it appropriate for GWAS and constitutes an originality of this project. Therefore, we will (i) identify candidate genes involved in the regulation of Pi accumulation in wheat grown under two Zn conditions (+Zn and -Zn) using GWAS; (ii) identify the causal genes associated with the accumulation of Pi in wheat under Zn deficiency, which will leads to investigate of their natural allelic variants, very important for genomic selection or editing to enable the molecular design of new cultivars; (iii) characterize the mutants/transgenic lines in causal genes at physiological and molecular levels by determining all parameters linked to Pi use efficiency. Finally, this project will be extended to study zinc and nitrogen homeostasis interaction with regard Pi use efficiency in model plants and crops using systems genetics approaches.

Annex 4 – Research products and activities

EVALUATION CAMPAIGN 2019-2020

GROUP A

Name of the unit / the team / the theme: Hormones, nutrients and development
Acronym: HoNuDe

Head of the unit / Team leader / Theme leader for the current contract: Benoit Lacombe
Head of the unit / Team leader / Theme leader for the next contract: Benoit Lacombe

I - PRODUCTION OF KNOWLEDGE AND ACTIVITIES CONTRIBUTING TO THE INFLUENCE AND SCIENTIFIC ATTRACTIVENESS OF THE TEAM

1- Articles

Scientific articles

L'équipe HONUDE a été créée en juillet 2015. Plusieurs publications de la période 2015-2018 correspondent à des travaux réalisés dans l'équipe INTEGRATION.

Most significant articles (20%)

1. Léran S, K.H. Edel, Pervent M., Hashimoto K., Corratge-Faillie C., Offenborn J.N., Tillard P., Gojon A., Kudla J., Lacombe B. (2015) Nitrate sensing and uptake in Arabidopsis are enhanced by ABI2, a phosphatase inactivated by the stress hormone abscisic acid. Sci Signaling. 8 (375): ra43.

2. Medici A., Marshall-Colon A., Ronzier E., Szponarski W., Wang R., Gojon A., Crawford N.M., Ruffel S., Coruzzi G.M., Krouk G. (2015) AtNIGT1/HRS1 integrates nitrate and phosphate signals at the Arabidopsis root tip. Nat Commun. 6: 6274.

3. Carré C., Mas A., Krouk G. (2017) Reverse engineering highlights potential principles of large gene regulatory network design and learning. NPJ Syst. Biol. Appl. 3 : 17. (

4. Pal S., Kisko M., Dubos C., Lacombe B., Berthomieu P., Krouk G., Rouached H. (2017) TransDetect identifies a new regulatory module controlling phosphate accumulation. Plant Physiol. 175(2):916-926.

5. Kisko M., Bouain N., Safi A., Medici A., Secco D., Fouret G., Krouk G., Aarts M.G., Busch W., Rouached A. (2018) LPCAT1 controls phosphate homeostasis in a zinc-dependent manner. eLIFE 7 pii: e32077.

6. Poitout A., Crabos A., Petřík I., Novák O., Krouk G., Lacombe B., Ruffel S. (2018) Responses to systemic nitrogen signaling in Arabidopsis roots involve trans-zeatin in shoots. Plant Cell. 30(6): 1243-1257.

7. Safi A., Medici A., Szponarski W., Marshall-Colon A., Ruffel S., Gaymard F., Coruzzi G., Lacombe B., Krouk G. (2018) HRS1/HHOs GARP transcription factors and reactive oxygen species are regulators of Arabidopsis nitrogen starvation response. bioRxiv. 2018/164277 (

8. Medici A., Szponarski W., Dangeville P., Safi A., Dissanayake I. M., Saenchai C., Emanuel A., Rubio V., Lacombe B., Ruffel S., Tanurdzic M., Rouached H., Krouk G. (2019) Identification of molecular integrators shows that nitrogen actively controls the phosphate starvation response in plants. Plant Cell. 31(5): 1171-1184.

2015

9. Krouk G., Carré C., Fizames C., Gojon A., Ruffel S., Lacombe B. (2015) GeneCloud reveals semantic enrichment in lists of gene descriptions. Mol Plant. 8(6): 971-973.

10. Léran S., Garg B., Boursiac Y., Corratge-Faillie C., Brachet C., Tillard P., Gojon A., Lacombe B. (2015) AtNPF5.5, a nitrate transporter affecting nitrogen accumulation in Arabidopsis embryo. Sci Rep. 5 : 7962.

2016

11. Ristova D, Carré C., Pervent M., Medici A., Kim G.J., Scalia D., Ruffel S., Birnbaum K. D., Lacombe B., Busch W., Coruzzi G.M., Krouk G. (2016) Combinatorial interaction network of transcriptomic and phenotypic responses to nitrogen and hormones in the Arabidopsis thaliana root. Sci. Signal. 9(451): rs13.

12. Ruffel S., Poitout A., Krouk G., Coruzzi G. M., Lacombe B. (2016) Long-distance nitrate signaling displays cytokinin dependent and independent branches. J Integr Plant Biol. 58(3): 226-229.

2017

13. Corratgé-Faillie C., Ronzier E., Sanchez F., Prado K., Kim J.-H., Lanciano S., Leonhardt N., Lacombe B., Xiong T. C. (2017) The Arabidopsis guard cell outward potassium channel GORK is regulated by CPK33. FEBS Lett. 591(13):1982-1992.

14. **Poitout A., Martinière A., Kucharczyk B., Queruel N., Silva-Andia J., Mashkoor S., Gamet L., Varoquaux F., Paris N., Sentenac H., Touraine B., Desbrosses G. (2017) Local signalling pathways regulate the Arabidopsis root developmental response to Mesorhizobium loti inoculation. J. Exp. Bot. 68 (5): 1199-1211.**

2018

15. **Belgaroui N., Lacombe B., Rouached H., Hanin M. (2018) Phytase overexpression in Arabidopsis improves plant growth under osmotic stress and in combination with phosphate deficiency. Scientific Reports . 8 (1): 1137**

16. **Bouain N., Satbhai S. B., Korte A., Saenchai C., Desbrosses G., Berthomieu P., Busch W., Rouached H. (2018) Natural allelic variation of the AZI1 gene controls root growth under zinc-limiting condition. PLoS Genet. 14 (4): e1007304.**

17. **Chaiwong N., Prom-u-thai C., Bouain N., Lacombe B., Rouached H. (2018) Individual versus Combinatorial Effects of Silicon, Phosphate, and Iron Deficiency on the Growth of Lowland and Upland Rice Varieties. Int. J. Mol. Sci. 19 (3): 899. (**

18. Varala K., Marshall-Colón A., Cirrone J., Brooks M.D., Pasquino A.V., Léran S., Mittal S., Rock T.M., Edwards M.B., Kim G.J., Ruffel S., McCombie W.R., Shasha D., Coruzzi G.M. (2018) Temporal transcriptional logic of dynamic regulatory networks underlying nitrogen signaling and use in plants. Proc Natl Acad Sci U S A.115(25): 6494-6499.

2019

19. Brooks M. D., Cirrone J., Pasquino A. V., Alvarez J.M ., Swift J., Mittal S., Juang C.L., Varala K., Gutiérrez R. A., Krouk G., Shasha D., Coruzzi G. M. (2019) Network Walking charts transcriptional dynamics of nitrogen signaling by integrating validated and predicted genome-wide interactions. Nat.Commun. 10 (1): 1569.

20. Falter-Braun P., Brady S. M., Gutiérrez R. A., Coruzzi G., Krouk G. (2019) iPlant Systems Biology (iPSB): An International Network Hub in the Plant Community. Mol Plant (in press).

Review articles

2015

1. Bhardwaj D., Medici A., Gojon A., Lacombe B., Tuteja N. (2015) A new insight into root responses to external cues: paradigm shift in nutrient sensing. Plant Signal Behav. 10(12): e1049791. Revue bibliographique

2016

2. **Bouain N., Doumas P., Rouached H. (2016) Recent advances in understanding the molecular mechanisms regulating the root system response to phosphate deficiency in Arabidopsis. Curr. Genomics 17(4): 308-314.** Revue bibliographique

3. **Krouk G. (2016) Hormones and nitrate: a two-way connection. Plant Mol. Biol. 91(6): 599-606.** Revue bibliographique

4. **Lacombe B., Achard P. (2016) Long-distance transport of phytohormones through the plant vascular system. Curr Opin Plant Biol. 34: 1-8.** Revue bibliographique

5. **Noguero M., Lacombe B. (2016) Transporters involved in root nitrate uptake and sensing by Arabidopsis. Front. Plant Sci. 7: 1391.** Revue bibliographique

6. O'Brien J.A., Vega A., Bouguyon E., Krouk G., Gojon A., Coruzzi G., Gutiérrez R.A. (2016) Nitrate transport, sensing and responses in plants. Mol. Plant 9(6): 837-856. Revue bibliographique

2017

7. **Chanclud E., Lacombe B. (2017) Plant Hormones: Key Players in Gut Microbiota and Human Diseases? Trends Plant Sci. 22(9): 754-758. Opinion**

8. **Corratge-Faillie C., Lacombe B. (2017) Substrate (un)specificity of Arabidopsis NRT1/PTR FAMILY (NPF) proteins. J. Exp. Bot. 68(12): 3107-3113.** Revue bibliographique

9. Friesner J., Assmann S. M., Bastow R., Bailey-Serres J., Beynon J., Brendel V., Buell C. R., Bucksch A., Busch W., Demura T., Dinneny J. R., Doherty C. J., Eveland A. L., Falter-Braun P., Gehan M. A., Gonzales M., Grotewold E., Gutierrez R. A., Kramer U., Krouk G., Ma S., Markelz R. J. C., Megraw M., Meyers B. C., Murray J. A. H., Provart N. J., Rhee S., Smith R. J., Spalding E. P., Taylor C., Teal T. K., Torii K. U., Town C., Vaughn M., Vierstra R., Ware D., Wilkins O., Williams C., Brady S. M. (2017) The Next Generation of Training for Arabidopsis Researchers: Bioinformatics and Quantitative Biology. Plant Physiol. 175 (4): 1499-1509. Opinion

10. **Heuer S., Gaxiola R., Schilling R., Herrera-Estrella L., López-Arredondo D., Wissuwa M., Delhaize E., Rouached H. (2016) Improving phosphorus use efficiency - a complex trait with emerging opportunities. Plant J. 90(5): 868-885.** Revue bibliographique

11. **Krouk G. (2017) Nitrate signalling: Calcium bridges the nitrate gap. Nature Plants. 3 (7): 17095. Commentaire**

12. **Medici A., Lacombe B., Ruffel S. (2017) Nitrate supply to grapevine rootstocks - new genome-wide findings. J.Exp.Bot. 68 (15): 3999-4001. Commentaire**

13. **Rouached H., Rhee Seung Y. (2017) System-level understanding of plant mineral nutrition in the big data era. Curr.Opin. Syst. Biol. 4: 71-77. Revue bibliographique**

14. **Ruffel S., Gojon A. (2017) Systemic nutrient signalling: On the road for nitrate. Nat. Plants. 3 (4): 17040.7 Commentaire**

15. **Safi A., Medici A., Szponarski W., Ruffel S., Lacombe B., Krouk G. (2017) The world according to GARP transcription factors. Curr Opin Plant Biol. 39 159-167.** Revue bibliographique

16. **Secco D., Bouain N., Rouached A., Promuthai C., Hanin M, Pandey AK, Rouached H. (2016) Phosphate, phytate and phytases in plants: from fundamental knowledge gained in Arabidopsis to potential biotechnological applications in wheat. Crit. Rev. Biotechnol. 37(7) 898-910.** Revue bibliographique

17. **Secco D., Whelan J., Rouached H., Lister R. (2017) Nutrient stress-induced chromatin changes in plants. Curr Opin Plant Biol. 39 1-7.** Revue bibliographique

2018

18. **Kisko M., Shukla V., Kaur M., Bouain N., Chaiwong N., Lacombe B., Pandey A., Rouached H. (2018) Phosphorus Transport in Arabidopsis and Wheat: Emerging Strategies to Improve P Pool in Seeds. Agriculture. 8 (2): 27.** Revue bibliographique

19. **Rouached H. (2018) Red light means on for phosphorus. Nat Plants. 4: 983-984. Commentaire**

20. **Ruffel S. (2018) Nutrient-related Long-Distance Signals: common players and possible crosstalk. Plant Cell Physiol. 59(9): 1723-1732.** Revue bibliographique

2019

21. **Bouain N., Krouk G., Lacombe B., Rouached H. (2019) Getting to the root of plant mineral nutrition. Trends Plant Sci 24(6): 542-552.** Revue bibliographique

Other articles (professional journals, etc.) (total number)

2018

1. **Bouain N., Korte A., Satbhai S. B., Rhee S. Y., Busch W., Rouached H. (2018) Systems approaches provide new insights into Arabidopsis thaliana root growth under mineral nutrient limitation. bioRxiv.**
2. **Noguero M., Leran S., Bouguyon E., Brachet C., Tillard P., Nacry P., Gojon A., Krouk G., Lacombe B. (2018) Revisiting the functional properties of NPF6.3/NRT1.1/CHL1 in xenopus oocytes; . bioRxiv. 2018/244467164277**
3. **Poitout A., Crabos A., Petřík I., Novák O., Krouk G., Lacombe B., Ruffel S. (2018) Root Responses to Heterogeneous Nitrate Availability are Mediated by trans-Zeatin in Arabidopsis Shoots. bioRxiv. 2018/242420**
4. **Safi A., Medici A., Szponarski W., Marshall-Colon A., Ruffel S., Gaymard F., Coruzzi G., Lacombe B., Krouk G. (2018) HRS1/HHOs GARP transcription factors and reactive oxygen species are regulators of Arabidopsis nitrogen starvation response. bioRxiv. 2018/164277**

2019

5. Kaur G., Shukla V., Kumar A., Kaur M., Goel P., Singh P., Shukla A., Kaur J., Singh J., Mantri S., Rouached H., Pandey A.K. (2019) Genome-wide expression analysis id 1 entifies core components during iron starvation in hexaploid wheat. BioRxiv.

2- Books

Monographs, critical editions, translations (total number)

Management and coordination of scientific books / Scientific book edition

Book chapters (total number)

Book chapters in English or another foreign language

2018

1. **Abualia R., Benkova E., Lacombe B. (2018) Chapter Five. Transporters and Mechanisms of Hormone Transport in Arabidopsis. Adv. Bot. Res. 87:115-138.**

Edited theses

3- Production in conferences / congresses and research seminars

Articles published in conference proceedings / congress

Other products presented in symposia / congress and research seminars

Invited oral presentations in international symposia/congress

2016

1. Lacombe B. (2016) Hormone and nutrient transport by NPF proteins (June 2016) International Workshop on Plant Membrane Biology, Annapolis (USA).

2. Lacombe B. (2016) NPF proteins are part of protein regulatory network involved in hormone-dependent nutrient sensing (21-26 June 2016) International Plant Growth Substance Association (IPGSA), Toronto (Canada).
3. Krouk G. (2016) A systems view of nitrogen signaling interactions. (22-26 Août 2016) International Congress "Nitrogen 2016" EMBO Conference: The Nitrogen Nutrition of Plant. Montpellier (France).

4. Ruffel S., Jacquot A., Fizames C., Shasha D., Tillard P., Santoni V., Schulze W., Gojon A., Gutierrez R., Lejay L. (2016) Regulation of root nitrate uptake by carbon and nitrogen using NRT2.1 as a target. (22-26 Août 2016) International Congress "Nitrogen 2016" EMBO Conference: The Nitrogen Nutrition of Plant. Montpellier (France).

2018

5. Krouk G. (2018) A Systems View of Nitrogen Signaling Interactions (21 au 24 janvie) Plant Signaling: Molecular Pathways and Network Integration. Granlibakken Tahoe, Tahoe City, California, (USA).

6. Krouk G. (2018) Gene Regulatory Network Dynamics: Lessons from Plants (June 10 - 15, 2018) Gordon Research Conference "Dynamic Plant Systems". Holderness School, Holderness, NH, (US).

7. Ruffel S. (2018) Regulatory Network Behind Systemic Nitrogen Signaling, in Arabidopsis. (Du 10 au 15 juin 2018) Gordon Research Conference :Plant Molecular Biology "Dynamic Plant Systems". Holderness School, Holderness, NH, (US).

8. Ruffel S., Poitout A., Crabos A., Petrik I, Novak O., Krouk G., Lacombe B. (2018) Interaction between systemic nitrogen signaling and hormones, in arabidopsis. (5 -10 août) 12th Congress of the International Plant Molecular Biology. Montpellier (France).

Invited oral presentations in national symposia/congress

2017

1. Krouk G. (2017) Gene Regulatory Networks: Lessons from plants. (16-17 novembre) LyonSysBio. Villeurbanne (France).

2. Krouk G. (2017) Réseaux de régulation génique : que nous apprennent les plantes ? (9 au 10 novembre) Les journées NETBIO AgroParisTech Toulouse (France).

Selected oral presentations in international symposia/congress

2016

1. Medici A., Sponarsky W., Dangeville P., Lacombe B., Rouached H., Krouk G. (2016) Nitrate drives the phosphate starvation response through IDR1. (22-26 Août 2016) International Congress "Nitrogen 2016" EMBO Conference: The Nitrogen Nutrition of Plant. Montpellier (France).

Selected oral presentations in national symposia/congress

Posters in international symposia/congress

2016

1. Léran S., Edel K., Pervent M., Hashimoto K., Corratgé-Faillie C., Offenborn N. J., Tillard P., Gojon A., Kudla J., Lacombe B. (2016) Nitrate sensing and uptake in Arabidopsis are enhanced by ABI$_{2, a}$ phosphatase inactived by the stress hormone abcisic acid. (22-26 Août 2016) International Congress "Nitrogen 2016" EMBO Conference: The Nitrogen Nutrition of Plant. Montpellier (France).

2018

2. Rouached H., Rhee S.Y.(2018) Interactions between plant mineral nutrients-phosphate, zinc and iron, a dynamic trio. (5 -10 août) 12th Congress of the International Plant Molecular Biology. Montpellier (France).

Posters in national symposia/congress

Invited seminars in foreign institutions

Invited seminars in French institutions

4- Electronic tools and products

Softwares

GeneCloud: Krouk et al 2015
TransDetect: Pal et al 2018
FRANK: Carre et al 2018
PAM: Depot Agence Protection des Programmes (inventors Krouk 50%, Carre 50%).

Databases

5- Instruments and methodology

Prototypes

Platforms and observatories

6- Other products

Theorised artistic creations, staging, movies

7- Editorial activities

Participation in editorial committees (books, collections, etc.)

-Faculty member: Faculty of 1000. Plant Biochemistry and Physiology Section (Since Dec. 2018)
-Associate Editor : -BMC Systems Biology. (Since 2013)
-Guest Editor : special issue on Plant Systems Biology of Molecular Plant (2019)

Collection and series management

8- Reviewing activities

Reviewing of articles

Members of Honude has reviewed around 20 MS each year for: Nature, Science, PNAS, Plant Cell, Trends in Plant Science, Molecular Plant Dev Cell, ELife, Nature Plants, Nature Comm, New Phytol, Plant Physdiol, Plant J, …

Grant evaluation (public or charities)

Members of Honude has reviewed around 10 grant application each year for: NSF (USA), ANR (France), SPS (France), FRS/FNRS (Belgium), DFG (Germany), NWO (Netheerland), ISF (Israel), NCN (Poland), INRA BAP

Reviewing of research institutes

DynaMo Center scientific advisory board (University of Copenhagen, Denmark)

Participation in institutional committees and juries (CNRS, Inserm, etc.)

2018 : Jury for the recruitment of an Associate professor at the University of Perpignan

Members of the Honude Team were involved in:
- 6 PhD committees
- 6 PhD Jury

Member of INRA GT1 (Groupe de Travail 1; Lead: Dr Francoise Medale) (INRA Think-tank on predictive biology)

9- Academic research grants

European (ERC, H2020, etc.) and international (NSF, JSPS, NIH, World Bank, FAO, etc.) grants – coordination

2013 – 2016 ; (CEFIPRA, Franco-Indian) Nutrient sensing in plants ; LACOMBE Benoît

2015 - 2018 ; (MSCA, H2020) **NITRO SYSTEMS (H2020)**; Reaching the roots of systemic nitrogen signaling in plants ; RUFFEL Sandrine

European (ERC, H2020, etc.) and international (NSF, JSPS, NIH, World Bank, FAO, etc.) grants - partnership

2014 – 2019 ; **NUTRINET (NSF)** ; A network-inspired approach to improving nuitrient use efficiency in crop plants ; RUFFEL Sandrine

Other European grants - coordination

2015 – 2016 ; **PROCOPE (Franco-Allemand) PHC (Partenariat Hubert Curien)** ; Analysis of CA2+ dependent of NO3 – transport and sensing in plants ; LACOMBE Benoit, KUDLA Jorg (University of Muenster, Allemagne)

Other European grants - partnership

National public grants (ANR, PHRC, FUI, INCA, etc.) - coordination

2012 – 2014 ; **NitroNet (ANR, retour Post-Doc)** ; Identification et modélisation des réseaux moléculaires impliqués dans la perception et la transduction du signal nitrate chez Arabidopsis ; KROUK Gabriel

2012 – 2016 ; **NUTSE (ANR JCJC)** ; Perception des nutriments chez les plantes ; LACOMBE Benoît

2014 – 2018 ; **HONIT (ANR, Franco-Autrichien)** ; Hormone cross-talk drives nutrient dependent plant development ; LACOMBE Benoît

2014 – 2015 ; **VARNET (Department BAP INRA)** ; Variabilité naturelle des réseaux de régulation génique impliqués dans la réponse des plantes à la disponibilité hétérogène en nitrate, chez Arabidopsis ; RUFFEL Sandrine

2016 – 2020 ; **CoopNet (LIA)** ; Franco-American Cooperation for understanding and modeling transcription factor Network interactions ; KROUK Gabriel

2017 – 2018 ; **N&Pwork (SupAgro incitative project)** ; Elaboration d'un modèle moléculaire de réponse à l'apport conbiné de nutriments (N, P) chez Arabidopsis thaliana via l'intégration transcriptomique et protéomique ; MEDICI Anna

2018 – 2019 ; **TOUT-Crops (Department BAP INRA)** ; TOwards Understanding and Translating to crops TGA1 mutant phenotype ; KROUK Gabriel

National public grants (ANR, PHRC, FUI, INCA, etc.) - partnership

2014 – 2019 ; IMANA **(ANR PRC)** ; Identification de régulations moléculaires majeures impliquées dans l'adaptation des plantes à la disponibilité en azote ; LACOMBE Benoît

Local grants (collectivités territoriales) - coordination

2015 – 2019 ; **BLE DURABLE (Région Languedoc Roussillon)** ; Génétique d'association, nouvelle approche de sélection de variétés de blé dur économes en fertilisation phosphatée pour une production durable ; ROUACHED Hatem

Local grants (collectivités territoriales) - partnership

PIA (labex, equipex etc.) grants - coordination

2017 – 2018 ; **NITROTOR (Labex Agro)** ; Unraveling TOR-mediated signaling in relation to nitrogen metabolism ; LACOMBE Benoît

2018 – 2018 ; **IPMB (Labex Agro)** ; International Plant Molecular Biology Congress 2018 - IPMB2018 ; LACOMBE Benoît

2018 – 2019 ; **HPCG (SATT - Labex Agro)** ; Projet de maturation High Performance Computing for Genetics ; KROUK Gabriel

PIA (labex, equipex etc.) grants - partnership

2018 – 2019 ; **GENFUN (Labex Agro)** ; Exploring a gene inducible system for functional studies related to flowering phenology in apple ; KROUK Gabriel

Grants from foundations and charities (ARC, FMR, FRM, etc.) - coordination

Grants from foundations and charities (ARC, FMR, FRM, etc.) - partnership

10- Visiting senior scientists and post-doc

Post-docs (total number)

Since 2014: 9

Foreign post-docs

Since2014: 3

Family Name	First name	Contract type & Employer	Nationality	Recruitment period	
BOUAIN	Nadia	CDD INRA ANR	TUNISIENNE	01/10/2016	30/09/2017
GARG	Bharti	Bourse campus France	INDIENNE	01/08/2013	30/11/2014
MEDICI	Anna	CDD INRA ANR	ITALIENNE	01/01/2012	30/11/2015

Visiting scientists (total number)

Since 2014 : 7

Foreign visiting scientists

Since 2014 : 6

Family Name	First name	Financing source	Nationality	Date of arrival	Date of departure	Status	University of origin
Alves	Ludovico	Bourse EMBO Short Term	Italien	07/01/2014	07/04/2014	Doctorant invité	Université de Naples
SECCO	David	Bourse jeune chercheur ARC	Australien	14/03/2016	13/03/2017	Post-doc invité	UWA acting through its ARC

							Centre of Excellence in Plant Energy Biology
SECCO	David	Bourse jeune chercheur ARC	Australien	14/03/2017	14/09/2017	Post-doc invité	UWA acting through its ARC Centre of Excellence in Plant Energy Biology
TANURDZIC	Milos	Bourse	Australien	04/07/2016	24/09/2016	Enseignant chercheur invité	University of Queensland Australie
CHAIWONG	Nantana	Bourse	Thailandais	01/02/2019	30/04/2019	Doctorant invité	Fac Chiang Mai bourse université
DE JAEGER	Geert	Agropolis Fondation/FNRS	Belgium	01/07/2017	31/08/2017	Professeur Invité	Ghent Uniiversity/VIB

11- Scientific recognition

Prizes and/or distinctions

IUF members

Chair of learned and scientific societies

Organisations of meetings and symposia (out of France)

Invitations to meetings and symposia (out of France)

Members of the honude teams have been invited 5 times/year

Members' long-term visits abroad

Hatem Rouached: 2016-2018: Carnegie Institute of Plant Biology (California, USA)

II - INTERACTION OF THE TEAM WITH THE NON-ACADEMIC WORLD, IMPACTS ON ECONOMY, SOCIETY, CULTURE OR HEALTH

1- Socio-economic interactions / Patents

Invention disclosures

Filed patents

Accepted patents

Licenced patents

2- Socio-economic interactions

Industrial and R&D contracts

Cifre fellowships

Creation of labs with private-public partnerships

Networks and mixed units

Start-ups

3- Expertise

Consulting

Participation in expert committees (ANSES etc.)

Legal expertise

Expert and standardization reports

4- Public outreach

Radio broadcasts, TV shows, magazines and newspaper

Journal articles, interviews, book edition, videos, other popularization outputs, debates on science and society, etc.

III - INVOLVEMENT OF THE TEAM IN TRAINING THROUGH RESEARCH

1- Educational outputs

Books

E-learning, MOOCs, multimedia lessons, etc.

2- Scientific productions (articles, books, etc.) from theses

Scientific productions (articles, books, etc.) from theses

Léran S, K.H. Edel, Pervent M., Hashimoto K., Corratge-Faillie C., Offenborn J.N., Tillard P., Gojon A., Kudla J., Lacombe B. (2015) Nitrate sensing and uptake in Arabidopsis are enhanced by ABI2, a phosphatase inactivated by the stress hormone abscisic acid. Sci Signaling. 8 (375): ra43.

Léran S., Garg B., Boursiac Y., Corratge-Faillie C., Brachet C., Tillard P., Gojon A., Lacombe B. (2015) AtNPF5.5, a nitrate transporter affecting nitrogen accumulation in Arabidopsis embryo. Sci Rep. 5 : 7962.

Kisko M., Bouain N., Safi A., Medici A., Secco D., Fouret G., Krouk G., Aarts M.G., Busch W., Rouached A. (2018) LPCAT1 controls phosphate homeostasis in a zinc-dependent manner. eLIFE 7 pii: e32077.

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Mean number of publications per student (Biology & Science and technology only)

3- Training

Habilitated (HDR) scientists

Since 2014 : 3

Family Name	First name	Position	Employer	Date of habilitation
KROUK	Gabriel	CR	CNRS	2015
LACOMBE	Benoit	CR	CNRS	2008
ROUACHED	Hatem	CR	INRA MONTPELLIER	2011

HDR obtained during the period

Since 2014 : 2 HDR

Family Name	First name	Position	Employer	Date of habilitation
KROUK	Gabriel	chercheur	CNRS	2015
SECCO	David	Chercheur invité	UWA, Australia	2016

PhD students (total number)

4 PhD students

PhD students benefiting from a specific doctoral contract

Family Name	First name	Starting date	Defense	Duration (months)	Funding
KISKO	Mushtak	24/09/2014	08/03/2018	39	ETR
LERAN	Sophie	01/09/2011	19/12/2014	38	CDO/CJS INRA
POITOUT	Arthur	01/10/2014	17/11/2017	37	CDO/CJS INRA
SAFI	Alaeddine	01/02/2015	08/03/2018	36	CDO/ANR

Defended PhDs

4 defended PhDs

Family Name	First name	Date of defense
KISKO	Mushtak	2018
LERAN	Sophie	2014
POITOUT	Arthur	2017
SAFI	Alaeddine	2018

Mean PhD duration

Mean duration: 35 months

Internships (M1, M2)

Master 1: 7

Master 2: 6

Family Name	First Name	University Degree	Period	
CHABANISs	Myriam	Master 1	03/03/2014	06/08/2014
PIERRE	Mathieu	Master 1	23/04/2014	23/07/2014
KELENJERIDZE	Eka	Master 1	27/02/2017	31/03/2017
NGUYEN	Hong Anh	Master 1	27/02/2017	31/03/2017
TETON	Guilhem	Master 1	27/02/2017	31/03/2017
THOMAS	Claire	Master 1	27/02/2017	31/03/2017
SKENAZI	Birgit	Master 1	05/03/2018	29/06/2018
SLAWINSKI	Lucie	Master 2	06/01/2014	30/06/2014
SOIDROUDINE	Abdou Salam	Master 2	17/02/2014	16/08/2014

PROTTO	Virginia	Master 2	21/11/2016	19/05/2017
KRYSIAK	Malgorzata	Master 2	23/01/2017	23/06/2017
EMANUEL	Amélie	Master 2	06/03/2017	30/06/2017
REBET	Kimberly	Master 2	12/06/2017	12/09/2017

People in charge for a mention or a master's degree course (total number)

Anna Medici is in charge of 2 modules in the pre-specialization Major1 - "Towards sustainable agriculture" and the Specialization "Plant Sciences" (M1 Level) at Montpellier SupAgro.

People in charge for a mention or a master's degree course with international certification (Erasmus mundus)

Integration team
Self-assessment document

Name of the team concerned by the current contract:	**Integration of Nutrient Signaling Pathways**
Name of the team concerned by the next contract (if different):	
Acronym of the current contract:	**Integration**
Acronym of the next contract (if different):	
Team leader for the current contract:	**Alain Gojon**
Team leader for the next contract:	**Alain Gojon**

RESULTS

1- Presentation of the team

Introduction

The team "Integration of Nutrient Signaling Pathways" was created in July 2015, and originates from the former team "Integration of Nutritive Functions" (under supervision by A. Gojon since 2000) that split to give birth to our new team and to the HoNuDe team. In addition, Philippe Nacry joined the Aquaporin team. We have highlighted below the projects specifically developed by our current team, and did not detail our contribution to what was conducted prior to the creation of the new teams, but continued in the HoNuDe team. Note that our team kept the responsibility of the Stable Isotopes Analysis facility (AIS).

Team's workforce and means

Since 2015, the team gathered 4 scientists (L. Bach, MdC UM; A. Gojon, DR INRA; L. Lejay-Lefebvre, CR INRA; and A. Martin, CR CNRS), and 2 technical assistants at 50% of their time (C. Fizames, IE INRA; and P. Tillard, IR INRA). A full-time technical assistant joined the team in June 2017 (J. Boucherez, TR INRA).

One post-Doc and 4 graduate students have worked or are working in the team: A. Jacquot (post-Doc 2014-2017), Fanny Bellegarde (PhD 2014-2017), Amel Maghiaoui (PhD 2017-), Valentin Chaput-Montagnac (PhD 2017-) and David Séré (PhD 2017-). Two foreign visitors were hosted: Jian-Fu Li (PhD student, Jiao Tong University, Shanghai, for 1 year in 2017-2018), and Takushi Hachiya (Assistant Professor, Nagoya University, for 4 months in 2018). In addition, the team hosted 11 L3, M1 or M2 students.

Concerning the financial means, our activity has been supported by several grants: One ANR PRC (IMANA, 2014-2018); two ANR PRCI with Germany (SIPHON, 2014-2017), and Taiwan (NitraSense, 2017-2020); One Labex AGRO grant (GENERICE, 2017-2019), one Montpellier iSITE project (eCO$_2$THREATS, 2019-2022); one grant from the BAP division of INRA (ClimNutr, 2017-2018), and one CNRS 80 PRIME grant (BREAK, 2019-2022).

Scientific policy

The aim of the team is to investigate the signaling mechanisms that control root nitrate (NO_3^-) acquisition in *Arabidopsis*. Uptake of NO_3^- in plants is regulated by signaling pathways acting either at the local level in the roots (e.g., NO_3^- sensing by root cells) or at the systemic level through shoot-to-root signal transduction (regulation by the N status of the whole plant and coordination with photosynthesis). These signaling pathways target both the membrane transporters (from the NRT1 and NRT2 families) that mediate NO_3^- influx into root cells, and the architecture of the root system that determines the area where this influx is possible.

In the past, the "Integration of Nutritive Functions" team has played a significant role in the functional characterization of NO_3^- transporter genes in Arabidopsis, and in the identification of regulatory mechanisms associated with the signaling pathways listed above. In particular, we have shown that: (i) the NRT1.1 NO_3^- transporter acts as a NO_3^- sensor regulating both root NO_3^- transport and lateral root development (Krouk et al. 2010, Gojon et al. 2011), (ii) the major high-affinity NO_3^- transporter NRT2.1 is regulated at multiple levels, from chromatin to protein (Widiez et al. 2011, Wirth et al. 2011, Laugier et al. 2012), and (iii) the control exerted by photosynthesis involves signaling from the Oxydative Pentose Phosphate Pathway (OPPP, Lejay et al. 2008). These findings structured the research project of the new "Integration of Nutrient Signaling Pathways" team. As a consequence, we stopped working on several questions previously addressed by the former team, such as the functional characterization of NO_3^- transporters, the development of systems biology approaches (that we only keep as a tool), and several aspects of hormone signaling (cytokinins, ABA) which are now part of the HoNuDe team's program. Furthermore, the new name of our team was chosen to highlight our wish to develop a more integrative approach on the signaling mechanisms. More precisely, we did this in three ways:

- Understanding the integrated role of a particular signaling pathway, through a coordinated investigation of the various functional and developmental processes it governs. This is illustrated by our work on the NRT1.1-dependent NO_3^- signaling.
- Understanding how regulatory mechanisms acting at various levels within a signaling pathway participate in the integrated control of one particular process. This is exemplified by our work on the regulation of NRT2.1 by N provision, from upstream mechanisms associated with chromatin dynamics down to posttranslational modifications of the protein.
- To understand how several distinct signaling pathways interact to give rise to an integrated response of their common molecular target. We addressed this question by investigating the mechanisms involved in the regulation of *NRT2* genes by C- and N- signaling pathways.

These three different aspects of the work are detailed in the three paragraphs of the following section.

2- Research products and activities for the team

Scientific track record

The team published 24 articles during 2014-2019 (including P. Nacry), with a weighted mean impact factor of the journals of 7.04.

Integrated regulation of root NO_3^- transporters and of lateral root development by NRT1.1-dependent NO_3^- signaling.

One main outcome of the 2009-2014 period was the characterization of the NO_3^- sensing role of the NRT1.1 NO_3^- transporter (Krouk et al. 2010), which was then proposed to act a "transceptor" (transporter-receptor, Gojon et al. 2011). NRT1.1 regulates both the expression of several hundred genes involved in N transport and metabolism, and the development of lateral roots in response to NO_3^-. This raised the question of how a single protein may trigger such different responses. Concerning root development, we found that NRT1.1 represses the emergence of lateral root primordia (LRP) at low NO_3^- availability by preventing auxin accumulation/signaling in these primordia. We proposed this is due to a NO_3^-–regulated auxin transport capacity of NRT1.1, which facilitates to the basipetal auxin transport out of the LRP. Nevertheless, this mechanism did not account for the role of NRT1.1 in regulating the expression of NO_3^-–responsive genes, including the *NRT2.1* transporter gene.

Using a set of transgenic plants expressing point mutants of NRT1.1, we demonstrated that regulation of LRP development and regulation of *NRT2.1* expression partly rely on separate signaling mechanisms, because point mutations in the T101 residue, a phosphorylation site of NRT1.1, could suppress one of these responses without affecting the other (Bouguyon et al. 2015). For instance, regulation of LRP development appeared to be a specific function of the phosphorylated form of NRT1.1, whereas the induction of *NRT2.1* by NO_3^- is controlled by the non-phosphorylated form. This suggests that NRT1.1 acts as a versatile NO_3^- sensor, with different forms of the protein triggering different signaling pathways. Further investigation unraveled a surprising posttranscriptional regulation of this protein. Although the *NRT1.1* gene is induced by NO_3^- in all root tissues, the expression of the protein is repressed by NO_3^- in LRP, but not in cortex or epidermis (Bouguyon et al 2016). Together with the negative effect of NO_3^- on auxin transport by NRT1.1, its repression by NO_3^- in LRP relieves the inhibitory effect on the development of these LRP, thereby explaining the stimulation of lateral root growth by NO_3^-. Recent results in collaboration with Dr. Eva Benkova (IST Vienna, Austria) indicate that NRT1.1 not only controls local auxin accumulation/signaling in LRP, but also auxin biosynthesis by the TAR2 enzyme in the root stele, and expression of the LAX3 auxin transporter in the tissues overlying the LRP (Maghiaoui et al., submitted). TAR2 mediates auxin biosynthesis in the primary root tissues for auxin supply to the LRP, and LAX3 is required for loosening primary root tissues in front of the LRP to favor its emergence. This shows that NRT1.1 controls a wide range of processes involved at various levels in LRP development. Finally, we were able to extend the range of NO_3^- transporters regulated by NRT1.1. Indeed, NRT2.4 and NRT2.5, contributing with NRT2.1 to the high-affinity NO_3^- uptake are also regulated by NRT1.1. This is especially significant because, at the opposite of NRT2.1 that is also under control by systemic signaling of the whole plant N status, NRT2.4 and NRT2.5 are predominantly regulated by the local external NO_3^- concentration through NRT1.1 (Chaput-Montagnac et al., in preparation).

Transcriptional and posttranscriptional regulation of root high-affinity NO_3^- transporters by N signaling: from epigenetic mechanisms to posttranslational modifications.

Previously, we showed that the chromatin factor HNI9 is involved in the repression of *NRT2.1* under high N supply (Widiez et al. 2011). This was associated with the enrichment of H3K27me3 at the *NRT2.1* locus, a chromatin modification associated with transcriptional repression, catalyzed by the Polycomb Repressive Complex 2 (PRC2). Therefore, we hypothesized that HNI9 triggers repression of *NRT2.1* through H3K27me3 deposition. However, we invalidated this hypothesis by analyzing mutants for CLF and SWN, which are the main H3K27 trimethyl-transferases of PRC2. Indeed, under high N condition, a decrease (in *clf* mutant) or the absence (in *clf swn* double mutant) of H3K27me3 at *NRT2.1* does not lead to its upregulation. However, we observed that *NRT2.1* is also labelled by H3K27me3 under low NO_3^- supply, a strongly inductive condition for *NRT2.1* under which HNI9 plays no role. Surprisingly, *NRT2.1* expression at low NO_3^- is higher in *clf* mutant than in WT. This led us to postulate an unexpected role for PRC2 in the modulation of *NRT2.1* expression under highly permissive conditions. From this point, the project evolved in two directions: i) characterizing the regulation of *NRT2.1* expression by PRC2, and ii) elucidating the mechanism of the HNI9-dependent repression of *NRT2.1*.

Concerning PRC2, we first demonstrated that CLF binds at the *NRT2.1* locus under low NO_3^- condition. We then performed tissue-specific analysis of H3K27me3 enrichment to show that *NRT2.1* is indeed targeted by H3K27me3 in cortex cells, where it is highly expressed. Finally, we used a *ProNRT2.1:GUS* reporter in WT and *clf* mutant to demonstrate that the high expression of *NRT2.1* in *clf* is due to its upregulation in the tissues where it is normally expressed (cortex and epidermis). Altogether, these results pointed an unexpected function of PRC2 in the modulation of one of the most highly expressed gene in the root transcriptome (Bellegarde et al. 2018). We thereafter observed that *NRT2.1* is also repressed by members of PRC1, such as LHP1. Accordingly, *NRT2.1* expression is upregulated in *lhp1* as in *clf* mutants, but strikingly, this expression is extremely low in a *clf lhp1* double mutant under low NO_3^- supply, suggesting that the concomitant release of repression by CLF and LHP1 leads to drastic silencing of *NRT2.1*. This hypothesis is under analysis in the frame of the PhD program of David Sere (2017-2020).

To examine the role of HNI9 in the response to high N provision, we compared the transcriptomic data of WT and *hni9-1* mutant. Because HNI9 was reported to be a transcriptional activator, we selected genes that were induced by high N in WT, but not in the *hni9-1* mutant. We obtained a list of 108 genes, into which biological functions related to redox processes were the most significantly represented. This observation led us to show that HNI9 is involved in preventing disruption of ROS homeostasis by high N provision. Indeed, high N provision leads to ROS production and *HNI9* mutation leads to higher ROS levels and to ROS-dependent phenotypes because HNI9 is required for activating a set of genes involved in cellular detoxification. Because ROS induce *NRT2.1* expression, this explained how HNI9 indirectly represses *NRT2.1* at high N supply. Furthermore, from the promoter sequence analysis on the 108 genes, we identified a role for the transcription factor HY5 in the induction of detoxification genes in response to high N. Therefore, we demonstrated how a detoxification program is induced by chromatin and transcription factors in response to high N supply (Bellegarde et al. 2019). This response is required to maintain plant redox status under physiological conditions, and lend support to the existence of an optimum balance between N nutrition and ROS homeostasis.

In addition to transcriptional control, we previously showed that post-translational regulation of NRT2.1 also plays an important role in modulating root NO_3^- uptake, and that one mechanism could correspond to NRT2.1 C-ter processing (Wirth et al. 2007; Laugier et al. 2012). To continue this work we performed a phospho-proteomic approach to determine NRT2.1 phosphorylation sites and we produced transgenic plants expressing truncated forms of NRT2.1 at C-ter. The combination of these approaches led us to highlight the importance of the NRT2.1 sequence located between residues 494-513. First, successive deletions in Cter showed that this sequence is essential for root high-affinity NO_3^- uptake. Second, this sequence contains one phosphorylation site at S501. Transgenic lines expressing point S501 mutants revealed that mimicking phosphorylation of S501 (S501D substitution) inactivates NRT2.1 function like the truncation of the 494-513 sequence. This could neither be explained by changes in the protein abundance of NRT2.1 or NAR2.1 (a partner protein required for NRT2.1 transport activity), nor by a lack of interaction between these two proteins. Furthermore, the relative phosphorylation of NRT2.1 at S501 decreases in response to a NO_3^- induction treatment, and correlates with the increase in root NO_3^- uptake. Collectively, these data led us to propose a model in which NO_3^- stimulates NRT2.1 synthesis and represses S501 phosphorylation, leading to the activation of a protein complex composed of NAR2.1 and NRT2.1 unphosphorylated form, maybe associated with other unknown proteins. This work was performed in collaboration with Prof. Waltraud Schulze (univ. Hohenheim, Germany) and is submitted for publication (Jacquot et al. 2019, http://biorxiv.org/cgi/content/short/583542v1).

Transcriptional regulation of root high-affinity NO_3^- transporters by C signaling and interaction with N signaling

Expression of *NRT2.1* is not only regulated by N supply, but also by photosynthesis to ensure the integration of both N acquisition by the roots and C acquisition by the shoot. Previous results of the team showed that *NRT2.1* upregulation by photosynthesis involves C signaling in the OPPP (Lejay et al. 2008). In roots, this pathway produces reducing power and is involved in resistance to oxidative stress. Therefore, this led to the hypothesis that *NRT2.1* regulation could be linked to the redox status of the root through a signaling pathway linked to OPPP. In this context we asked two questions: (i) Is *NRT2.1* regulated by the redox status ?, and (ii) which step of the OPPP is involved in the regulation of *NRT2.1*?

To answer the first question we studied the effects of menadione and ascorbate (oxidant and anti-oxidant, respectively). Menadione increases the induction of *NRT2.1* in response to light and sucrose, while ascorbate represses it. Furthermore, the induction of *NRT2.1* by light and sucrose is increased in the *vtc2* mutant, which is impaired in ascorbate production. This suggests that the upregulation of *NRT2.1* by photosynthates is mediated by changes in redox status of the roots through the production of reducing power. This made an interesting parallel with the work done on HNI9 (see above). Therefore, part of these results was included in the Bellegarde et al. (2019) paper.

To determine which step of the OPPP is involved in the regulation of *NRT2.1* we used a mutant under-expressing *PGL3*, encoding the second enzyme of the OPPP. Induction of *NRT2.1* by sucrose and light is not impaired in this mutant and can even be stronger than in the WT. It suggests that the signaling mechanism depends on the first step of the OPPP, catalyzed by the glucose-6-phosphate dehydrogenase (G6PDH). In roots, 3 isoforms of G6PDH are expressed, G6PD2 and G6PD3 in plastids and G6PD6 in the cytosol. KO mutants for *G6PD3* and *G6PD6* do not seem to be affected in the regulation of *NRT2.1*. KO mutants for *G6PD2* could not be obtained, suggesting lethality of this mutation. To circumvent this problem, we started the production of transgenic plants expressing artificial miRNA under the control of an estradiol inducible promoter. This will allow us to obtain conditional KO mutants for *G6PD2*, *G6PD3*, *G6PD6* and both *G6PD2/G6PD3*. This work is done in collaboration with Yves Gibon in Bordeaux to characterize the mutants at the metabolic level.

C and N signaling pathways strongly interact to regulate *NRT2.1* expression. We showed that this interaction results in emerging properties that cannot be predicted by the simple combination of the known effects of these signaling pathways. Therefore, we conducted a systems biology approach to identify new regulatory elements involved in the regulation of *NRT2* genes in response to combined C and N treatments. A series of trancriptomic experiments allowed us to identify a gene regulatory network of 26 genes, co-regulated and predicted to have a functional link with *NRT2.1*. Among these, three transcription factor genes, *TGA3*, *MYC1* and *BHLH093* were predicted to impact directly the expression of *NRT2.1*, *NRT2.4* and *NRT2.5*, and we characterized their role using KO mutants. We found BHLH093 is involved mainly in the upregulation of *NRT2.4* by light, while MYC1 and TGA3 contribute to the upregulation of *NRT2.4* and *NRT2.5* in response to N starvation. This work is in collaboration with Prof. Rodrigo Gutierrez (Univ. Santiago, Chile) and we are currently performing ChIP experiments combined with Y1H experiments to validate the predicted interaction of these transcription factors with *NRT2* promoters. A manuscript will be submitted in May/June 2019.

References not listed in Annex 4:

Gojon et al. 2011 Nitrate transceptor(s) in plants. J Exp Bot 62: 2299-2308.

Krouk et al. 2010 Nitrate-regulated auxin transport by NRT1.1 defines a mechanism for nutrient sensing in plants. Dev Cell 18: 927-937

Laugier et al. 2012 Regulation of high-affinity nitrate uptake in roots of Arabidopsis depends predominantly on posttranscriptional control of the NRT2.1/NAR2.1 transport system. Plant Physiol 158: 1067-1078

Lejay et al. 2008 Oxidative pentose phosphate pathway-dependent sugar sensing as a mechanism for regulation of root ion transporters by photosynthesis. Plant Physiol 146: 2036-2053

Widiez et al. 2011 HIGH-NITROGEN-INSENSITIVE 9 (HNI9)-mediated systemic repression of root NO3- uptake is associated with changes in histone methylation. Proc Natl Acad Sci USA 108: 13329-13334.

Wirth et al. 2007 Regulation of root nitrate uptake at the NRT2.1 protein level in Arabidopsis thaliana. J Biol Chem 282: 23541-23552

Key events

- Organization of the EMBO Conference "NITROGEN2016" (https://colloque.inra.fr/nitrogen2016), which was the Third International Symposium on the Nitrogen Nutrition of Plants, following NITROGEN2010 in Japan and NITROGEN2013 in Chile. The Conference was also supported by the NSF, and gathered 197 attendees from 22 countries.

- The key role of NRT1.1 as a nitrate transceptor has been extensively documented through five highly complementary publications (Mounier et al. 2014 PCE, Bouguyon et al. 2015 Nat Plants, Léran et al. 2015 Sci. Signaling, Medici et al. 2015 Nat Commun, Bouguyon et al. 2016 Plant Physiol). These publications are shared with the HoNuDe team as they resulted from the work initiated in the former "Integration of Nutritive Functions" team.

- A new permanent CNRS scientist (Sandra Cortijo) has been recruited and will join our team at the end of 2019.

FIVE-YEAR PROJECT AND STRATEGY

1- SWOT analysis

Strengths - Complementarity of expertise and knowledge - Regular funding acquisition - Renewal of scientific projects and leaders assured	**Weaknesses** - Scientific valorization not completed yet
Opportunities - Strong national and international collaborative network - Close links with the University/Regular intervention in Master classes	**Threats** - Highly competitive field - Transition of team leader in the coming years - Slight decrease in technical support to come

2- Structure, workforce and scientific orientations

The Integration team wishes to maintain its overall structure for the next 2021-2025 contract. However, to do so, the replacement of A. Gojon as the team leader will have to occur during the first half of the contract because A. Gojon is planning to retire in 2023 at latest. A. Martin has expressed his interest to become the next team leader. The other changes in the team workforce will be the recruitment of S. Cortijo (CR CNRS) in 2019, and the retirement of P. Tillard in 2020.

The research project of the Integration team is structured into 2 main axes: 1. NO_3^- sensing and signaling: from chromatin to protein, 2. Regulation of NO_3^- transport by photosynthesis (Interconnection of C and N signaling). Inside these 2 axes, complementary projects are leaded by each scientist of the team, with the aim of providing a comprehensive and integrated view of a common scientific question.

1. Nitrate sensing and signaling: from chromatin to protein

The objective of this part is to identify key regulatory mechanisms controlling regulation of root NO_3^- transporters and of root system architecture in response to NO_3^-. This will be done at different levels of regulation, from chromatin-based mechanisms to post-translational protein modifications.

Epigenetic mechanisms in the control of NO_3^--regulated genes (A. MARTIN).

Our analysis of the epigenetic control of *NRT2.1* expression leads to the conclusion that chromatin-based mechanisms significantly contribute to the regulation of NO_3^--responsive genes. We found that PRC complexes are important regulators of *NRT2.1* and of genes highly expressed under low NO_3^- supply and, more recently, that chromatin remodelers are involved in the dynamic response of NO_3^- starvation genes. We now aim at providing a global view of the epigenetic mechanisms controlling NO_3^--responsive genes, using a combination of two main approaches:

(i) Targeted approach on already identified genes. This will first encompass the investigation of how PRC complexes modulate the expression of different alleles of *NRT2.1* and other NO_3^--responsive genes (*NRT1.1*, *NIA1*, etc.) in endoreplicated cells in order to fine-tune their expression (co-existence of both repressive and permissive chromatin modifications in the same cell?). In addition, we will investigate the relationships between known transcription factors involved in the response to NO_3^- (NLPs, HRS1, LBD37, LBD38 and LBD39) and chromatin remodeling factors. More precisely, we will study how TFs can physically interact with chromatin remodelers and specify their target loci, or can participate in chromatin-based processes of the induction of NO_3^--responsive genes (e.g., formation of chromatin loops).
(ii) In parallel to these hypothesis-driven approaches, we will identify epigenomic and transcriptomic regulatory circuits in the root in response to NO_3^- starvation. To do this, we aim at performing an ambitious and highly resolutive systems biology approach, combining dynamics cell-type specific epigenomics and transcriptomics. We will perform ATAC-seq (identifying open chromatin regions and TFs occupancy inside them) and RNA-seq using an Arabidopsis line where root cortex nuclei are specifically labelled by GFP, and can be isolated by FACS. Analysis of these genomic datasets, and most importantly inference of regulatory circuits, will be done in collaboration with Dr. Sophie Lebre, mathematician from the Alexander Grothendieck Institute in Montpellier.

These projects concerning the epigenetic mechanisms involved in the control of NO_3^--regulated genes are part of an ANR project submitted in 2019 (ongoing evaluation) in collaboration with F. Pontvianne (LGDP Perpignan) and M. Ingouff (DIADE Montpellier), and of an 80 PRIME project funded by CNRS from 2019 to 2022 (in collaboration with S. Lebre, IMAG Montpellier, and J. Chamieh, IBMM Montpellier), which will include a PhD fellowship from October 2019.

Regulation of NO_3^--responsive genes at the inter-individual level (S. CORTIJO)

A recent report showed that *NRT2.1* expression level can display significant variations between genetically identical individuals, under precisely controlled environment. This inter-individual variation in gene expression may have significant value for plant adaptation in the wild because it may differently prime separate plants to a subsequent change in NO_3^- provision. Sandra Cortijo, a CNRS researcher newly recruited in the team (end of 2019), will investigate the mechanisms leading to the inter-individual expression variability of genes involved in N nutrition in Arabidopsis, as well as the consequences for plant physiology. In particular, we will question (i) whether *NRT2.1* inter-individual expression variability leads to inter-individual variations in root NO_3^- uptake, (ii) if TFs and chromatin-based mechanisms can determine *NRT2.1* and genome-wide inter-individual expression variability, and (iii) if inter-individual expression variability can be explained by cell to cell variability inside individuals.

NRT2.1 regulation at the protein level and role in root NO_3^- sensing (L. LEJAY)

The results concerning the regulation of NRT2.1 at the protein level raise two fundamental questions: (i) What are the mechanisms linked to the phosphorylation of S501 and leading to NRT2.1 inactivation? and (ii) What is the role of NRT2.1 in root NO_3^- sensing and is it linked to NRT2.1 post-translational modifications? To investigate the first point we will combine different biochemical approach with mass spectrometry to identify NRT2.1 protein partners and the protein kinase responsible for S501 phosphorylation. This will be followed by a reverse genetic approach to characterise the functional impact of the proteins identified. The second point is based on preliminary data indicating that NRT2.1 is rapidly cleaved at C-ter after provision of NO_3^-. This leads to the release in the soluble protein fraction of a peptide phosphorylated at S501. Since previous results obtained in our team and by others suggest a role for NRT2.1 in NO_3^- sensing we want to explore the hypothesis that the processing of the C-ter part of NRT2.1 could correspond to a NO_3^- signaling mechanism. To do this, the size, regulation and localisation of NRT2.1 C-ter cleaved peptide will be further characterized. Thereafter, we will determine if this peptide can regulate the expression of NO_3^-–responsive genes, using synthetic versions or transgenic plants. This work is part of an ANR project submitted in collaboration with Waltraud Schulze in Germany.

Regulation of root growth and development by NO_3^- and cell cycle (L. BACH, A. GOJON).

Balance between cell proliferation and cell expansion is important for shaping root system architecture (RSA). In particular, nutrient availability can be perceived at different cell cycle stages to determine whether cells must divide, expand or differentiate. Although crosstalk between NO_3^- sensing/signaling and hormones such as auxin have been reported to act on RSA, the cellular and molecular mechanisms underlying these pathways remain unclear. The first aim of the project is to further investigate whether NO_3^- acts via cell-cycle to modulate/shape primary root tip and LR development. This will be done by characterizing the regulation of different cell cycle Arabidopsis regulators such as CDK (cyclin dependent kinases), cyclins or CKI (cyclin kinase inhibitors, known to switch between cell cycle and endocycle). Using a collection of reporter lines for these regulators, we will investigate their transcription under various NO_3^- conditions. For some of these regulators, we will further characterize the root system architecture of their KO mutants using an available root phenotyping platform. Then, to test whether NO_3^- impacts the different cell cycle transitions in primary root and lateral roots, a dual-color marker for *in vivo* visualization of cell cycle progression will be introduced in a set of mutants for NO_3^- transporters or regulators, and the resulting lines will be analyzed under an extended range of NO_3^- concentrations. In addition, we will pay particular attention to the LRPs, because we have demonstrated that under severe NO_3^- deficiency, the development of most of the LRPs is blocked at stage IV that corresponds to the breakout of Casperian strip in endodermis tissue and to the initiation of the LRP meristem. In addition to that, mutants for cell wall remodeling will be crossed with *nrt1.1* mutant to decipher whether NO_3^- can regulate the looseness of tissue surrounding LRP in a NRT1.1 dependent manner. This project will be the basis of a Young Researcher ANR proposal in 2019.

2. Regulation of NO_3^- transport by photosynthesis. Interconnection of C and N signaling

Investigating the coordinated regulation of root NO_3^- uptake by N provision and photosynthesis is of special interest for understanding how the N nutrition of plants will respond to the elevation of atmospheric CO_2. In this context our goal is to decipher the mechanisms involved in the regulation of NO_3^- transport by photosynthesis and elevated CO_2.

Regulation of root NO_3^- uptake by photosynthesis (L. LEJAY)

Following the results on the role of OPPP in the upregulation of *NRT2.1* expression by photosynthesis, the first goal is to confirm that the signalling mechanism is linked to the first step of the OPPP, using plants of Arabidopsis expressing inducible miRNAs for the three isoforms of G6PDH. This will be followed by transcriptomic and metabolomic analyses to decipher the signalling pathway linked to OPPP and to characterize its impact on C and N metabolism, the production of NADPH and the redox status of the plant. Since we also identified the transcription factor BHLH093, as involved in the regulation of NRT2.1 and NRT2.4 by photosynthesis, we will perform similar analysis on KO mutants and 35S overexpressors to define its role and position in the signalling pathway. This work is planned to be part of an ANR project in collaboration with Yves Gibon in Bordeaux for metabolomics. Furthermore, because oxidative stress seems to be an important player in the regulation of N metabolism by C and N signalling, this work will also be part of a larger project, in collaboration with Yves Gibon and Rodrigo Gutierrez in Chile, to determine the implication of such signalling pathways for plants adaptation to extreme environment in the context of the characterisation of plants growing in the Atacama Desert.

Regulation of N nutrition and signaling by elevated CO_2 concentration (A. MARTIN, A. GOJON)

Several reports have demonstrated recently that elevated atmospheric CO_2 concentrations (eCO_2) that are expected in the middle of the century (*i.e.*, 550-700 ppm), will lead in most C3 species to an alteration of plant nutritional status, in particular for N. This is in total contrast to what has been observed for short-term CO_2 treatments, which leads to an increase of NO_3^- uptake and assimilation. On this aspect, the plants response to eCO_2 is therefore not understood, although it represents a major socio-economic issue with repercussions in agronomy and human health. We thus aim at investigating the causes of the negative effect of eCO_2 on Arabidopsis N status. To do this, we will perform (i) a gene regulatory network approach, combining ATAC-seq and RNA-seq in order to identify regulatory modules involved in the response to eCO_2, and (ii) a combination of physiological, developmental phenotyping and GWAs to identify genes at the basis of the plant response to eCO_2. These approaches are part of an ANR project submitted in 2019 in collaboration with M. Hodges (IPS2 Saclay), and of a Montpellier I-SITE MUSE funded project (2019-2022).

Annex 4 – Research products and activities

EVALUATION CAMPAIGN 2019-2020

GROUP A

Name of the team:	**Integration of Nutrient Signaling Pathways**
Acronym:	**INTEGRATION**
Theme leader for the current contract:	**Alain Gojon**
Theme leader for the next contract:	**Alain Gojon**

I - PRODUCTION OF KNOWLEDGE AND ACTIVITIES CONTRIBUTING TO THE INFLUENCE AND SCIENTIFIC ATTRACTIVENESS OF THE TEAM

1- Articles

Scientific articles

Most significant articles (20%)

1. Alvarez J. M., Riveras E., Vidal E. A., Gras D. E., Contreras-Lopez O., Tamayo K. P., Aceituno F., Gomez I., Ruffel S., Lejay L., Jordana X., Gutierrez R. A. (2014) Systems approach identifies TGA1 and TGA4 transcription factors as important regulatory components of the nitrate response of Arabidopsis thaliana roots. Plant J. 80(1):1-13.

2. **Bouguyon E., Brun F., Kubeš M., Meynard D., Pervent M., Léran S., Lacombe B., Krouk G., Guiderdoni E., Zažímalová E., Hoyerová K., Nacry P., Gojon A. (2015) Multiple mechanisms of nitrate sensing by Arabidopsis nitrate transceptor NRT1.1. . Nat. Plants. 1 : 15015.**

3. **Bellegarde F., Herbert L., Séré D., Caillieux E., Boucherez J., Fizames C., Roudier F., Gojon A., Martin A. (2018) Polycomb Repressive Complex 2 attenuates the very high expression of the Arabidopsis gene NRT2.1. Sci. Rep. 8: 7905.**

2014

4. De Jong F., Thodey K., Lejay L.V., Bevan M.W. (2014) Glucose elevates NRT2.1 protein levels and nitrate transport activity independently of its HXK1-mediated stimulation of NRT2.1 expression. Plant physiol 164(1): 308-320.

5. Guan P., Wang R., Nacry P., Breton G., Kay S. A., Pruneda-Paz J. L., Davani A., Crawford N. M. (2014) Nitrate foraging by Arabidopsis roots is mediated by the transcription factor TCP20 through the systemic signaling pathway. Proc. Natl. Acad. Sci. USA 111(42): 15267-15272.

6. **Mounier E., Pervent M., Ljung K., Gojon A., Nacry P. (2014) Auxin-mediated nitrate signalling by NRT1.1 participates in the adaptive response of Arabidopsis root architecture to the spatial heterogeneity of nitrate availability. Plant Cell Environ. 37: 162-174.**

2015

7. Krouk G., Carré C., Fizames C., Gojon A., Ruffel S., Lacombe B. (2015) GeneCloud reveals semantic enrichment in lists of gene descriptions. Mol Plant. 8(6): 971-973.

8. Léran S, K.H. Edel, Pervent M., Hashimoto K., Corratge-Faillie C., Offenborn J.N., Tillard P., Gojon A., Kudla J., Lacombe B. (2015) Nitrate sensing and uptake in Arabidopsis are enhanced by ABI2, a phosphatase inactivated by the stress hormone abscisic acid. Sci Signaling. 8 (375): ra43.

9. Léran S., Garg B., Boursiac Y., Corratge-Faillie C., Brachet C., Tillard P., Gojon A., Lacombe B. (2015) AtNPF5.5, a nitrate transporter affecting nitrogen accumulation in Arabidopsis embryo. Sci Rep. 5 : 7962.

10. Medici A., Marshall-Colon A., Ronzier E., Szponarski W., Wang R., Gojon A., Crawford N.M., Ruffel S., Coruzzi G.M., Krouk G. (2015) AtNIGT1/HRS1 integrates nitrate and phosphate signals at the Arabidopsis root tip. Nat Commun. . 6: 6274.

2016

11. **Bouguyon E., Perrine-Walker F., Pervent M., Rochette J., Cuesta C., Benkova E., Martiniere A., Bach L., Krouk G., Gojon A., Nacry P. (2016) Nitrate Controls Root Development through Post-Transcriptional Regulation of the NRT1.1/NPF6.3 transporter/sensor. Plant physiol 172(2): 1237-1248.**

12. Li G., Tillard P., Gojon A., Maurel C. (2016)Dual regulation of root hydraulic conductivity and plasma membrane aquaporins by plant nitrate accumulation and high-affinity nitrate transporter NRT2.1 . Plant Cell Physiol. 57(4): 733-742.

2017

13. Merret R., Carpentier M.C., Favory J.J., Picart C., Descombin J., Bousquet-Antonelli C., Tillard P., Lejay L., Deragon J.M., Charng Y.y. (2017) Heat Shock Protein HSP101 Affects the Release of Ribosomal Protein mRNAs for Recovery after Heat Shock. Plant Physiol. 174 (2): 1216-1225.

2018

14. Dubois M., Selden K., Bediée A., Rolland G., Baumberger N., Noir S., Bach L., Lamy G., Granier C., Genschik P. (2018) SIAMESE-RELATED1 is regulated post-translationally and participates in repression of leaf growth under moderate drought. Plant Physiol. 176(4): 2834-2850.

2019

15. **Bellegarde F., Maghiaoui A., Boucherez J., Krouk G., Lejay L., Bach L., Gojon Al., Martin A. (2019) The Chromatin Factor HNI9 and ELONGATED HYPOCOTYL 5 Maintain ROS Homeostasis under High Nitrogen Provision. Plant physiol. 180(1): 582-592.**

Review articles

Most significant articles (20%)

1. **Bellegarde F., Gojon A., Martin A. (2017) Signals and players in the transcriptional regulation of root responses by local and systemic N signaling in Arabidopsis thaliana. J Exp Bot. 68(10): 2553-2565. (review)**

2. **Jacquot A., Li Z., Gojon A., Schulze W., Lejay L. (2017) Post-translational regulation of nitrogen transporters in plants and microorganisms. J. Exp. Bot. 68(10): 2568-2580. (review)**

2014

3. Genschik P., Marrocco K., Bach L., Noir S. ,Criqui M.C. (2014) Selective protein degradation: a rheostat to modulate cell-cycle phase transitions. J. Exp. Bot.65(10): 2603-2615. (review)

4. Léran S., Varala K., Boyer J.C., Chiurazzi M., Crawford N.M., Daniel-Vedele F., David L., Dickstein R., Fernandez E., Forde B.G., Gassmann W., Geiger D., Gojon A., Gong J.M., Halkier B.A., Harris J.M., Hedrich R., Limami A.N., Rentsch D., Seo M., Tsay Y.F., Zhang M., Coruzzi G.M., Lacombe B. (2014) A unified nomenclature of NITRATE TRANSPORTER 1/PEPTIDE TRANSPORTER family members in plants. Trends Plant Sci. 19(1):5-9. (opinion, review)

5. **Ruffel S., Gojon A., Lejay L. (2014) Signal interactions in the regulation of root nitrate uptake. J Exp Bot. 65(19):5509-55177. (review)**

2015

6. Bhardwaj D., Medici A., Gojon A., Lacombe B., Tuteja N. (2015) A new insight into root responses to external cues: paradigm shift in nutrient sensing. Plant Signal Behav. 10(12): e1049791. (review)

2016

7. O'Brien J.A., Vega A., Bouguyon E., Krouk G., Gojon A., Coruzzi G., Gutiérrez R.A. (2016) Nitrate transport, sensing and responses in plants. Mol. Plant 9(6): 837-856. (review)

2017

8. **Gojon A. (2017) Nitrogen nutrition in plants: rapid progress and new challenges. J. Exp. Bot. 68 (10): 2457-2462. (editorial)**

9. **Ruffel S., Gojon A. (2017) Systemic nutrient signalling: On the road for nitrate. Nat. Plants. 3 (4): 17040.7 (comment)**

Other articles (professional journals, etc.) (total number)

2018

1. **Bellegarde F., Maghiaoui A., Boucherez J., Krouk G., Lejay L., Bach L., Gojon A., Martin A. (2018) HNI9 and HY5 maintain ROS homeostasis under high nitrogen provision in Arabidopsis. BioRXiv.**
2. Noguero M., Leran S., Bouguyon E., Brachet C., Tillard P., Nacry P., Gojon A., Krouk G., Lacombe B. (2018) Revisiting the functional properties of NPF6.3/NRT1.1/CHL1 in xenopus oocytes; . BioRxiv.

2019

3. **Jacquot A., Chaput V., Mauries A., Li Z., Tillard P., Fizames C., Bonillo P., Bellegarde F., Laugier E., Santoni V., Hem S., Martin A., Gojon A., Schulze W., Lejay L. NRT2.1 phosphorylation prevents root high-affinity nitrate uptake activity in** *Arabidopsis thaliana*. **BioRXiv**

2- Books

Monographs, critical editions, translations (total number)

Management and coordination of scientific books / Scientific book edition

Book chapters (total number)

Book chapters in English or another foreign language

2016

1. Azouaoui H., Montigny C., Jacquot A., Barry R., Champeil P., Lenoir G. (2016) (Chapter 6):Coordinated Overexpression in Yeast of a P4-ATPase and Its Associated Cdc50 Subunit: The Case of the Drs2p/Cdc50p Lipid Flippase Complex. *In : P-Type ATPases, Methods and Protocols - Springer New York.* Methods Mol Biol 1377 : 37-55.

2018

2. Lejay L., Gojon A. (2018) Chapter Six. Root Nitrate Uptake. In Adv. Bot. Res. 87:139-169.

Edited theses

3- Production in conferences / congresses and research seminars

Articles published in conference proceedings / congress

Other products presented in symposia / congress and research seminars

Invited oral presentations in international symposia/congress

2014

1. Gojon A. The Arabidopsis transceptor NRT1.1/NPF6.3 triggers independent responses to nitrate through multiple sensing/transduction mechanisms. Workshop "Roots of sustainable agriculture: from concepts to practice", October 11-15 2014. Zhejiang University, Hangzhou, China.

2015

2. Gojon A. Transceptor-mediated nitrate signaling in plants. International Plant Molecular Biology Congress, October 25-30 2015, Iguazu, Brazil.

3. Gojon A. The role of NRT1.1 as a nitrate transceptor in Arabidopsis. First trilateral CRAG-INRA-UPSC Workshop. October 6-8, 2015. Nancy, France.

2016

4. Ruffel S., Jacquot A., Fizames C., Shasha D., Tillard P., Santoni V., Schulze W., Gojon A., Gutierrez R., Lejay L. (2016) Regulation of root nitrate uptake by carbon and nitrogen using NRT2.1 as a target. (22-26 Août 2016) International Congress "Nitrogen 2016" EMBO Conference: The Nitrogen Nutrition of Plant. Montpellier, France.

2018

5. Gojon A. Nitrate signaling mechanisms in Arabidopsis roots. International Symposium on Root Development and Nutrients/Water absorption. November 26-30, 2018. Zhejiang University, Hangzhou, China.

6. Gojon A., Tsay YF. Integration of physiological and developmental responses of the plant to nitrate. France-Taiwan Scientific festival. September 13-14, 2018. Taipei, Taiwan.

2019

7. Gojon A. Nitrate signaling mechanisms in Arabidopsis roots. International Symposium on "Plants Responses to Abiotic Stresses and Environmental Signals". June 14-15, 2019. China Agricultural University, Beijing, China.

8. Lejay L. (2019) Response of nitrogen nutrition to challenging environment in Arabidopsis. EMBO Conference Integrative Biology: From molecules to ecosystems in extreme environments. 22-25 April 2019, Santiago, Chile.

Invited oral presentations in national symposia/congress

Selected oral presentations in international symposia/congress

2015

1. Bellegarde F., Gojon A., Martin A. CHROMATIN AND NUTRITION: PRC2-MEDIATED REGULATION OF THE ROOT NITRATE TRANSPORTER GENE NRT2.1. The 26th International Congress on Arabidopsis Research (5-9 July 2015) Paris, France.

2. Bellegarde F., Gojon A., Martin A. PRC2-MEDIATED CHROMATIN CONTROL OF ADAPTATION TO NUTRITIONAL ENVIRONMENT IN ARABIDOPSIS. European Workshop on Plant Chromatin 2015 (24-27 June 2015) Upsala, Sweden.
3. Lejay L. Regulation of root NO3- uptake in Arabidopsis thaliana using NRT2.1 as a target. (2015, July 05-09) 26th International Conference on Arabidopsis Research. Paris (France).

2016

4. Bellegarde F, Gojon A., Martin A. (2016) Polycomb repressive complex2 modulates *NRT2.1* expression under nitrogen limiting conditions. (22-26 Août 2016) International Congress "Nitrogen 2016" EMBO Conference: The Nitrogen Nutrition of Plant. Montpellier (France).

Selected oral presentations in national symposia/congress

2017

1. Bellegarde F., Herbert L., Alcon C., Caillieux E., Roudier F., Gojon A., Martin A. A non-canonical role for Polycomb Repressive Complex 2 in the modulation of gene expression. French Loop of Chromatin (15-16 May 2017) Saint-Beauzille de Putois, France.

2. Bellegarde F., Herbert L., Alcon C., Caillieux E., Roudier F., Gojon A., Martin A. A non-canonical role for Polycomb Repressive Complex 2 in the modulation of gene expression. Plant Epigenetics Meeting (22-23 June 2017) Gif sur Yvette, France.

Posters in international symposia/congress

2014

1. Jacquot A., Mauries A., Laugier E., Hem S., Rofidal V., Tillard P., Santoni V., Schulze W., Gojon A., Lejay L. (2015) Post-translational regulation of the root nitrate uptake transporter NRT2.1 in *Arabidopsis thaliana*. (5-9 July 2015) 26th International Conference on Arabidopsis Research. Paris (France).

2016

2. Bellegarde F., Bach L., Nacry P., Gojon A. and Martin A. Regulation of root architecture by the nitrate transporter NRT2.1 (22-26 August 2016) International Congress "Nitrogen 2016" EMBO Conference: The Nitrogen Nutrition of Plant. Montpellier (France).

3. Bouguyon E., Pervent M., Perrine-Walker F., Rochette J., Krouk G., Bach L., Gojon A., Nacry P. (2016). The Arabidopsis nitrate transceptor NRT1.1/NPF6.3 that governs distinct signaling pathways is subjected to a complex post transcriptional regulation. (22-26 August 2016) International Congress "Nitrogen 2016" EMBO conference: The Nitrogen nutrition of plants. Montpellier (France)

4. Jacquot A., Mauries A., Laugier E., Hem S., Rofidal V., Tillard P., Santoni V., Schulze W., Gojon A., Lejay L. (2016) Post-translational regulation of the root nitrate uptake transporter NRT2.1 in *Arabidopsis thaliana*. (22-26 August 2016) International Congress "Nitrogen 2016" EMBO Conference: The Nitrogen Nutrition of Plant. Montpellier (France).

5. Léran S., Edel K., Pervent M., Hashimoto K., Corratgé-Faillie C., Offenborn N. J., Tillard P., Gojon A., Kudla J., Lacombe B. (2016) Nitrate sensing and uptake in *Arabidopsis* are enhanced by ABI$_{2,\ a}$ phosphatase inactived by the stress hormone abcisic acid. (22-26 August 2016) International Congress "Nitrogen 2016" EMBO Conference: The Nitrogen Nutrition of Plant. Montpellier (France).

2018

6. Chaput V., Ruffel S., Przybyla-Toscano J., Fayos Y., Moyano T. Fizames C., Tillard P., Gutierrez R., Gojon A., Lejay L. (2018) Identification of new transcription factors regulating root transporters in response to combined nitrogen/carbon treatments (5 -10 août) 12th Congress of the International Plant Molecular Biology. Montpellier (France).

7. Jacquot A., Chaput V., Mauries A., Li Z., Bonillo P., Tillard P., Fizames C., Santoni V., Gojon A., Schulze W., Lejay L. (2018) Post-translational regulation of the root nitrate uptake transporter NRT2.1 in *Arabidopsis thaliana*. (5 -10 août) 12th Congress of the International Plant Molecular Biology. Montpellier (France).

8. Jacquot A., Chaput V., Mauries A., Li Z., Bonillo P., Tillard P., Fizames C., Santoni V., Gojon A., Schulze W., Lejay L. (2018) Post-translational regulation of the root nitrate uptake transporter NRT2.1 in *Arabidopsis thaliana*. (12-14 Septembre) Plant Adapt 2018. Banyuls-sur-mer (France).

9. Séré D., Bellegarde F., Schivre G., Herbert L., Cailleux E., Boucherez J., Fizames C., Tillard P., Roudier F., Gojon A. and Martin A. Flying too close to the sun: The expression of a highly transcribed gene is safeguarded by Polycomb complexes (5 -10 août) 12th Congress of the International Plant Molecular Biology. Montpellier (France).

10. Séré D., Bellegarde F., Schivre G., Herbert L., Cailleux E., Boucherez J., Fizames C., Tillard P., Roudier F., Gojon A. and Martin A. Flying too close to the sun: The expression of a highly transcribed gene is safeguarded by Polycomb complexes (10-14th September 2018) International Plant Systems Biology meeting, Roscoff (France).

Posters in national symposia/congress

Invited seminars in foreign institutions

2014

1. Gojon A. The Arabidopsis transceptor NRT1.1/NPF6.3 triggers independent responses to nitrate through multiple sensing/transduction mechanisms. College of Resources and Environmental Sciences, Nanjing Agricultural University, China

2017

2. Gojon A. N signaling in Arabidopsis: the role of NRT1.1 as a nitrate transceptor. School of Agriculture and Biology, Shanghai Jiao Tong University

3. Gojon A. Regulators of root nitrate acquisition in Arabidopsis. College of Resources and Environmental Sciences, Nanjing Agricultural University, China

Invited seminars in French institutions

2019

1. Gojon A. Physiologie moléculaire de la nutrition azotée des plantes: quels liens entre mécanismes moléculaires et approches agronomiques ? Académie d'Agriculture de France, séance du 10 avril 2019

4- Electronic tools and products

Softwares

Databases

5- Instruments and methodology

Prototypes

Platforms and observatories

6- Other products

Theorised artistic creations, staging, movies

7- Editorial activities

Participation in editorial committees (books, collections, etc.)

Review Editor for Frontiers in Plant Science (A. Martin)

Guest Editor of a special issue of the Journal of Experimental Botany on “The Nitrogen Nutrition in Plants” (2017, A. Gojon)

Collection and series management

8- Reviewing activities

Reviewing of articles

Reviewers for Science, Nature, Nature Plants, The Plant Cell, Molecular Plant, New Phytologist, Plant Cell and Environment, Journal of Experimental Botany, Plant Physiology, Plant Biotechnology Journal

Grant evaluation (public or charities)

Reviewers for INRA BAP department, Labex SPS, National Science Center (Poland)

Reviewing of research institutes

Members of the CoNRS section for Plant Science (L. Lejay 2009-2016, A. Martin 2017-2021), member of AERES committee for evaluation of RDP Lyon (L. Lejay, 2015) and EBI Poitiers (A. Gojon, 2017).

Participation in institutional committees and juries (CNRS, Inserm, etc.)

Alain Gojon:

- President of one jury for INRA recruitment (AI, 2016).
- Member of the Council of the “Biology-Agrosciences” Scientific Department of the Montpellier University
- Member of the Council of the GAIA Doctoral School

9- Academic research grants

European (ERC, H2020, etc.) and international (NSF, JSPS, NIH, World Bank, FAO, etc.) grants – coordination

European (ERC, H2020, etc.) and international (NSF, JSPS, NIH, World Bank, FAO, etc.) grants - partnership

2012 – 2015 ; **EUROOT (H2020)** ; Enhancing resource uptake from roots under stress in cereal crops ; NACRY Philippe

Other European grants - coordination

Other European grants - partnership

National public grants (ANR, PHRC, FUI, INCA, etc.) - coordination

2014 – 2015 ; **ACSES (Department BAP INRA)** ; Analyse Cellule spécifique de l'Epigénome en réponse au stress ; MARTIN Antoine

2014 – 2017 ; **SIPHON (ANR PRCI)** ; Voies de signalisation impliquées dans la phosphorylation des protéines membranaires en réponse à N et C ; LEJAY-LEFEBVRE Laurence

This project allowed the identification of a crucial domain in the C-terminus of the NRT2.1 nitrate transporter, which includes the S501 phosphorylation site, and which plays a key role in controlling the root nitrate uptake in Arabidopsis (Jacquot et al., submitted).

2016 – 2020 ; **NITRASENSE (ANR PRCI)** ; Intégration des réponses physiologiques et développementales de la plante au nitrate : rôle de la signalisation dépendante de NRT1.1 dans l'efficence d'utilisation de l'azote ; GOJON Alain

This project allowed unraveling a much wider impact of the Arabidopsis NRT1.1 nitrate transceptor on the overall auxin signaling in roots than previously described. Indeed we were able to show that NRT1.1 not only controls auxin distribution in lateral root primordia, but also governs part of the auxin biosynthesis pathway in the roots (Maghiaoui et al., submitted).

2017 – 2018 ; **ClimNutr (Department BAP INRA)** ; Changement climatique et nutrition minérale des plantes ; GOJON Alain, MARTIN Antoine

National public grants (ANR, PHRC, FUI, INCA, etc.) - partnership

2014 – 2019 ; **IMANA (ANR PRC)** ; Identification de régulations moléculaires majeures impliquées dans l'adaptation des plantes à la disponibilité en azote ; GOJON Alain

This project allowed demonstrating that chromatin factors are key players in the transcriptional regulation of the NRT2.1 root nitrate transporter in Arabidopsis (Bellegarde et al. 2018, Bellegarde et al. 2019).

Local grants (collectivités territoriales) - coordination

Local grants (collectivités territoriales) - partnership

PIA (labex, equipex etc.) grants - coordination

2011 – 2015 ; **RHIZOPOLIS (Labex AGRO)** ; A federative project for plant root research ; GOJON Alain

This project allowed major advances on the nitrate signaling role of the Arabidopsis NRT1.1 nitrate transceptor, which included regulation of a wide range of both functional and developmental responses of the plant to nitrate (Bouguyon et al. 2015, Bouguyon et al. 2016). It also set the ground for successful proposal of the NITRASENSE ANR project (see above).

2016 – 2016 ; **NITROGEN (Labex AGRO)** ; Conférence EMBO : 3ème Symposium international sur la nutrition en azote des plantes- Nitrogen 2016 ; GOJON Alain

2018 – 2018 ; **NRT1/1 (Labex AGRO)** ; Elucidation of mechanism for ammonium toxicity caused by nitrate sensor NRT1.1 / NPF6.3 in Arabidopsis thaliana (NRT1.1-included ammonium toxicity) ; GOJON Alain

2018 – 2021; **eCO2THREATS (iSITE Montpellier)** ; Alteration of plant nutrient contents by elevated CO2 : consequences for crop quality, human health and environmental protection ; GOJON Alain

PIA (labex, equipex etc.) grants - partnership

2017 – 2019 ; **GENERICE (Labex AGRO)** ; Generation & Deployment of Genome-Edited, Nitrogen-useEfficient Rice Varieties ; LEJAY Laurence

Grants from foundations and charities (ARC, FMR, FRM, etc.) - coordination

Grants from foundations and charities (ARC, FMR, FRM, etc.) - partnership

10- Visiting senior scientists and post-doc

Post-docs (total number)

Since 2014: 1

Foreign post-docs

Since 2014: 0

Visiting scientists (total number)

Since 2014 : 2

Foreign visiting scientists

Since 2014 : 2

Name	**First Name**	**Funding**	**Nationality**	**Arrival Date**	**Departure Date**	**Status**	**University of origin**
Hachiya	Takushi	JSPS + Labex AGRO	Japanese	01/05/2018	31/08/2018	Assistant Professor	Institute for advanced research (IAR), Nagoya University
Li	Jianfu	CSC Fellowship	Chinese	10/10/2017	09/10/2018	Graduate student	SJTU-Nottingham-Fudan Joint Center, Plant Biotech Center, School of Agriculture and Biology

11- Scientific recognition

Prizes and/or distinctions

IUF members

Chair of learned and scientific societies

Organisations of meetings and symposia (out of France)

L. Lejay: Member of organization committee for the EMBO Workshop Integrative Biology: from molecules to ecosystems in extreme environments, Santiago (Chile, 2019).

Invitations to meetings and symposia (out of France)

L. Lejay: EMBO Workshop Integrative Biology: from molecules to ecosystems in extreme environments, Santiago (Chile, 2019).

A. Gojon: International Workshop “Roots of sustainable agriculture: from concepts to practice”, October 11-15 2014, Zhejiang University, Hangzhou, China.

A. Gojon: 11th International Plant Molecular Biology Congress, October 25-30 2015, Iguazu, Brazil.

A. Gojon: International Symposium on Root Development and Nutrients/Water absorption. November 26-30, 2018, Zhejiang University, Hangzhou, China.

A. Gojon: France-Taiwan Scientific Festival. September 13-14, 2018, Taipei, Taiwan.

A. Gojon: International Workshop on Responses of Plants to abiotic stresses and environmental signals, June 14-15, 2019, China Agricultural University, Beijing, China.

II - INTERACTION OF THE TEAM WITH THE NON-ACADEMIC WORLD, IMPACTS ON ECONOMY, SOCIETY, CULTURE OR HEALTH

1- Socio-economic interactions / Patents

Invention disclosures

Filed patents

Accepted patents

Licenced patents

2- Socio-economic interactions

Industrial and R&D contracts

Cifre fellowships

Creation of labs with private-public partnerships

Networks and mixed units

Start-ups

3- Expertise

Consulting

Participation in expert committees (ANSES etc.)

Legal expertise

Expert and standardization reports

4- Public outreach

Radio broadcasts, TV shows, magazines and newspaper

Journal articles, interviews, book edition, videos, other popularization outputs, debates on science and society, etc.

Winner of the regional competition MT180 (V. Chaput, https://we.tl/t-vo4kzpvLOF).

A.Gojon: Interview for the newspaper "Le Monde":
https://www.lemonde.fr/planete/article/2018/08/27/les-emissions-de-co2-menacent-l-alimentation-humaine_5346733_3244.html

III - INVOLVEMENT OF THE TEAM IN TRAINING THROUGH RESEARCH

1- Educational outputs

Books

E-learning, MOOCs, multimedia lessons, etc.

Creation of a MOOC dedicated to basic microscopy (L. Bach, ongoing).

2- Scientific productions (articles, books, etc.) from theses

Scientific productions (articles, books, etc.) from theses

Scientific articles

Mounier E., Pervent M., Ljung K., Gojon A., Nacry P. (2014) Auxin-mediated nitrate signalling by NRT1.1 participates in the adaptive response of Arabidopsis root architecture to the spatial heterogeneity of nitrate availability. Plant Cell Environ. 37: 162-174

Bouguyon E., Brun F., Kubeš M., Meynard D., Pervent M., Léran S., Lacombe B., Krouk G., Guiderdoni E., Zažímalová E., Hoyerová K., Nacry P., Gojon A. (2015) Multiple mechanisms of nitrate sensing by Arabidopsis nitrate transceptor NRT1.1. . Nat. Plants. 1 : 15015.

Bouguyon E., Perrine-Walker F., Pervent M., Rochette J., Cuesta C., Benkova E., Martiniere A., Bach L., Krouk G., Gojon A., Nacry P. (2016) Nitrate Controls Root Development through Post-Transcriptional Regulation of the NRT1.1/NPF6.3 transporter/sensor. Plant physiol 172(2): 1237-1248

Bellegarde F., Herbert L., Séré D., Caillieux E., Boucherez J., Fizames C., Roudier F., Gojon A., Martin A. (2018) Polycomb Repressive Complex 2 attenuates the very high expression of the Arabidopsis gene NRT2.1. Sci. Rep. 8: 7905.

Bellegarde F., Maghiaoui A., Boucherez J., Krouk G., Lejay L., Bach L., Gojon Al., Martin A. (2019) The Chromatin Factor HNI9 and ELONGATED HYPOCOTYL 5 Maintain ROS Homeostasis under High Nitrogen Provision. Plant physiol. 180(1): 582-592.

Review articles

O'Brien J.A., Vega A., Bouguyon E., Krouk G., Gojon A., Coruzzi G., Gutiérrez R.A. (2016) Nitrate transport, sensing and responses in plants. Mol. Plant 9(6): 837-856.

Bellegarde F., Gojon A., Martin A. (2017) Signals and players in the transcriptional regulation of root responses by local and systemic N signaling in *Arabidopsis thaliana*. J Exp Bot. 68(10): 2553-2565. (review).

Mean number of publications per student (Biology & Science and technology only)

The only PhD student who defended her thesis during the reporting period is Fanny Bellegarde. She has 3 publications (see above).

3- Training

Habilitated (HDR) scientists

Since 2014 : 3

Last Name	First Name	Status	Employer	Date of HDR
GOJON	Alain	DRr	INRA MONTPELLIER	2000
LEJAY-LEFEBVRE	Laurence	CR	INRA MONTPELLIER	2014
MARTIN	Antoine	CR	CNRS	2017

HDR obtained during the period

Since 2014 : 2 HDR

Last Name	First Name	Status	Employer	Date of HDR
LEJAY-LEFEBVRE	Laurence	CR	INRA MONTPELLIER	2014
MARTIN	Antoine	CR	CNRS	2017

PhD students (total number)

4 PhD students

PhD students benefiting from a specific doctoral contract

Last Name	First Name	Starting Date	Defense Date	Duration of PhD	Funding
BELLEGARDE	Fanny	01/10/2014	08/12/2017	36	CDE
CHAPUT	Valentin	01/10/2017		36	CDE
MAGHIAOUI	Amel	01/10/2017		36	CDE
SERE	David	01/10/2017		36	CDE

Defended PhDs

1 defended PhD

Last Name	First Name	Defense date
BELLEGARDE	Fanny	2017

Mean PhD duration

Mean duration: 36 months

Internships (M1, M2)

Master 1: 5

Master 2: 6

Last Name	First Name	Level	Period	
Rougier	Marianne	Master 1	01/03/2015	31/07/2015
Chaput Montagnac	Valentin	Master 1	01/03/2016	26/08/2016
Maghiaoui	Amel	Master 1	01/03/2016	22/07/2016
Bal	Maxime	Master 1	04/03/2019	28/06/2019
Tan	Yiyi	Master 1	04/03/2019	28/06/2019
Gayraud	Thomas	Master 2	06/01/2014	06/07/2014
Fayos	Ian	Master 2	05/01/2015	05/07/2015
Chantrenne	Juliette	Master 2	09/03/2015	09/09/2015
Bonillo	Pauline	Master 2	03/01/2017	30/06/2017
Herbert	Leo	Master 2	03/01/2017	30/06/2017
Maghiaoui	Amel	Master 2	03/01/2017	30/06/2017

People in charge for a mention or a master's degree course (total number)

3: (L. Bach, L. Lejay, A. Martin).

L. Bach is responsible for 2 modules of M1 and M2 Plant Functional Biology at the University of Montpellier.

L. Lejay and A. Martin contribute to courses in M1 and M2 Plant Functional Biology at the University of Montpellier.

People in charge for a mention or a master's degree course with international certification (Erasmus mundus)

Influx team
Self-assessment document

Name of the team concerned by the current contract: Ion Flux Coordination and Signaling in Plant Cells
Name of the team concerned by the next contract (if different):
Acronym of the current contract: Influx
Acronym of the next contract (if different):
Team leader for the current contract: Alexis de Angeli
Team leader for the next contract: Alexis de Angeli

RESULTS

1- Presentation of the team

Introduction

The team **“Ion Flux Coordination and signaling in plant cells”** was created in january 2019. The team orginates from an ATIP-Avenir CNRS-INSERM project obtained by the team leader De Angeli Alexis to start a new reserch goup. Prior to the creation of the team in B&PMP, A. De Angeli was CNRS staf scientisct in the Institute ofr Integrative Biology of the Cell in Gif sur Yvette;

Team’s workforce and means

The **“**Ion **Flux Coordination and signaling in plant cells”** is so far formed by 1 permanent scientist A. De Angeli (CRCN CNRS). A request for a full time technical assitant has submitted to INRA.
One post-Doc, Paloma Cubero-Font, joined the group in February 2019 after obtaining a 2 years grant from a Spanish Foundation (Aflonso Martin Escudero). A second 2 years’ post-doc will be hired in the next months based on the ATIP-Avenir funding for 2 years. A L3 student (Roxane Doireau) joined the tem of a two-month internship.
The financial means are guaranteed by the ATIP-Avenir funding for 3 years (2019-2021) with a possible extension for 2 years.

Scientific policy

The aim of the group is to understand the molecular mechanisms involved in the regulation and coordination of ion fluxes across the cellular membranes of plant cells. The name of the team reflects this objective. We mainly use the stomata and guard cells as a cellular model system to address this question. To Indeed, stomata are illustrative of the importance of the control and coordination of ion fluxes in cells. The control of the stomata pore depends on the capacity of guard cells to induce massive and controlled ion fluxes across the plasma and the vacuolar membranes to change their volume and consequently the stomata aperture.
To reach our objectives and understand the mechanisms underlying the coordination of the plant cell membranes we use different approaches. We investigate the mechanisms of ion fluxes coordination from the biophysical properties of ion channels, to their function within the cell and their role at a whole plant level.

- We focus on the ALMT (Aluminum activated Malate Transporters) ion channels, a family important in stomata. We investigate the molecular basis of the regulation of the ALMTs (ion selectivity, activation by malate and nucleotide block, secondary modifications) and its impact in plant cells.
- We use genetically encoded biosensors expressed in different knock out backgrounds of ion transporters and signaling pathways to study *in vivo* flux coordination and they are important for plant adaptation to the environment we use this cell type as a model for our study.
- We want to develop mathematical models of intracellular ion fluxes.

The different aspects of the work we plan will be detailed in the Five-year project and strategy paragraph.

FIVE-YEAR PROJECT AND STRATEGY

1- SWOT analysis

- ***Strong points;*** ***originality of the project, Financial support.***

- ***Needs for improvements;*** **insertion in the local environment; collaboration; visibility**

- ***Possibilities brought in by the context / surrounding environment;*** **collaborations, scientific environment**

- ***Risks linked to the context / environment.*** **Need to reach a “critical mass”, permanent staff recruitment,**

2- Structure, workforce and scientific orientations

The INFLUX team started in January 2019 in BPMP institute based on an ATIP-Avenir project. The team is interested in understanding the mechanisms coordinating ion fluxes at the different cellular membranes of plant cells. Guard cells are a good model to address this question since their physiological function, the opening and closure of the stomata pore, relies on the coordination of ion fluxes across cellular membranes, mainly the tonoplast and the plasma membrane. Intracellular ion channels and transporters and the signaling cascades they are involved in are the main actors involved in these processes. Despite the importance of ion flux coordination, we still have an extremely limited knowledge on the mechanisms involved in this process. Finally, guard cells are important for the control of gas exchanges between the leaf and the atmosphere and they are involved in the regulation of CO_2 uptake and water evaporation. Thus, understanding the mechanisms underlying the responses of guard cells to the environment is important for understanding the mechanisms regulating biomass production and drought tolerance in plants.

In this context the INFLUX team will develop different approaches to address its objectives:

1) investigating the biophysical properties of ion transport systems using electrophysiological approaches. For this we will mainly focus on the ion channel family of the ALMTs that are important for guard-cell functions and are present in both the plasma and the vacuolar membranes.
2) Use genetically encoded biosensors and live imaging to visualize *in vivo* the dynamics of the ion concentrations in the cytosol of guard cells. We will use and develop genetically encoded biosensors to dynamically measure anion concentrations and pH in the two major cellular compartments (cytosol and vacuole).
3) Develop *in silico* modelling of ion fluxes in plant cells.

The platforms in BPMP (electrophysiology and imaging) will be main partners in the host institute to reach our objectives. For the biosensor part a collaboration with N. Paris (team KaliPHruit) will be kept and developed, as well as with D. Arosio (CNR, Trento Italy). One important aspect is to establish collaborations to develop the flux modelling part, for this I plan to contact at a national level with groups at the INRIA.

Annex 4 – Research products and activities

EVALUATION CAMPAIGN 2019-2020

GROUP A

Name of the unit / the team / the theme: Ion Flux Coordination and signaling in plant cells
Acronym: INFLUX

Theme leader for the current contract: Alexis DE ANGELI
Theme leader for the next contract: Alexis DE ANGELI

I - PRODUCTION OF KNOWLEDGE AND ACTIVITIES CONTRIBUTING TO THE INFLUENCE AND SCIENTIFIC ATTRACTIVENESS OF THE TEAM

1- Articles

Scientific articles

2014

1. Wege S., De Angeli A., Droillard M.J., Kroniewicz L., Merlot S., Cornu D., Gambale F., Martinoia E., Barbier-Brygoo H., Thomine S., Leonhardt N., Filleur S. (2014) Phosphorylation of the vacuolar anion exchanger AtCLCa is required for the stomatal response to abscisic acid. Sci Signal. 7 (333): ra65.
2. Zhang J., Martinoia E., De Angeli A. (2014) Cytosolic Nucleotides Block and Regulate theArabidopsisVacuolar Anion ChannelAtALMT9. J Biol Chem. 289 (37): 25581-25589.

2016

3. Baetz U., Eisenach C., Tohge T., Martinoia E., De Angeli A. (2016) Vacuolar Chloride Fluxes Impact Ion content and Distribution during Early Salinity Stress. Plant Physiol. 172 1167-1181.

2017

4. Eisenach C., Baetz Ulrike, Huck N. V., Zhang J., De Angeli A., Beckers G.J. M., Martinoia E. (2017) ABA-Induced Stomatal Closure Involves ALMT4, a Phosphorylation-Dependent Vacuolar Anion Channel of Arabidopsis. Plant Cell. 29 (10): 2552-2569.

Review articles

2016

1. De Angeli A., Thomine S., Frachisse J.M. (2016) Anion Channel Blockage by ATP as a Means for Membranes to Perceive the Energy Status of the Cell. Mol Plant. 9 (3): 320-322. (Editorial)

2017

2. Eisenach C., De Angeli A. (2017) Ion Transport at the Vacuole during Stomatal Movements. Plant Physiol. 174 (2): 520-530. (Editorial)

Other articles (professional journals, etc.) (total number)

2- Books

Monographs, critical editions, translations (total number)

Management and coordination of scientific books / Scientific book edition

Book chapters (total number)

Book chapters in English or another foreign language

Edited theses

3- Production in conferences / congresses and research seminars

Articles published in conference proceedings / congress

Other products presented in symposia / congress and research seminars

Invited oral presentations in international symposia/congress

1. 05/2014 International Symposium FOR 1061 "Dynamic storage function plant vacuoles" Heidelberg, Germany
2. 06/2015 Gordon Conference: Organellar Channels & Transporters Bentley University in Waltham MA United States
3. 05/2016 IWPMB Annapolis
4. 05/2017 EMBO/FEBS Lecture Course "Channels and transporters" Erice, Italy.

Invited oral presentations in national symposia/congress

1. 07/06/2019 Pacth-Club Montpellier

Selected oral presentations in international symposia/congress

1. 05/2016 Communication orale au 6th Biosensor Meeting Orsay.
2. 06/2018 Communication orale, International Conference "Plant Physiology & Biochemistry", Vienna (Austria)
3. 06/2018 Communication International meeting SPS, Orsay (France).
4. 07/2018 Communication orale à International Plant Molecular Biology Congress (IPMB), Montpellier (France).

Selected oral presentations in national symposia/congress

Posters in national symposia/congress

Invited seminars in foreign institutions

1. De Angeli Alexis Vacuolar anion transport, novel insights in the ALMT anion channel family. 06/06/2014 University of Heidelberg (Germany).
2. De Angeli Alexis Visualizing the activity of anion transporters and channels in living cells : CLC, ALMTs and some others…23/05/2019 University of Wuerzbrg (Germany)

Invited seminars in French institutions

4- Electronic tools and products

Softwares

Databases

5- Instruments and methodology

Prototypes

Platforms and observatories

6- Other products

Theorised artistic creations, staging, movies

7- Editorial activities

Participation in editorial committees (books, collections, etc.)

Collection and series management

8- Reviewing activities

Reviewing of articles

Grant evaluation (public or charities)

Reviewing of research institutes

Participation in institutional committees and juries (CNRS, Inserm, etc.)

9- Academic research grants

European (ERC, H2020, etc.) and international (NSF, JSPS, NIH, World Bank, FAO, etc.) grants – coordination

European (ERC, H2020, etc.) and international (NSF, JSPS, NIH, World Bank, FAO, etc.) grants - partnership

Other European grants - coordination

Other European grants - partnership

National public grants (ANR, PHRC, FUI, INCA, etc.) - coordination

2019 – 2021 ; **ATIP - Avenir Program 2018 (CNRS-INSERM)** ; Intracellular ion flux coordination, a novel perspective on the mechanisms regulating ion transport in *A. thaliana* guard cells ; DE ANGELI Alexis

National public grants (ANR, PHRC, FUI, INCA, etc.) - partnership

Local grants (collectivités territoriales) - coordination

Local grants (collectivités territoriales) - partnership

PIA (labex, equipex etc.) grants - coordination

PIA (labex, equipex etc.) grants - partnership

Grants from foundations and charities (ARC, FMR, FRM, etc.) - coordination

Grants from foundations and charities (ARC, FMR, FRM, etc.) - partnership

10- Visiting senior scientists and post-doc

Post-docs (total number)

Depuis 2014: 0

Foreign post-docs

Depuis 2014: 0

Visiting scientists (total number)

Depuis 2014 : 1

Foreign visiting scientists

Depuis 2014 : 1

Name	First Name	Funding	Nationality	Arrival Date	Departure Date	Status	University of origin
Cubero Font	Paloma	Bourse	Espagne	30/01/2019	30/01/2021	Doctorant invité	Espagne Alfonso Escudero

11- Scientific recognition

Prizes and/or distinctions

IUF members

Chair of learned and scientific societies

Organisations of meetings and symposia (out of France)

Invitations to meetings and symposia (out of France)

II - INTERACTION OF THE TEAM WITH THE NON-ACADEMIC WORLD, IMPACTS ON ECONOMY, SOCIETY, CULTURE OR HEALTH

1- Socio-economic interactions / Patents

Invention disclosures

Filed patents

Accepted patents

Licenced patents

2- Socio-economic interactions

Industrial and R&D contracts

Cifre fellowships

Creation of labs with private-public partnerships

Networks and mixed units

Start-ups

3- Expertise

Consulting

Participation in expert committees (ANSES etc.)

Legal expertise

Expert and standardization reports

4- Public outreach

Radio broadcasts, TV shows, magazines and newspaper

Journal articles, interviews, book edition, videos, other popularization outputs, debates on science and society, etc.

III - INVOLVEMENT OF THE TEAM IN TRAINING THROUGH RESEARCH

1- Educational outputs

Books

E-learning, MOOCs, multimedia lessons, etc.

2- Scientific productions (articles, books, etc.) from theses

Scientific productions (articles, books, etc.) from theses

Mean number of publications per student (Biology & Science and technology only)

3- Training

Habilitated (HDR) scientists

Since 2014 : 1

Family Name	First name	Position	Employer	Date of habilitation
DE ANGELI	Alexis	CR	CNRS	2016

HDR obtained during the period

Since 2014 : 1 HDR

Family Name	First name	Position	Employer	Date of habilitation
DE ANGELI	Alexis	CR	CNRS	2016

PhD students (total number)

0 PhD student

PhD students benefiting from a specific doctoral contract

Defended PhDs

0 defended PhD

Mean PhD duration

Mean duration: 0 months

Internships (M1, M2)

Master 1: 0

Master 2: 0

People in charge for a mention or a master's degree course (total number)

People in charge for a mention or a master's degree course with international certification (Erasmus mundus)

TICER team
Self-assessment document

Name of the team concerned by the current contract: Ion Transport and Adaptation to the Environment in Cereals

Name of the team concerned by the next contract (if different):

Acronym of the current contract: TICER

Acronym of the next contract (if different):

Team leader for the current contract: Anne-Aliénor Véry

Team leader for the next contract: Anne-Aliénor Véry

RESULTS

1- Presentation of the team

Introduction

The team "Ion transport in cereals and adaptation to the environment" (TICER) was created in July 2015. It originates from empowerment of 3 researchers from the former team "Ionic channels" directed by H. Sentenac: Anne-Aliénor Véry, Jean-Benoît Peltier and Jean-Christophe Boyer. In January 2016, a researcher from the "Aquaporin" team, Doan Luu, joined the TICER group. In January 2017, Claire Corratgé-Faillie (IR CNRS) was recruited in BPMP to work on the electrophysiology platform of the UMR, and was affected at 50% of her time to TICER for technical support. At last, in January 2019, Pierre Berthomieu (Pr., SupAgro) (previously leading the "Metals" group of BPMP) integrated the TICER team. The research line of the TICER group was defined in 2015 as an extension of the current projects in the "Ionic channels" team of Anne-Aliénor Véry (since 2006) and Jean-Benoît Peltier (since 2013) on mechanisms of Na^+ and K^+ transports. The work already performed on this thematic in the "Ionic channels" team before the TICER creation has therefore been included to the below report and publication list. The TICER group is directed by Anne-Aliénor Véry since it has been created.

Team's workforce and means

The TICER team currently gathers 4 researchers (J-C Boyer, CR INRA; DT Luu, CR CNRS; J-B Peltier, CR INRA; A-A Véry, CR CNRS), one teacher-researcher (P Berthomieu, Pr., SupAgro), and one engineer (C Corratgé-Faillie, CDI IR CNRS) at 50% of her time. For most of the TICER scientists, the project they develop within the TICER team corresponds to a new project started at their arrival in the group: *i.e.*, from 3 months (P Berthomieu), 3,25 years (DT Luu), and 3,75 years (J-C Boyer).

Two post-docs and seven graduate students have worked in the "Ionic channels" thereafter in TICER or are currently working in TICER: Manuel Nieves-Cordones (post-Doc 2014-2016), Thanh Hao Nguyen (PhD 2013-2016; co-direction with H Sentenac in "Ionic channels" then "Elsa", then post-doc in 2017), Fouad Al Shiblawi (PhD 2014-2017), Sonia Mohamed (PhD 2014-2017; co-direction with Emmanuel Guiderdoni UMR AGAP CIRAD), Jing Zhou (PhD 2017-), Houssein Zhour (2019-), La Hoang Ahn (2019-). Four foreign PhD students were hosted in the TICER team for few months: Amal Ben Amar from Casablanca University, Morocco (4 months per year in 2015, 2016, and was hosted in 2014 in the "Ionic channels" group, in the framework of a PHC PRAD project), Kumkum Kumari (PhD student from Madras University, India, benefiting from an EMBO fellowship for 3 months in 2018), Lina Zhao (PhD student from Jiao Tong University, Shanghai, China, benefiting from a Chinese Science Council fellowship for 6 months; from June 2019) and Mohamed Tebini (PhD student at Tunis University, benefiting from a 3 month fellowship from Tunisia; from June 2019). In addition, the team hosted 10 L3, M1 or M2 students since 2015.

Our activity has been supported by several grants: an ANR IA grant (Demeterres, 2014-2019); a PHC Maghreb project (Aladin 2018-2020); an Iraqi government grant (bench fee support for F Al Shiblawi PhD, 2014-2016); two grants from Agropolis Foundation (Cultivar, 2018 and 2019).

Scientific policy

The scientific strategy that was chosen, when the team TICER was created, was to investigate ionic mechanisms involved in plant adaptation to abiotic stresses, especially salt stress. Focus was made on K^+ nutrition and K^+/Na^+ homeostasis control. The project targeted cereals, particularly rice as the cereal model species. Rice, which is the most salt (Na^+ especially) sensitive cereal, is particularly subjected to soil salinization threat owing to its cultivation habitat (high occurrence in river deltas). Our interest has covered especially salt tolerance mechanisms inexistent in Arabidopsis or probably more complex in cereals than in Arabidopsis due to a higher number of involved genes with diversified functions (see below).

An initial research line already developed within the "Ionic channels" team was the analysis of functions of K^+ and Na^+ transporters from the HKT family. A great interest for this family of transporters in the field of salt stress tolerance has emerged about 15 years ago after the discovery that the unique gene of this family in Arabidopsis is a strong determinant of salt tolerance and that major QTL of salt tolerance previously mapped in cereals correspond to members of the same family ([113, 17]). The HKT family is particularly diversified in cereals as compared to Arabidopsis with up to 9 genes present in their genomes. QTL of salt tolerance, K^+/Na^+ homeostasis or K^+ use efficiency are to date highlighted for 4 of the nine cereal *HKT* genes (e.g., [9, 11, 17], Campbell et al. 2017). On this transporter family, the TICER group has worked on two aspects:

- Functional analyses of HKT transporters involved in salt tolerance QTL to get additional clues on the physiological bases of these QTL

- *In vitro* production and purification of HKT transporters toward structure-function analyses of these systems.

Advances in the biochemical work for HKT production and purification and the arrival of new members in TICER led to reexamine our scientific strategy and to decide to invest a new domain of research in order to take into account the biotic conditions and interactions with beneficial soil microbes which can deeply affect the development and functioning of the root system and thereby the plant mineral nutrition and adaptation to abiotic stress. Two new lines of research were thus initiated. (i) The first one takes into account endomycorrhizal symbiosis and aims at developing cellular biology analyses of the mechanisms of nutrient (K^+ in our case) acquisition from the fungus at the arbuscular interface, and the equipment in membrane transport system and functioning of the plant cell membrane at this interface. (ii) The second one aims at taking into account the presence of rhizobacteria that affect the root system architecture and the development of root hairs. Evidence is available in the literature that these two biotic interactions can play a role in plant adaptation to environmental conditions, including to salinity.

2- Research products and activities for the team

Scientific track record

Functional analysis of HKT transporters involved in salt tolerance QTL in rice and wheat

AA Véry, PhD student F Al Shiblawi, Invited PhD student S Tounsi (CBS Sfax, Tunisia)

Based on knowledge we had gained in previous years on the functional behavior of different HKT transporters, we engaged collaborative work with foreign groups interested in the functional analysis of HKTs corresponding to salt tolerance QTL. One of such projects concerned a QTL of root Na^+ accumulation involving *HKT1;1*-type genes in rice identified by GWAS by the group of Harkamal Walia (Nebrasca-Lincoln University, USA). This QTL targeted for the first time a *HKT1;1*-type gene. We compared the functional properties of the two variants of OsHKT1;1, OsHKT1;1-Ni and OsHKT1;1-Zh, from the *japonica* variety Nipponbare and the *indica* one Zhenshan respectively, since the two varieties contrasted for the trait of interest. The two transporters were expressed in *Xenopus* oocytes and we compared the amount of protein at the oocyte membrane (using GFP tags and confocal fluorescence imaging analyses), the level of currents, the current activation threshold, the cationic selectivity and the apparent affinity for Na^+ of the two transporters. We revealed a difference in the current activation threshold, which shed light on the genetic analysis (Campbell *et al.*, 2017).

A similar study concerned a QTL corresponding to another type of HKT transporters, HKT1;4, in durum wheat, in collaboration with a Tunisian group from the Biotechnology Center of Sfax (CBS) (Ben Amar et al., 2014; Tounsi et al., 2016).

Towards HKT transporter crystallization

J-B Peltier, J-C Boyer, 3 M1 students (2017, 2018, 2019)

Two classes of HKT transporters are found in plants: class I gathers HKT transporters that mediate Na^+-selective transport, and class II HKT transporters that are permeable to both Na^+ and K^+ and able to mediate Na^+-K^+ symport. In bacteria and fungi, homologs of plant HKT named TRK or Ktr have been shown to mediate active K^+ uptake from low concentrations (µM range) which is thought to involve H^+-K^+ symport. Investigating the structure-function relationship of plant HKT transporters is a highly exciting objective, at the biological level, to understand how these systems which derived from simple (2 transmembrane segments) ion channels like their counterparts in bacteria and fungi, can behave as transporters and mediate Na^+-K^+ symport. Gaining knowledge about the structure function relationship of these plant transporters is furthermore crucial for understanding precisely the mechanism of ion conduction and discrimination (between Na^+ and K^+) and how HKT transporters enhance crop performance under salinity stress. The HKT/Trk/Ktr-type K^+ transporter superfamily is present in non-animal organisms (plant, bacteria, fungi) only. X-ray crystal structures of TrkH from *V. parahaemolyticus* and KtrB from *B. subtilis* have given insights into the molecular bases of ion permeation through these transporters [3, 23]. Nevertheless, major differences distinguish bacteria and plant HKTs. For instance, no functional homolog of the class I HKTs selective for Na^+, among which most salt tolerance QTL concerning plant HKTs, has been found in bacteria. Also, bacterial transporters comprise a cytosolic octameric ATP-binding regulatory subunit, which is absent in plant HKTs [5]. We have thus decided to overexpress and crystallize two HKT proteins representative of the 2 HKT classes present in plants, both from cereals, TmHKT1;4-A2 from the wheat relative species *T. monococcum* (QTL Nax1; [11], Tounsi et al., 2016) and OsHKT2;2 (K^+ use efficiency QTL; [9]).

In vitro translation is an attractive method to overexpress functional membrane proteins [19]. Because transcription and translation are realized *in vitro*, such expression systems circumvent potential drawbacks like cell toxicity and protein insolubility fairly common when membrane proteins are overexpressed in living pro- or eu-karyotic systems. OsHKT2;2 and TmHKT1;4-A2 have been overexpressed using wheat germ extract (WEPRO, CellFree Sciences) because of its high performance for eukaryotic proteins and because of its close genetic relationship with our proteins (no codon optimization required) [8, 21]. Moreover, this system has been shown to allow obtaining of protein amounts (mg) compatible with crystallization requirements [15].

Two versions of each HKT protein have been produced. The first one contains a N-term 6xHis tag, a C-term TwinStreptag and a TEV site between each tag and the HKT protein to facilitate purification (tags) and ii) tag removal (TEV sites) before crystallization. The second version does not harbor any tag. The first version was originally used to produce both HKT proteins in presence of detergent(s) during the translation, in order to help keeping membrane proteins soluble in an aqueous environment. A screen of 10 different detergents (alone or in combinations) was performed allowing us to select 3 best solubilizing conditions compatible with high translation efficiency (Brij58, Tween80/C12E8 and Brij58/C12E8). In parallel, the second version with no tag was produced in presence of synthetic liposomes, which led to HKT-containing proteoliposomes. Different lipids have been tested to form liposomes (DMPC, POPC, POPE, DPPC, DOPC). Liposomes were extruded to homogenize their size. Iodixanol floatation step cushion was used to purify proteoliposomes. HKT purity was somehow equivalent between the tagged version purified on column and the untagged version in proteoliposomes purified with step cushion. Untagged purified proteoliposomes show the advantage to be closer to the native form in its native environment than the detergent solubilized version. Moreover, we tested the functionality of the proteoliposome version of HKTs using the “Black lipid membrane” (BLM) technique. In this technique, proteoliposomes are fused to an artificial lipid bilayer separating two compartments in which are placed electrodes for electrical conductivity measurements. HKT were shown to be functional in BLM with a likely unitary conductance close to 50 pS in 200 mM NaCl. Thereafter, about 100 µg of TmHKT1;4-A2 were produced for pre-crystallization assays. Crystallization steps are underway in collaboration with Sébastien Granier (IGF) using Lipidic Cubic Phase (LCP), a membrane mimetic matrix suitable to crystallize membrane proteins. Nine hundred pre-crystallization conditions have been recently tested on an on-going process.

Analysis of K^+ uptake by rice roots and cell biology of the beneficial effect of endomycorrhization on plant K^+ nutrition upon salt stress

DT Luu, AA Véry, Post-doc M Nieves-Cordones, PhD student S Mohamed

Beside the analysis of HKT transporters, a new research line has been initiated with respect to salt tolerance mechanisms in the TICER group, targeting root K^+ transport and beneficial interactions with arbuscular mycorrhizae, owing to recent evidence for the improved plant K^+ nutrition in conditions of mycorrhization, especially upon salt stress (see for instance [16]). It is important to note that maintenance of a high cellular K^+/Na^+ ratio is a common mechanism developed by plants to withstand salt stress, and efficient K^+ uptake is crucial in this objective [10].

We therefore aimed at characterizing the main systems involved in root K^+ uptake in rice. In the framework of the ANR project Demeterres, we have characterized the high affinity pathway of root K^+ uptake in rice. In the model plant Arabidopsis, the high-affinity K^+ uptake by the root is mediated essentially by the transporter AtHAK5 [7]. In rice, 8 homologs of AtHAK5 exist (Véry et al., 2014). Comparison of root K^+ uptake rate in wild type Nipponbare plants and in a *Tos17* insertional mutant in the *OsHAK5* gene or in CRISPR-CAS9 lines that we produced to disrupt *OsHAK1* activity, evidenced that OsHAK1 is the major K^+ uptake system in the 1 µM to 1 mM external K^+ concentration range (Nieves-Cordones et al., 2017). OsHAK1 being a major transporter, its activity had a strong cell depolarizing effect with a slope of 29 mV per concentration decade in the 1 to 300 µM K^+ concentration range, suggesting that K^+ uptake by this system involved a H^+-K^+ symport. OsHAK1 apparent K_M for K^+ was found to be close to 15 µM. We also showed that OsHAK1 was as well highly permeable to Cs^+, with an apparent K_M of transport close to 30 µM (Nieves-Cordones et al., 2017).

In the field of plant salinity tolerance, studies aiming at identifying key determinants have so far rarely taken into account mycorrhizal symbiotic interactions, although beneficial effects of arbuscular mycorrhizal (AM) fungi in the plant response to salt stress is now widely acknowledged [4, 6]. Improvement of plant mineral nutrition upon mycorrhizal inoculation, including increased K^+ uptake, promoting a higher K^+/Na^+ in the host plants, has been consistently reported, but the molecular mechanisms are still to be elucidated [4, 18]. In rice, AMF infection naturally occurs [14] and recent studies have identified in this species AMF-dependent expression of cation transport systems involved in cell/tissue Na^+ sequestration upon salinity stress[16]. Preliminary data of our group confirmed beneficial effect of AM fungus inoculation on salt tolerance in rice. In these experiments, rice cv. Nipponbare was grown in soil watered by sub-irrigation, and inoculated or not with the AM fungus species *R. irregularis*. Eight weeks after germination, plants were watered with 100 mM NaCl. After 2 weeks of salt stress, tillers exhibited fewer youngest leaf burning (one of the most visible responses to salt stress) in AM-fungus inoculated plants than in non-inoculated control plants. This effect was related to a 25% increase in K^+ content, and a 50% increase of the K^+/Na^+ ratio in shoots. Altogether, the results indicated that the AM fungus interaction increased K^+ nutrition upon salt stress. Our model proposes that K^+ is selectively taken up from the soil by fungal K^+ transport systems before being secreted towards cortical cells of the colonized root where it is efficiently taken up by plant transporters and channels. The higher K^+ status of colonized cortical cells leads to increased K^+ loading into the xylem vessels and higher K^+ to Na^+ flux ratios transported towards the shoot tissues. Our future goal for investigating the mechanisms by which mycorrhizal symbiosis improves rice K^+ nutrition upon salt stress is to develop a cell biology approach, focusing on the fate of main root K^+ transport system (OsHAK1, see above, together with a K^+ channel from the Shaker family for "low affinity" uptake), at the symbiotic interface in cortical cells colonized by the arbuscules (*Cf.* project section).

Responses to beneficial rhizobacteria in cereals: development of plant growth devices for phenotyping of root system architecture and root hair development

J-B Peltier, collaboration with H Sentenac (ELSA team), PhD student T Rongsawat

A first step in our objective to take into account the effects of beneficial rhizobia on mineral nutrition and adaptation to soil conditions, including salinity, has been to develop plant growth devices allowing to get high resolution images of root system architecture and root hair development in 2-3 week-old cereals (rice and wheat). Two devices have been developed. The first one obtained by 3-D printing especially dedicated to the observation (microscopy) of the root hair zone, *in situ* without root transfer. The second one, developed from the rhizobox technique, allows to get image (high resolution A3 scanner) of the whole root architecture, the root hairs remaining clearly visible. These methodological developments have been carried out (over ca. 2,5 years) within the framework of a collaborative work between TICER (J-B Peltier) and ELSA (H. Sentenac) and the co-supervision of a PhD thesis (T. Rongsawat). Examples of images of root systems/root hairs recently obtained are available at https://www1.montpellier.inra.fr/wp-inra/bpmp/recherche/les-equipes/elsa/.

FIVE-YEAR PROJECT AND STRATEGY

1- SWOT analysis

In perspective of a five-year project, the unit (team / theme) shall list:

- its strong points;

Plants of agronomical major importance as models, strong internal skills in most developed lines, attractiveness for students (PhD students to support at least 3 of our 4 lines in 2020), focused team project

- needs for improvements;

Moderate financial means, moderate technical support (0.5 etp, i.e. 0.1 etp per researcher/teacher-researcher)

- possibilities brought in by the context / surrounding environment;

Local possibilities of collaboration on rice (Cirad, IRD, INRA) and durum wheat (INRA), involvement in the National Agrosym network (for biotic interactions), strong opportunities of international collaborations in particular with “South regions” (China, Vietnam & Maghreb).

- risks linked to the context / environment.

Young group (quite new project for most group scientists), still (moderate) methodological/technical developments required for several projects, time for transporter crystallization, which constitutes a major step for the structure-function relationship program, difficult to predict

2- Structure, workforce and scientific orientations

The project of the team TICER is essentially in continuity with the research work we have progressively developed since the creation of the team. Four research lines will be coordinated to target different mechanisms and integrated processes, including biotic interactions with soil microbes, involved in mineral nutrition and adaptation to abiotic conditions in cereals. Each line is mainly developed by one researcher of the group, and will benefit from the support from at least 1 PhD student in the 2-3 following years. Claire Corratgé-Faillie (engineer) will contribute to these programs with respect to electrophysiology and molecular biology aspects and supervision of BTS/M1/M2 students.

Structure-function analysis of HKT transporters

J-C Boyer & J-B Peltier - C Corratgé-Faillie

Directly in line with the work of the team when it was created, focused on Na^+ and K^+ HKT transporters, the work on the structure-function relationship of HKT transporters will be continued. Resolving the structure of these transporters would (i) constitute a major achievement toward the fundamental understanding of Na^+ *vs* K^+ selectivity in ion transport systems (since HKT transporters offer a unique opportunity to analyze opposite Na^+/K^+ selectivity within a similar structure) and (ii) strongly help structure-function analyses by site-directed mutagenesis in order to identify new molecular determinants of salt tolerance associated with such systems (structural modeling cannot be done with confidence based on available distant bacterial structures).

Crystallization tries will be continued in collaboration with S. Granier (Institut de Génomique Fonctionnelle, Montpellier). The level of purification already obtained by J-B Peltier and J-C Boyer, and assessment of functionality of the purified proteins allow to be quite confident on the success of crystallization in the medium term. However, since the time which will be required until achievement cannot be predicted, no PhD student will be hired on this project before the step of the structure is reached.

Crystallization tests will be pursued with different strategies to enhance the protein concentration and the likelihood to obtain crystals in LCP and vapor diffusion method: e.g., solubilization of tagged proteins in detergents, insertion of proteins in polymer-bounded lipid nanodiscs [2]. Also, alternative approaches like cryo-EM and NMR could be considered if HKT are recalcitrant to crystallization [12]. Once the HKT structures will be solved, structure/function studies will be initiated (in oocytes and/or BLMs) using HKT versions mutagenized on specific sites suggested from the structure models. Finally, the physiological consequences of Crispr-Cas 9 mediated modification of HKT ion conduction properties *in planta* will be assessed, in particular under salt stress conditions.

Stomatal movements and leaf rolling: osmotic processes in leaves and transpiration control

AA Véry, P Berthomieu, J Zhou (PhD student, 2017-2021)- C Corratgé-Faillie

The knowledge and experience we have gained during the last period on the mechanisms of K^+ and Na^+ transport in cereals will be used to investigate two osmocontractility processes involved in control of plant transpiration, and thereby of Na^+ translocation to leaves (where this cation is highly toxic) and plant adaptation to salinity as well as to drought stress: (i) stomatal regulation and (ii) leaf rolling. This work will be carried out using rice as a cereal model.

(i) Rice stomata (like those of other cereals) are anatomically more complex than those of dicot plants since they comprise in addition to guard cells, subsidiary cells that boarder the guard cells and display coordinated osmotic regulation. Stomatal functioning in rice has been yet weakly analyzed. As a background for stomatal functioning analyses, a setup for transpiration rate measurements at the whole plant level, allowing precise kinetics analyses of stomatal reactivity to environmental cues (e.g., light/dark transition), has been developed in the group (Nguyen et al., 2017). Furthermore, collaboration has been engaged for the analysis of ion transport fluxes through the guard cell membrane in intact stomates with R Roelfsema at the University of Würzburg, Germany (Nguyen et al., 2017).

(ii) Leaf rolling, which occurs in grass leaves in response to water deficit, leads to decreased transpiration rates by reducing the leaf area exposed to sunlight. Since it both controls the photosynthetic area and contributes to the regulation of the plant water status, leaf rolling is considered as a major determinant of cereal yield. For instance, regarding rice, it is taken into account in breeding programs, e.g., in CGIAR such as the International Rice Research Institute ("IRRI", Philippines; eg, [20, 22]). Our own work on leaf rolling is carried out in durum wheat in collaboration (PHC program) with Morrocan partners (Keltoum El Bouhmadi, Saïd Mahboub, University of Casablanca). Using a collection of Moroccan durum wheat accessions, we have observed a strong positive correlation between the level of leaf rolling upon drought stress and the preservation of yield. Leaf rolling is known to strongly involve osmoregulation in the large epidermal bulliform cells, but this mechanism has not been described at the ion transport level.

In both stomate and bulliform cells, we are now interested in the identification of key transport systems involved in the osmotic regulation, based on candidate genes expressed in these cell types and reverse genetics analyse. We will analyze the impact of these transport systems on leaf transpiration rate and leaf rolling upon osmotic (drought/salt) stress and the accumulation of Na^+ and K^+ in rice leaf tissues upon salt stress. In addition, taking advantage of local durum wheat resources for genetic analyses (EPO collection, J David, UMR AGAP, Montpellier), we will check the possibility to initiate a QTL analysis of leaf rolling in collaboration with the Morrocan group of Casablanca University.

Analysis of the beneficial effect of endomycorrhization on plant K^+ nutrition upon salt stress

DT Luu and HA La (PhD student, 2019-2022) - C Corratgé-Faillie

This line will be the continuation of the project initiated on this topic in the three last years by DT Luu (*Cf.* above for rationale). Focus will now be made on the mechanisms by which K^+ is transferred from the AM fungus to root cells, paying special attention to the symbiotic interface in cortical cells colonized by the arbuscules. The project will take advantage of collaborations already established with the UMR Agro-ecologie at INRA Dijon (PE Courty) on endomycorrhization, and with the Agricultural Genetics Institute/LMI-RICE at Hanoi, Vietnam (TH Giang), *e.g.*, for rice transformation. The analysis of the fate and activity of K^+ transport systems (OsHAK1 and OsHKT2;1 transporters, OsAKT1 Shaker channel) at the root plasma membrane of the arbuscular interface, in particular upon salt stress, will comprise expression studies (by qRT-PCR and *in situ* hybridization), imaging of the transport systems at the membrane (using fluorescent tag or by immunocytochemistry), endocytosis and exocytosis dynamics, possible lateral diffusion and re-localisation, and root K^+ uptake assays. Using loss-of-function mutants will allow to identify key system(s) responsible for the improved K^+ nutrition upon mycorrhization.

Analysis of the role of PGPR rhizobia on root hair development and plant nutrition

JB Peltier, P Berthomieu and H Zhour (PhD student, 2019-2022; co-tutelle with Beirut University, Lebanon)

This line of research will be aimed at developing "high throughput quantitative" analyses of the changes in root architecture and development of root hairs induced in cereals by interacting PGPR, paving the way to GWAS approaches. The working hypotheses is that such changes are likely to contribute to improved plant nutrition in challenging abiotic conditions (low nutrient availability, salt stress, water stress) and that plant breeding under artificial conditions (high fertilization inputs) could have reduced the ability of modern varieties to interact with PGPR. The previous work (in collaboration with the ELSA team) has aimed at developing experimental procedures allowing to get high resolution images of root system architecture and root hair zones in 2-3 week-old cereals. The objective now is to develop a dedicated software (in collaboration with a bioimage analyst, Volker Baecker, CNRS-INSERM Montpellier RIO Imaging) allowing to describe such images with quantitative parameters. Different PGPR strains, some of them having been already shown to differ in their capacity to promote root hair development (density and length) in young seedlings grown *in vitro* on agar plates, will be tested on a series of wheat accessions selected from a transect of wheat domestication (in collaboration with J David and P Roumet wheat geneticists, UMR AGAP Montpellier). Root exudates will also be collected from the corresponding plants grown under nutrient deficiency (using an experimental procedure and setup that we have already developed). Ongoing collaborations with wheat geneticists from Montpellier and microbiologists from Lebanon ensure the availability of the biological material for this project (transect of wheat domestication, PGPR strains collected from wheat plants collected in the "Fertile Crescent" where wheat domestication took place).

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Annex 4 – Research products and activities

EVALUATION CAMPAIGN 2019-2020

GROUP A

Name of the team **Ion transport and adaptation to environment in cereals**
Acronym: TICER

Theme leader for the current contract: Anne-Aliénor VERY
Theme leader for the next contract: Anne-Aliénor VERY

I - PRODUCTION OF KNOWLEDGE AND ACTIVITIES CONTRIBUTING TO THE INFLUENCE AND SCIENTIFIC ATTRACTIVENESS OF THE TEAM

1- Articles

Scientific articles

20% of most significant ones:

1. **Taochy C., Gaillard I., Ipotesi E., Oomen R., Leonhardt N., Zimmermann S., Peltier J.B., Szponarski W., Simonneau T., Sentenac H., Gibrat R., Boyer J.C. (2015) The Arabidopsis Root Stele Transporter NPF2.3 Contributes to Nitrate Translocation to Shoots under Salt Stress. Plant J. 83(3): 466-479. (fr)**

2. Charpentier M., Sun J., Vaz Martins T., Radhakrishnan G.V., Findlay K., Soumpourou E., Thouin J., Véry A.A., Sander D., Morri R.J., Oldroyd G.E.D. (2016) Symbiotic calcium oscillations require nuclear-localised cyclic nucleotide gated channels. Science 352(6289): 1102-1105. (eu)

3. **Nguyen T.H., Huang S., Meynard D., Chaine C., Michel R., Roelfsema R., Guiderdoni E., Sentenac H., Véry A.A. (2017) A dual role for the OsK5.2 ion channel in stomatal movements and K+ loading into xylem sap. Plant Physiol. 174(4): 2409-2418.**

4. **Chu T.T.H., Hoang T.G., Trinh D.C., Bureau C., Meynard D., Vernet A., Ingouff M., Do N.V., Périn C., Guiderdoni E., Gantet P., Maurel C., Luu D.T.(2018) Sub-cellular markers highlight intracellular dynamics of membrane proteins in response to abiotic treatments in rice. RICE. 11 (1): 23.**

2014

5. **Ben Amar S., Brini F., Sentenac H., Masmoudi K., Véry A.A. (2014) Functional characterisation in Xenopus oocytes of Na+ transport systems from durum wheat reveals diversity among two HKT1;4 transporters. J. Exp. Bot. 65: 213-222.**

6. Jammes F., Leonhardt N., Tran D., Bousserouel H., Véry A. A., Renou J. P., Vavasseur A., Kwak J. M., Sentenac H., Bouteau F., Leung J. (2014) Acetylated 1,3-diaminopropane antagonizes abscisic acid-mediated stomatal closing in Arabidopsis (97). Plant J. 79(2):322-333. (

2015

7. Ragel P., Rodenas R., Garcia-Martin E., Andres Z., Villalta I., Nieves-Cordones M., Rivero R. M., Martinez V., Pardo J. M., Quintero F. J., Rubio F. (2015) The CBL-Interacting Protein Kinase CIPK23 Regulates HAK5-Mediated High-Affinity K+ Uptake in Arabidopsis Roots. Plant physiol. 169(4):2863-2873.

8. Yang G., Sentenac H., Véry A. A., Su Y (2015) Complex interactions among residues within pore region determine the K+ dependence of a KAT1-type potassium channel AmKAT1. Plant J. 83(3): 401-412.

2016

9. **Damiani I., Drain A., Guichard M., Balzergue S., Boscari A., Boyer J.C., Brunaud V., Cottaz S., Rancurel C., Da Rocha M., Fizames C., Fort S., Gaillard I., Maillol V., Danchin E. G. J., Rouached H., Samain E., Su Y.H., Thouin J., Touraine B., Puppo A., Frachisse J.M., Pauly N., Sentenac H. (2016) Nod Factor Effects on Root Hair-Specific Transcriptome of Medicago truncatula: Focus on Plasma Membrane Transport Systems and Reactive Oxygen Species Networks. Front Plant Sci. 7 : 794.**

10. **Lefoulon C., Boeglin M., Moreau B., Véry A.A., Szponarski W., Dauzat M., Michard E., Gaillard I., Chérel I. (2016) The Arabidopsis AtPP2CA protein phosphatase inhibits the GORK K+ efflux channel and exerts a dominant suppressive effect on phosphomimetic activating mutations.** *J. Biol. Chem.* **291(12) : 6521-6533.**

11. Mouradi M., Bouizgaren A., Farissi M., Makoudi B., Kabbadj A., Véry A.-A., Sentenac H., Qaddoury A., Ghoulam C. (2016) Osmopriming improves seeds germination, growth, antioxidant responses and membrane stability during early stage of Moroccan alfalfa populations under water deficit. Chi.J.Agr.Res. 76 (3): 265-272.

12. Mouradi M., Farissi M., Bouizgaren A., Makoudi B., Kabbadj A., Véry A.A., Sentenac H., Qaddourya A., Ghoulam C. (2016) Effects of water deficit on growth, nodulation and physiological and biochemical processes inMedicago sativa-rhizobia symbiotic association. Arid.LandRes.Mana. 30 (2): 193-208.

13. **Tounsi S., Ben Amar S., Masmoudi K., Sentenac H., Brini F., Véry A. A. (2016) Characterisation of two HKT1;4 Transporters from Triticum monococcum to elucidate the Determinants of the Wheat Salt Tolerance Nax1 QTL. Plant Cell Physiol. 57(10): 2047-2057.**

14. Wang L., Yang S. Y., Guo M. Y., Huang Y. N., Sentenac H., Véry A. A., Su Y. H. (2016) The S1-S2 linker discriminates the distinct pH sensitivity between ZmK2.1 and KAT1. Plant J. 85(5): 675-685.

2017

15. Campbell M. T., Bandillo N., Al Shiblawi F. R.A., Sharma S., Liu K., Du Q., Schmitz A. J., Zhang C., Véry A.A., Lorenz A. J., Walia H. (2017) Allelic variants of OsHKT1;1 underlie the divergence between indica and japonica subspecies of rice (Oryza sativa) for root sodium content. PLOS Genetics.13 (6): e1006823.

16. **Corratgé-Faillie C., Ronzier E., Sanchez F., Prado K., Kim J.-H., Lanciano S., Leonhardt N., Lacombe B., Xiong T. C. (2017) The Arabidopsis guard cell outward potassium channel GORK is regulated by CPK33. FEBS Lett. 591(13):1982-1992.**

17. Garriga M., Raddatz N., Véry A.-A., Sentenac H., Rubio-Meléndez M. E., González W., Dreyer I. (2017) Cloning and functional characterization of HKT1 and AKT1 genes of Fragaria spp.—Relationship to plant response to salt stress. J Plant Physiol. 210: 9-17.

18. **Nieves-Cordones M., Mohamed S., Tanoi K, Kobayashi N., Takagi K., Vernet A., Guiderdoni E., Perin C., Sentenac H., Véry A. A. (2017) Production of low-Cs+ rice plants by inactivation of the K+ transporter OsHAK1 with the CRISPR-Cas system. Plant J. 92(1): 43-56.**

2018

19. Huang L.T., Zhao L.N., Gao L.W., Véry A.A., Sentenac H., Zhang Y.D. (2018) Constitutive expression of CmSKOR , an outward K + channel gene from melon, in Arabidopsis thaliana involved in saline tolerance. Plant Sci. 274: 492-502. (etr)

20. **Mohamed S., Sentenac H., Guiderdoni E, Véry A. A., Nieves-Cordones M. (2018) Internal Cs+ inhibits root elongation in rice. Plant Signal Behav. 13(2): e1428516.**

2019

21. **Wang L, Guo M-Y, Thibaud J-B, Véry A-A, Sentenac H. A repertoire of cationic and anionic conductances at the plasma membrane of** *Medicago truncatula* **root hairs. Plant J. 98(3): 418-433.**

Review articles

2014

1. Ahmadi N., Audebert A, Bennett M.J., Bishopp A., Costa de Oliveira A., Courtois B., Diedhiou A., Diévart A, Gantet P., Ghesquière A., Guiderdoni E., Henry A., Inukai Y, Kochian L., Laplaze L., Lucas M., Luu D.T., Manneh B., Mo X., Muthurajan R., Périn C., Price A., Robin S., Sentenac H., Sine B., Uga Y., Véry A.A., Wissuwa M., Wu P., XuJ. (2014) The roots of future rice harvests. Rice. 7: 29 (

2. **Véry A.A., Nieves-Cordones M., Daly M., Khan I., Fizames C., Sentenac H. (2014) Molecular biology of K+ transport across the plant cell membrane: What do we learn from comparison between plant species? J. Plant Physiol. 171(9):748-769.**

2016

3. **Nieves-Cordones M., Martinez V., Benito B., Rubio F. Comparison between** *Arabidopsis* **and rice for main pathways of K+ and Na+ uptake by roots. Front.Plant Sci. 7 : 992.**

4. **Nieves-Cordones M., Ródenas R., Chavanieu A., Rivero R. M., Martinez V., Gaillard I., Rubio F. (2016) Uneven HAK/KUP/KT Protein Diversity Among Angiosperms: Species Distribution and Perspectives. Front. Plant Sci. 7 : 127.**

2017

5. **Corratge-Faillie C., Lacombe B. (2017) Substrate (un)specificity of Arabidopsis NRT1/PTR FAMILY (NPF) proteins. J. Exp. Bot. 68(12): 3107-3113.**

Other articles (professional journals, etc.) (total number)

2017

1. Gantet P., Luu D. T. (2017) Les rizières du Mékong et du fleuve Rouge menacées par le sel In Science Du Sud (P: 8) Le journal de l'IRD Eds.- http://es.calameo.com/read/00331938484fa4a9a54e9?authid=2SPIpSwVikpM

2- Books

20% most sifnificant ones:

1. Peltier J.B., Fares A., Rossignol M. (2014) Identification of nitrosylated proteins (SNO) and applications in plants. *In Nitric Oxide in Plants: Metabolism and Role in Stress Physiology* (M. M. M. Nasir Khan, Firoz Mohammad, Francisco J. Corpas, eds) (p. 85-125).Springer International Publishing (Switzerland)

Monographs, critical editions, translations (total number)

Management and coordination of scientific books / Scientific book edition

Book chapters (total number)

Book chapters in English or another foreign language

2016

1. Nieves-Cordones M., Al Shiblawi F. R., Sentenac H. (2016) Roles and Transport of Sodium and Potassium in Plants. Met. Ions Life Sci. 16 291-324.

2017

2. Takano J., Yoshinari A., Luu D.T. (2017) Plant aquaporin trafficking. In: Plant Aquaporins/From Transport to Signaling, ed Chaumont F & Tyermann S. (Springer International Publishing), pp 47-81.

Edited theses

3- Production in conferences / congresses and research seminars

1. Chagvardieff P, Blin V, Charton F, Fornier A, Grange D, Guiderdoni E, Henner P, Messalier M, Paillard H, Prevost T, Rennesson M, Sarrobert C, Vavasseur A, Véry A-A (2016). DEMETERRES project: development of innovative technologies for removing radionuclides from contaminated solid and liquid matrices. 13th International Conference on Radiation Shielding (ICRS-13) & 19th Topical Meeting of the Radiation Protection & Shielding Division of the American Nuclear Society -2016 (RPSD-2016), Paris, France (3-6 October 2016). Published in conference proceedings: EPJ Web of Conferences, 153. Editions Presses de Sciences 9, DOI : 10.1051/epjconf/201715305026

2. Luu DT, Véry A-A. Hydromineral nutrition and adaptation to abiotic stresses in rice: Analysis of root aquaporins and Na^+/K^+ transport systems. (15.05.2014-16.05.2014) The Global Rice Partnership Root (GRISP) Workshop. Montpellier (France). Invited oral presentation

3. Sentenac H., Peltier J.-B. (2018) Interactions between rhizobia and root hairs: from *Medicago truncatula* to wheat via *Arabidopsis thaliana.* The 3rd *Molecular Plant* International Symposium. June 12-15, 2018·Xi'an, China. Invited oral presentation

4. Nguyen T-H, Meynard D, Guiderdoni E, Sentenac H, Véry A-A. Control of leaf transpiration in rice under salt stress: functional characterization and roles of potassium channels expressed in rice stomata. (26.09.2016-28.09.2016) 14th International Symposium on Rice Functional Genomics. Montpellier (France). Selected oral presentation

5. Nieves-Cordones M., Mohamed S, Meynard D, Périn C, Sentenac H, Guiderdoni E, Véry A-A. Functional and molecular analysis of Cs^+ uptake in rice, and its interaction with K^+ uptake. (26.09.2016-28.09.2016) 14th International Symposium on Rice Functional Genomics. Montpellier (France). Selected oral presentation

6. Guo M.Y., Wang L., Sentenac H., Véry A.A. (2018) Building a functional repertoire of ion channels active at the plasma membrane of apical regions of medicago truncatula legume root hairs. (5 -10 août) 12th Congress of the International Plant Molecular Biology. Montpellier (France). Selected oral presentation

7. Ronzier E., Corratgé-Faillie C., Sanchez F., Brière C., Thibaud J.B., Xiong T.C. (2018) Two Ca2+-dependent protein kinases (cpks) oppositely regulate a ubiquitous k+ channel in arabidopsis thaliana. (5 -10 août) 12th Congress of the International Plant Molecular Biology. Montpellier (France). Selected oral presentation

8. Peltier J.-B., Sentenac H. (2017) Réponses des poils absorbants à l'environnement abiotique et biotique, et développement d'un programme de phénotypage automatique. 5th Meeting on Nitrogen-Fixing Root Endosymbioses. (12.10.2017-13.10.2017) Toulouse. Selected oral presentation

Articles published in conference proceedings / congress

Other products presented in symposia / congress and research seminars

Invited oral presentations in international symposia/congress

2015

1. Véry A.-A., Cayrol B, Guiderdoni E, Mohamed S, Nieves-Cordones M, Sentenac H. Cs transport, root architecture and HKT transporters in rice. (15.10.2015) Biological Workshop on Cs Bioremediation. French Embassy in Tokyo. Tokyo (Japan)

2017

2. Véry A-A. Production of low-Cs^+ rice plants by inactivation of the K^+ transporter OsHAK1 with the CRISPR-Cas system. (16.10.2017-18.10.2017) Symposium Net4FS: an interdisciplinary training network to address key questions in plant development for food security. Montpellier (France).

Invited oral presentations in national symposia/congress

2014

1. Luu D.T., Véry A.A. Hydro-mineral nutrition and adaptation to abiotic stresses in rice: Analysis of root aquaporins and Na+/K+ transport systems. (May 15, 2014) Global Rice Science Partnership (GRiSP) meeting. Montpellier (France).

2018

2. Véry A-A, Luu DT. Analyse de mécanismes adaptatifs du riz à la contrainte saline. (06.06.2018) Visite de l'Académie d'Agriculture de France sur la thématique des avancées sur les recherches en biologie et génétique au service de la riziculture. Montpellier (France).

2019

3. Berthomieu P. (2019) Analyse de la teneur en minéraux dans le grain de blé dur. (9-20 mars 2019) Conférence orale aux Journées du groupe Céréales à paille. Montpellier (France).

Selected oral presentations in international symposia/congress

2016

1. Ben-Amar A, El Bouhmadi K, Sentenac H, Véry A-A, Mahboub S. Study of leaf rolling upon abiotic stress in wheat and rice. (18.12.2016-22.12.2016) Journées Internationales de Biotechnologie (JIB 2016) de l'Association Tunisienne de Biotechnologie. Sousse (Tunisia).

Selected oral presentations in national symposia/congress

2014

1. Wang L. Characterisation of legume root hair ion channels signalling the presence of symbiotic bacteria. Colloque annuel du Patch Club de Montpellier. (06.06.2014) Castelnau le Lez.

2017

2. Mohamed S., Nieves-Cordones M., Sentenac H., Véry A.-A., Guiderdoni E. (2017) Contrôle de l'accumulation de cations toxiques chez le riz par modification des transporteurs, de l'architecture et de l'anatomie racinaires: application à une culture sur des sols contaminés par le césium 137. "Rencontres Céréales" (03.03.2017) Montpellier.

3. Nguyen T.H. (2017) Role of outward Shaker channels in the control of leaf transpiration in rice. (30 juin) Colloque annuel du Patch Club de Montpellier. Castelnau le Lez.

4. Peltier J.-B., Sentenac H. (2017) Phénotypage des réponses racinaires du blé dur à l'inoculation par des bactéries diazotrophes. Workshop "*Interactions céréales micro-organismes bénéfiques du sol*" (Organis: Benoît Lefebvre, LIPM). (16.11.2017) Paris.

5. Rongsawat T., Peltier J.-B., Sentenac H. (2017) Phénotypage du développement de la zone pilifère en réponse à des bactéries rhizosphériques chez le blé: dispositif expérimental et analyse d'image automatisée. Journées du Réseau PhytoMic 2017 "*Diversité et rôle du microbiote dans la performance et l'adaptation de la plante*", Round Table "*Génétique et amélioration des plantes au sein du réseau PhytoMic*". (9.11.2017-10.11.2017) Rennes

2018

1. Fristot E. Caractérisation fonctionnelle en bicouche lipidique d'un transporteur de Na^+ de blé produit *in vitro*. Colloque annuel du Patch Club de Montpellier. (8 juin 2018) Montpellier.

Posters in international symposia/congress

2014

1. Drain A, Wang L., Véry A.-A., Fizames C., Pauly N., Gaillard I., Sentenac H. Early steps in nodulation signaling: role of ion channels in Medicago root hair. XVI International congress on Molecular Plant-Microbe Interactions.(July 6-10), Rhodes Island (Greece).

2. Leung J., Leonhardt N., Tran D., Véry A.A., Vavasseur A., Renou J.P., Kwak J., Sentenac H., Bouteau F., Jammes F. (2014). Metabolite switch with anti abscisic acid actvity (28 juillet-1 août 2014). 25th International Conference on Arabidopsis Research (ICAR), Vancouver (Canada).

3. Tounsi S, Véry A-A, Sentenac H, Masmoudi K, Brini F. Caractérisation fonctionnelle par électrophysiologie d'un transporteur HKT1;4-A1 de *Triticum monococcum*. (20.12.2014-24.12.2014) Journées Internationales de Biotechnologie 2014 (Association Tunisienne de Biotechnologie). Yasmine Hammamet (Tunisie)

2015

4. Ben Amar S, Masmoudi K, Véry A-A, Sentenac H, Brini F. Isolement et étude de l'activité des promoteurs des gènes *TdHKT1;4-1* et *TdHKT1;4-2* chez des plantes transgéniques de blé. (20.12.2015-24.12.2015) Journées Internationales de Biotechnologie 2015 (Association Tunisienne de Biotechnologie). Djerba (Tunisie).

5. Hmidi D, Messedi D, Sentenac H, Véry A-A, Abdelly C. Clonage et caractérisation électrophysiologique d'un transporteur HKT chez l'orge sauvage *Hordeum maritimum*. (20.12.2015-24.12.2015) Journées Internationales de Biotechnologie 2015 (Association Tunisienne de Biotechnologie). Djerba (Tunisie).

6. Leung J, Leonhardt N, Tran D, Véry A-A, Bouteau F, Jammes F. How C3 plants balance competing priorities between H_2O and CO_2. (29.06.2015-02.07.2015) 3rd International Symposium on Plant Signaling and Behavior. Paris (France).

7. Nguyen T-H, Meynard D, Guiderdoni E, Sentenac H, Véry A-A. Identification and functional characterization of inward Shaker channels expressed in rice stomata. (13.09.2015-16.09.2015) 26ème colloque "Canaux Ioniques", Sète, France.

8. Tounsi S, Véry A-A, Sentenac H, Masmoudi K, Brini F. Etude fonctionnelle de deux transporteurs HKT1;4 de *Triticum monococcum*. (20.12.205-24.12.2015) Journées Internationales de Biotechnologie 2015 (Association Tunisienne de Biotechnologie), Djerba, Tunisie.

9. Wang L, Sentenac H, Véry A-A. Electrophysiological characterization of *Medicago truncatula* root hair conductances potentially involved in the early Nod factor signaling in rhizobial symbiosis. (13.09.2015-16.09.2015) 26ème colloque "Canaux Ioniques". Sète (France).

2016

10. Al Shiblawi FR, Meynard D, Périn C, Guiderdoni E, Sentenac H, Véry A-A. Production of loss-of-function rice mutant lines in Na^+ transporter genes by using the CRISPR-Cas9 biotechnology. (26.09.2016-28.09.2016) 14th International Symposium on Rice Functional Genomics. Montpellier (France).

11. Ben-Amar A, El Bouhmadi K, Sentenac H, Véry A-A, Mahboub S. Enroulement foliaire chez le blé dur en conditions de stress hydrique. (18.10.2016-19.10.2016) Colloque International sur "L'Afrique face aux changements climatiques" labellisé "COP22", Faculté de Médecine et de Pharmacie. Casablanca (Maroc).

12. Mohamed S, Audebert A, Dardou A, Chaine C, Bureau C, Cayrol B, Vernet A, Uga Y, Courtois B, Véry A-A, Périn C, Guiderdoni E. DRO1 natural and induced alleles to explore the influence of root system architecture for reducing the uptake of toxic ions in contaminated paddy fields. (26.09.2016-28.09.2016) 14th International Symposium on Rice Functional Genomics. Montpellier (France).

2017

13. Doccula F.G., Candeo A., Luoni L., Grenzi M., Bassi A., Véry A.A, Costa A. Is the plasma membrane localization of GLR3.3 required for the aminoacid-induced cytosolic Ca^{2+} increase in Arabidopsis root cells? (05.09.2017-07.09.2017) Plant Calcium Signalling conference. Norwich (UK).

14. Suji S., Véry A.A., Vinekar R., Venkataraman G., Kumaresan K., Sowdhamini R., Parida A. The sodium transporter OcHKT1;5 from the halophytic wild rice *Oryza coarctata* Roxb. Tateoka shows distinct transport and structural determinants compared to rice OsHKT1;5. (21.02.2017-25.02.2017) InterDrought V conference hosted by ICRISAT (International Crop Research Institute for the Semi-Arid Tropics). Hyderabad (India).

Posters in national symposia/congress

2017

1. Mohamed S., Nieves-Cordones M., Meunier A.C., Vernet A., Périn C., Guiderdoni E., Sentenac H., Véry A.A. Production of low-Cs^+ rice plants by inactivation of the K^+ transporter OsHAK1 with the CRISPR-Cas9 system. Colloque Inter-LabEx CRISPR-Cas9. (17.05.2017) Montpellier (France).
2. Al Shiblawi F.R., Meynard D., Périn C., Guiderdoni E., Sentenac H., Véry A.A. Production of loss-of-function rice mutant lines in Na^+ transporter genes by using the CRISPR-Cas9 biotechnology. Colloque Inter-LabEx CRISPR-Cas9. (17.05.2017) Montpellier (France).

2018

3. Houdinet G., Guerrero-Galan C., Garcia K., Delteil A., Corratgé-Faillie C., Conéjéro G., Gaillard I., Touraine B., Sentenac H., Zimmermann S.D. (2018) Unravelling nutrient exchange in ectomycorrhizal symbiosis contributing to plant potassium nutrition. (5 -10 août) 12th Congress of the International Plant Molecular Biology. Montpellier (France).

Invited seminars in foreign institutions

2015

1. Véry A.-A. Rice adaptation to salinity: characterization of K^+ and/or Na^+ transporters from the HKT family. (25.09.2015) LMI-Rice, Hanoi (Vietnam).

2016

2. Véry A.-A.. Characterization of *Medicago* root hair K^+ conductances potentially involved in early steps of rhizobial symbiosis establishment. (30.06.2016) University of Würzburg, Würzburg (Germany).

2019

3. Véry A.-A. Molecular and functional analysis of K^+ transport in rice: HAK transporters and Shaker channels. (12.04.2019) IBMCP, Universitat Politecnica de Valencia-CSIC, Valencia (Spain).

Invited seminars in French institutions

2014

1. Luu D.T., Véry A.A. Water and K+ transports by roots: comparison between Arabidopsis and rice. (May 14 2014) Rhizopolis meeting, Montpellier (France).

4- Electronic tools and products

Softwares

Databases

5- Instruments and methodology

Prototypes

2019

Patch-clamp set-up equipped with cell-wall laser ablation device built for in situ patch clamp experiments on external root cells (Cf. Wang et al. 2019)

Platforms and observatories

6- Other products

Theorised artistic creations, staging, movies

7- Editorial activities

Participation in editorial committees (books, collections, etc.)

Collection and series management

8- Reviewing activities

Reviewing of articles

10 (Anne-Aliénor Véry), 10 (Doan Luu)

Grant evaluation (public or charities)

CAPES-COFECUB 2019 (Doan Luu)

Reviewing of research institutes

Participation in institutional committees and juries (CNRS, Inserm, etc.)

Member of Thesis committee: 10 (Doan Luu + Anne-Aliénor Véry)

Member of Thesis jury (reporter): 6 (Doan Luu + Anne-Aliénor Véry).

9- Academic research grants

European (ERC, H2020, etc.) and international (NSF, JSPS, NIH, World Bank, FAO, etc.) grants – coordination

European (ERC, H2020, etc.) and international (NSF, JSPS, NIH, World Bank, FAO, etc.) grants - partnership

Other European grants - coordination

2012 – 2014 ; PHC PRAD avec le Maroc ; Sélection et caractérisation de symbioses Medicago sativa/rhizobia tolérantes aux conditions salines : analyses moléculaires et approches agro-physiologiques ; Coordinateur : SENTENAC Hervé ; participante : VERY Anne-Aliénor ; responsable macocain : Pr Cherki Ghoulam (Université de Marrakesch)

2014 – 2012 ; PHC PRAD avec le Maroc ; Enroulement des feuilles et tolérance au stress hydrique chez le blé et le riz : Rôle de transporteurs membranaires de K+ et Na+ ; coordinateur : VERY Anne-Aliénor ; participant SENTENAC Hervé ; responsable marocain : Pr. Saïd Mahboub (Université de Casablanca)

2015 – 2017 ; PHC Hoa Sen Lotus avec le Vietnam ; Application of functional genomics and association genetics to characterize genes involved in abiotic stress tolerance in rice ; coordinateur : GANTET Pascal (IRD UMR Diade, Montpellier) ; participants : LUU Doan, VERY Anne-Aliénor, SENTENAC Hervé ; responsable Vietnamien : Hoang Thi Giang (LMI Rice, Hanoi)

2018-2020 ; **PHC ALADIN Maghreb (Partenariat Hubert Curien)** ; Adaptation des légumineuses au déficit hydrique et impact sur la culture de blé en rotation ; Coordinateur : BOSCARI Alexandre (Institut Sophia Agrobiotech, Nice Sophia Antipolis ; VERY Anne-Aliénor

Other European grants - partnership

National public grants (ANR, PHRC, FUI, INCA, etc.) - coordination

National public grants (ANR, PHRC, FUI, INCA, etc.) - partnership

Local grants (collectivités territoriales) - coordination

Local grants (collectivités territoriales) - partnership

PIA (labex, equipex etc.) grants - coordination

2018 – 2018 ; **(Labex AGRO - Projet Cultivar)** ; Rôle des systèmes de transport du potassium du riz colonisé par des champignons mycorhiziens à arbuscules et sous stress salin ; LUU Doan

2019 – 2019 ; **(Labex AGRO - Projet Cultivar)** ; Biologie cellulaire des systèmes de transport du potassium chez le riz ; LUU Doan

PIA (labex, equipex etc.) grants - partnership

2013 – 2019 ; **DEMETERRES (ANR RSNR)** ; Développement de méthodes bio-et Ecotechnologies pour la remédiation raisonnée des effluents et des sols en appui à une stratégie de réhabilitation post-accidentelle ; SENTENAC Herve, VERY Anne-Aliénor

Grants from foundations and charities (ARC, FMR, FRM, etc.) - coordination

CultiVar program n°1504-07 funded by Agropolis Fondation (2018 and 2019). 14.5kEUR (Doan Luu).

Grants from foundations and charities (ARC, FMR, FRM, etc.) - partnership

10- Visiting senior scientists and post-doc

Post-docs (total number)

Since 2014: 2

Foreign post-docs

Since 2014: 2

Family Name	First name	Contract type & Employer	**Nationality**	**Périod**	
Nguyen	Thanh Hao	CDD INRA ANR	VIETNAMIEN	30/01/2017	29/07/2017
Nieves Cordones	Manuel	CDD INRA ANR	ESPAGNOL	01/06/2014	31/05/2016

Visiting scientists (total number)

Since 2014 : 14

Foreign visiting scientists

Since 2014 : 15

Family Name	First name	Financing source	Nationality	Date of arrival	Date of departure	Status	University of origin
Ben Amar	Amal	Bourse campus France	Marocain	01/09/2014	31/12/2014	Doctorante invitée	Faculté des Sciences Ben M'sik Maroc
Ben Amar	Amal	Bourse campus France	Marocain	05/09/2015	31/12/2015	Doctorante invitée	Faculté des Sciences Ben M'sik Maroc
Ben Amar	Amal	Bourse campus France	Marocain	01/07/2016	18/11/2016	Doctorante invitée	Faculté des Sciences Ben M'sik Maroc
Doccula	Fabrizio Gandolfo	Bourse erasmus	Italien	01/02/2017	30/04/2017	Doctorant invité	Université Degli Studi Di Milano Milan
Hmidi	Dorsaf	Bourse d'alternance tunisienne	Tunisien	21/04/2014	15/08/2014	Doctorant invité	Centre de Biotechnologies de Borj Cédria (CBBC) Tunisie
Hmidi	Dorsaf	Bourse d'alternance tunisienne	Tunisien	15/03/2015	15/09/2015	Doctorant invité	Centre de Biotechnologies de Borj Cédria (CBBC) Tunisie
Kumari	KumKum	Bourse EMBO	Indien	15/06/2018	13/09/2018	Doctorante invitée	M S Swaminathan Research Foundation
Mouradi	Mohamed	Bourse campus France	Marocain	01/09/2014	31/12/2014	Doctorant invité	Université Cadi AYYAD Maroc
Tebini	Mohamed	Bourse tunisienne	Tunisien	01/05/2019	31/07/2019	Doctorant invité	Fac des sciences de Tunis
Tounsi	Sana	Bourse tunisienne	Tunisien	12/05/2014	15/08/2014	Doctorant invité	Centre de biotechnologie de Sfax
Tounsi	Sana	Bourse tunisienne	Tunisien	15/04/2015	31/07/2015	Doctorant invité	Centre de biotechnologie de Sfax
Wang	Limin	Bourse	Chinoise	01/11/2015	10/12/2015	Doctorant invité	Shanghai Jiao Tong University chine
Wang	Limin	Contrat BBSRC	Chinoise	12/08/2018	18/08/2018	Post-doctorante invité	Université de Cambridge UK
Wang	Limin	Contrat BBSRC	Chinoise	30/05/2018	20/06/2018	Post-doctorante invité	Université de Cambridge UK
Zhang	Yidong	Bourse chinoise CSC	Chinoise	01/12/2013	31/10/2014	Professeur invité	Université Jiaotong Shanghai Chine

11- Scientific recognition

Prizes and/or distinctions

IUF members

Chair of learned and scientific societies

Organisations of meetings and symposia (out of France)

Invitations to meetings and symposia (out of France)

Invitation to give a talk at the “Biological Workshop on Cs Bioremediation”, French Embassy in Tokyo, Japan (15.10.2015) (Anne-Aliénor Véry)

II - INTERACTION OF THE TEAM WITH THE NON-ACADEMIC WORLD, IMPACTS ON ECONOMY, SOCIETY, CULTURE OR HEALTH

1- Socio-economic interactions / Patents

Invention disclosures

Filed patents

Accepted patents

Licenced patents

2- Socio-economic interactions

Industrial and R&D contracts

Cifre fellowships

Creation of labs with private-public partnerships

Networks and mixed units

Start-ups

3- Expertise

Consulting

Participation in expert committees (ANSES etc.)

Legal expertise

Expert and standardization reports

4- Public outreach

Radio broadcasts, TV shows, magazines and newspaper

TV Shows : Gantet P., Luu D. T. (2017) Les rizières du Mékong et du fleuve Rouge menacées par le sel. Science au Sud 84: 8.

Journal articles, interviews, book edition, videos, other popularization outputs, debates on science and society, etc.

III - INVOLVEMENT OF THE TEAM IN TRAINING THROUGH RESEARCH

1- Educational outputs

Books

E-learning, MOOCs, multimedia lessons, etc.

2- Scientific productions (articles, books, etc.) from theses

Scientific productions (articles, books, etc.) from theses

8 (Anne-Aliénor Véry + Doan Luu)

Scientific articles

Ben Amar S., Brini F., Sentenac H., Masmoudi K., Véry A.A. (2014) Functional characterisation in Xenopus oocytes of Na+ transport systems from durum wheat reveals diversity among two HKT1;4 transporters. J. Exp. Bot. 65: 213-222. (

Campbell M. T., Bandillo N., Al Shiblawi F. R.A., Sharma S., Liu K., Du Q., Schmitz A. J., Zhang C., Véry A.A., Lorenz A. J., Walia H. (2017) Allelic variants of OsHKT1;1 underlie the divergence between indica and japonica subspecies of rice (Oryza sativa) for root sodium content. PLOS Genetics.13 (6): e1006823.

Nguyen T.H., Huang S., Meynard D., Chaine C., Michel R., Roelfsema R., Guiderdoni E., Sentenac H., Véry A.A. (2017) A dual role for the OsK5.2 ion channel in stomatal movements and K+ loading into xylem sap. Plant Physiol. 174(4): 2409-2418

Nieves-Cordones M., Mohamed S., Tanoi K, Kobayashi N., Takagi K., Vernet A., Guiderdoni E., Perin C., Sentenac H., Véry A. A. (2017) Production of low-Cs+ rice plants by inactivation of the K+ transporter OsHAK1 with the CRISPR-Cas system. Plant J. 92(1): 43-56.

Mohamed S., Sentenac H., Guiderdoni E, Véry A. A., Nieves-Cordones M. (2018) Internal Cs+ inhibits root elongation in rice. Plant Signal Behav. 13(2): e1428516.

Review articles

Véry A.A., Nieves-Cordones M., Daly M., Khan I., Fizames C., Sentenac H. (2014) Molecular biology of K+ transport across the plant cell membrane: What do we learn from comparison between plant species? J. Plant Physiol. 171(9):748-769. (doct)

Book chapters in English or another foreign language

Nieves-Cordones M., Al Shiblawi F. R., Sentenac H. (2016) Roles and Transport of Sodium and Potassium in Plants. Met. Ions Life Sci. 16 291-324.

Mean number of publications per student (Biology & Science and technology only)

1,4

3- Training

Habilitated (HDR) scientists

Since 2014 : 2

Family Name	First name	Position	Employer	Date of habilitation
LUU	Doan Trung	chercheur	CNRS	2014
VERY	Anne-Aliénor	chercheur	CNRS	2006

HDR obtained during the period

Since 2014 : 1 HDR

Family Name	First name	Position	Employer	Date of habilitation
LUU	Doan Trung	chercheur	CNRS	2014

PhD students (total number)

6 PhD students

PhD students benefiting from a specific doctoral contract

Family Name	First name	Starting date	Defense	Duration (months)	Funding
AL-SHIBLAWI	Fouad Razzaq	12/09/2014	15/11/2017	37	ETR
DALY	Mariem	01/06/2011	22/12/2014	42	ETR
MOHAMED	Sonia	01/10/2014	13/09/2017	36	CDE
NGUYEN	Thanh Hao	01/01/2014	16/12/2017	36	ETR
WANG	Limin	07/01/2013	16/07/2015	31	ETR
ZHOU	Jing	06/10/2017		36	ETR

Defended PhDs

5 defended PhDs

Family Name	First Name	Date of defense
AL-SHIBLAWI	Fouad Razzaq	2017
DALY	Meriem	2014
MOHAMED	Sonia	2017
NGUYEN	Thanh Hao	2017
WANG	Limin	2015

Mean PhD duration

Mean duration: 36 months

Internships (M1, M2)

Master 1: 5

Master 2: 2

Family Name	First Name	University Degree	Period	
Guyot	Valentin	Master 1	06/03/2017	30/06/2017
Przyszczypkowski	Marc	Master 1	20/03/2017	10/05/2017
Fristot	Elsa	Master 1	19/03/2018	03/08/2018
Raud	Elena	Master 1	04/03/2019	28/06/2019
Beaumont	Benoit	Master 1	25/03/2019	21/05/2019
Seck	Maguette	Master 2	08/01/2018	06/07/2018
Bui	Thai Xuan	Master 2	04/03/2019	30/08/2019

People in charge for a mention or a master's degree course (total number)

People in charge for a mention or a master's degree course with international certification (Erasmus mundus)

Member of PhD selection panel of Doctoral School GAIA (2016 and 2017) and Sciences du Végétal (Paris Orsay, 2018) (Doan Luu).

TSF team
Self-assessment document

Name of the team concerned by the current contract: Iron Transport and Signaling
Name of the team concerned by the next contract (if different): Metal Mobility
Acronym of the current contract: TSF
Acronym of the next contract (if different): MeMo
Team leader for the current contract: Catherine Curie
Team leader for the next contract: Catherine Curie

RESULTS

1- Presentation of the team

Introduction

TSF has emerged as an independent team in January 2008 from the split of the historic “Iron team” led by Jean-François Briat. From that time on, Catherine Curie has been the group leader of TSF. It was initially composed of 4 researchers and its main objective was to unravel the molecular mechanisms of iron uptake by the root, using the model plant *Arabidopsis thaliana*. We had published seminal papers identifying the main high affinity Fe transporters at the root surface of dicots and monocots and were then focusing on dissecting the regulatory pathways controlling root Fe acquisition. In parallel, we sought to understand how Fe is distributed within the plant, characterizing key transporters and refining the role of Fe ligands through fruitful collaborations with analytical chemists. Two of the 4 scientists of the team left in 2010 and 2011, among whom Grégory Vert who took with him the work on the regulation of Fe uptake. This left Catherine Curie and Stéphane Mari alone in charge of the projects of TSF. Fortunately, the team had been quite successful at obtaining ANR grants, so postdocs compensated for the loss of permanent scientists to support the different research programs. One of the key events of that period (2008-2012) was the development in the team of a histochemical method to stain and visualize Fe in plant tissues. With it we could investigate Fe mobility within the plant and identify the responsible transporters and ligands. The method, now largely used in our scientific community, undoubtedly increased the team’s international reach. Around that time, we described the presence of a large pool of Fe stored in the endodermal vacuoles of the embryo, which led us to focus on **Fe biology of the seed**, a line of work that **Stéphane Mari** has developed ever since. In particular we investigated the mechanisms of Fe loading during embryogenesis and Fe remobilization during germination, trying to identify transporters and Fe ligands acting in these processes. During the same period, through the characterization of NRAMP transporters with multiple metal specificity, we started some work on Mn transport and homeostasis. **Catherine Curie was in charge of the Mn project.** Thanks to two ANR postdocs**,** we characterized the main high affinity Mn transporter at the root surface on one hand, and the actors of Mn distribution on the other hand.

What distinguishes most the period that is under evaluation from the previous one is the arrival of 4 researchers in the team, which brought the number of permanent researchers of TSF from 2 in 2014 to 6 at the end of 2019 and which greatly reinforced our research programs on Fe and Mn homeostasis.

Given the growing interest of the team for Mn and more generally for other metals than iron, we have collectively opted for changing the team’s name from “Iron transport and Signaling” (TSF: 2008-2019) to “Metal Mobility” (Memo: 2019-).

Unit’s workforce and means

The team is now composed of 8 permanent members:

- 5 researchers (Catherine Curie, DR2 CNRS; Stéphane Mari, DR2 INRA; Léon Dirick, CR1 CNRS; Odile Richard, CR1 CNRS; Tou-Cheu Xiong, CR1 INRA) and by the end of the year 2019, 1 assistant professor (Loren Castaings, MdC),

- 0.5 ETP engineer (Carine Alcon, IE CNRS; Carine is 50% of her time responsible of the imaging platform of the lab), 1 technician (Sandrine Chay, TRSup INRA; 40% of her time is dedicated to the SAME platform), 0.5 ETP University technician (Stéphanie Loubet, 2 years extended leave 2017-2019).

2 non-permanent members: 1 postdoc ANR (Diego Almeida), 1 PhD student (Alexis Brun).

During the evaluated period, TSF hosted a total of 4 postdocs (Loren Castaings, 4 ½ years; Santiago Alejandro, 2 ½ years; Lili Wei, 2 ½ years, Diego Almeida, 3 years), 2 PhD students (Minh Hoang and Hai Le Minh whose thesis was unfortunately interrupted after 18 month), as well as two contractual technicians supported by private and/or University salaries (Antoine Caquot, 1 year; David Correia, 3 years).

TSF also received the visit of invited scientists including Talita Oliveira de Araujo (Brazilian PhD, 12 months), Angela Carrio (Spanish PhD, 6 months), Shailender Kumar (Indian, EMBO fellowship, 1 month), Akihiro SAITO (Japanese fellowship, 12 months), Alexandra Leskova (Slovakian fellowship, 4 months)

5 Masters students

The team's funding relied on **4 ANR contracts**, 3 as coordinator (MANOMICS 2012-2015, SUBCELIF 2013-2017, ISISTOR 2016-2020) and 1 as partner (PLANTMAN 2012-2015), **3 industrial and R&D contracts** (NUTRIPOL1 2015-2016; NUTRIPOL 2 2016-2018; AGROMATRIX 2019-2020), **1 project from the BAP INRA department** (IRONSEED 2016-2017).

Scientific policy

In cells, iron (Fe) and manganese (Mn) are precious trace metals that are used as cofactors in a range of enzymatic reactions. Importantly Fe and Mn are core-components of the photosynthetic apparatus and play a crucial role in oxidative stress responses or pathogen defense. Like all transition metals however, they become harmful in excess by catalyzing the production of reactive oxygen species. Fe and Mn deficiencies represent important agronomic issues since they occur in the widely represented alkaline soils where most of the metals are present as insoluble compounds, and since they greatly impact plant growth. To cope with Fe and Mn shortage, plants rely on the synthesis and exposure at the plasma membrane of high affinity transporters that were characterized in the TSF group: The Fe transporter IRT1 (Vert et al., 2002) and the Mn transporter NRAMP1 (Cailliatte et al., 2010). Historically, our team has been interested in understanding how plants regulate metal uptake in order to adapt to metal availability in the environment. In particular, earlier work of our team showed that IRT1 expression results from complex transcriptional and post-translational controls; The *IRT1* gene is transcriptionally activated by Fe deficiency via short and long-distance signaling (Vert et al., 2003) as well as post-translationally inhibited in Fe replete conditions through removal of the protein from the cell surface by endocytosis (Barberon et al., 2011); The actors of Fe uptake regulation have now been for a large part identified (Dubeaux et al., and ref therein). On the contrary, **very little is known about the mechanisms that regulate Mn uptake**.

Later on, our group has shifted its focus from metal uptake at the root surface to metal distribution to organs, cells and organelles. To that aim, we developed a specific, highly sensitive and easy to set up method to image Fe in plant tissues with a resolution reaching the subcellular level (Roschzttardtz et al., 2009a). This so-called Perls-DAB method has enabled us to establish an atlas of the major Fe pools in the main organs of the plant (Roschzttardtz et al., 2009b, 2013) and represents a long-awaited, invaluable tool to study Fe movement and Fe transporters activity within a plant. For instance, it helped us characterize Fe transport activity of YSL4 and YSL6 at the surface of the chloroplast (Divol et al., 2013). **For Mn however, there is no such tool available to probe Mn content and distribution in plant tissues.**

Understanding Fe movement within the plant is more complicated than just identifying its transporters in the different membranes. Indeed, Fe exists under a range of species, each one recruiting its own set of specific transporters; In addition to having two possible redox forms, reduced Fe^{2+} ions and oxidized Fe^{3+} ions, Fe will systematically react with organic ligands to form complexes. The identity of these Fe complexes, still little described, will vary in the different cell compartments according to the pH and the relative presence of ligands and chelatable metals. Therefore **Fe chelation is a central process in Fe homeostasis albeit poorly understood**. It involves Fe reductases and oxidases to control Fe redox state as well as enzymes of ligands biosynthesis pathway and transporters of these ligands. For example, by studying the citrate effluxer FRD3, we have previously shown that citrate, through the citrate-Fe complex, promotes apoplastic Fe mobility (Roschzttardtz et al., 2011).

Plants and seeds in particular represent the main dietary source of micronutrients for humans. The consumption of grains with low quantity of micronutrients is considered the main cause of mineral deficiencies in humans. Dietary Fe deficiency accounts for 90% of world anemia and is prevalent even in developed countries. In addition, Fe and Mn amounts in the seed are crucial for the vigor of the germinating plant because upon germination, photosynthesis resumes and relies on Mn and Fe reserves to feed the photosystems. In this context **understanding the molecular bases of Fe and Mn entry and storage in seeds** will help design **biofortification strategies** to produce plants with more concentrated and more available metals.

In the frame of this current contract (2014-2019), **the main objectives of the TSF team** have remained in line with what was initiated in the previous contract, i.e. to unravel the mechanisms that govern Fe and Mn homeostasis, with a stronger emphasis and work force still dedicated to Fe.

I. To study the mechanisms of Fe homeostasis, we investigated the process of Fe entry in the embryo during embryogenesis **(1)**, carried out a candidate gene approach to identify a root low affinity Fe transporter **(2)** and examined the role of Fe ligands in Fe distribution and Fe storage in seed through the use of alternative plant models **(3)**. We also launched a GWAS analysis to identify new actors of Fe remobilization from the seed during germination **(4)**.

II. To study the mechanisms of Mn homeostasis, we characterized a novel transporter involved in intracellular distribution of Mn **(1)** and unraveled the regulatory process at play in root Mn uptake, showing that NRAMP1 is post-translationally regulated at the level of membrane trafficking **(2)**.

III. In addition, during this evaluated period, the TSF team has invested part of its time to develop **applied research on agropolymers** in collaboration with an industrial partner, the Solvay company.

Actions taken in response to recommendations of the previous evaluation

At the time of the previous evaluation, the team was only composed of two researchers, with only one HDR. The committee of experts recommended that the team have at least one more HDR to avoid limitations in the number of thesis supervisions. This problem has now been solved since S. Mari obtained his HDR in 2014. Moreover, despite being judged excellent, the project appeared too ambitious to the committee relative to the available work forces. In particular, initiating a new line of work on Mn seemed risky in the context of such a small team. With the successive arrival of 4 scientists in the last 4 years, we have now gathered enough work force to develop in parallel two strong scientific programs on Fe and Mn homeostasis.

2- Research products and activities for the team

Scientific track record

I. Fe homeostasis

1. Ascorbate pathway: identification and cloning of TASC1 (ANR ISISTOR 2017-2020)

This work is supervised by Stéphane Mari. During the PhD thesis of Louis Grillet, the molecular bases of Fe acquisition by the embryo during its development were investigated. In collaboration with the LCABIE lab of analytical chemists in Pau, we have demonstrated that it involves a step of reduction of the Fe^{3+}-citrate-malate predominantly present in the embryo sac (**Flis et al., 2016**) to produce Fe^{2+} that is taken up by the embryo. This reduction is catalyzed by the release of ascorbate by the embryo (**Grillet et al., 2014**). These results served as ground work to ask which transporter could mediate ascorbate efflux. Our Vietnamese PhD student, Minh Hoang, carried out a yeast functional screening designed to replace a defective yeast ferric reductase activity by an ascorbate efflux activity encoded by an Arabidopsis cDNA. Minh isolated TASC1, a MATE family member. TASC1 is a tonoplast ascorbate transporter (collaboration intra-BPMP with the HoNuDe team), the inactivation of which results in decreased tolerance to Fe deficiency (**Hoang et al., under revision**). Since a *tasc1* knockout mutant fails to remobilize Fe stores during early germination, an appealing hypothesis is that **TASC1 transports ascorbate into the vacuole to reduce Fe^{3+}** and provide Fe^{2+} to the NRAMP3 and NRAMP4 tonoplast transporters that together remobilize Fe upon germination.

2. IRT1 and NRAMP1 have overlapping roles in low affinity Fe transport (ANR France-Taiwan MANOMICS 2012-2016)

When scarce in the medium, Fe and Mn are respectively taken up by the high affinity transporters IRT1 and NRAMP1 (Vert et al., 2002; Cailliatte et al., 2010). The molecular bases for low affinity transport however remained unknown. Since IRT1 and NRAMP1 have a broad range of possible metal substrates, we tested the hypothesis that they might have overlapping functions by generating an *irt nramp1*double mutant. These plants showed extreme Fe-deficiency symptoms even in the absence of Fe shortage. Physiological and molecular characterization of the double mutant, together with the analysis of promoter-swap lines between the two genes suggested that **NRAMP1 and IRT1 cooperate in the low affinity range to take up Fe in the root** (**Castaings et al., 2016**).

3. Identification and role of Fe ligands (Curie and Mari, 2017)

- Role of phytate in Fe storage in seeds (ANR SUBCELIF 2013-2017)

In the Arabidopsis embryo, iron is stored in vacuoles of endodermal cells and this particular pool serves as the primary source of Fe during the early steps of post-germinative growth (Roschzttardtz et al 2009). To unravel the overall process of Fe remobilization we first focused on the chemical characterization of this specific pool that was proposed to correspond to Fe-phytate. In a first approach, we could show in collaboration with Hatam Rouached in BPMP, that the **overexpression of a bacterial phytase in Arabidopsis did accelerate the remobilization of Fe from vacuoles** (**Belgaroui et al 2014**). We then confirmed the identity of Fe-phytate by synchrotron radiation XRF imaging and X-ray spectroscopy in collaboration with MP Isaure (LCABI, Pau). By a candidate gene approach, we could then identify two phytases belonging to the Purple Acid Phosphatase family, **PAP15 and PAP17, as key players involved in the hydrolysis of phytate and the release of vacuolar Fe** in the early steps of seedling growth (**Chay et al, in prep**).

- Role of nicotianamine in Fe mobility in the moss *Physcomitrella patens*

Nicotianamine (NA) is a plant-specific Fe ligand, found in particular in phloem and xylem saps and known to play a role in long distance transport in higher plants (Curie et al., 2009). NA-metal complexes cross cellular membranes via members of the YSL family of transporters, 8 in total in Arabidopsis. In our group, we discovered that YSL4 and YSL6 are transporters of the plastid envelope and mediate Fe efflux from the chloroplast in dry seed embryo (Divol et al., 2013). This suggested for the first time that in addition to its role in long-distance Fe mobility, NA also has a cellular function. With the idea to uncouple the two functions of NA, we have **introduced in the group the non-vascular plant model *Physcomitrella patens***, the genome of which contains only 1 gene for NA synthase and 2 YLS genes highly homologous to YSL4 and YSL6 from Arabidopsis. In collaboration with Alessandro Alboresi (University of Padova, Italy), we have generated **knockouts of PpNAS, PpYSLa and PpYSLb, to characterize their metal homeostasis defects.** The *Ppnas* mutant is impaired in gametophore growth and Fe content, and fails to develop reproductive organs, especially under Fe deficiency; the *ysla yslb* double mutant has reduced Fe concentration in the sporophyte (Dirick et al., unpublished). Together these results suggest a role of NA in Fe homeostasis of the moss.

4. Natural variation as a tool to discover new actors of Fe homeostasis (ANR ISISTOR)

Odile Richard, CR1 CNRS, joined the team in September 2016. Odile is an accomplished geneticist and distinguished herself in the past by cloning a QTL of Zn tolerance in Arabidopsis (Pineau et al., 2012). We have therefore taken the opportunity of Odile's arrival in the team to launch a campaign of gene discovery through quantitative genetics studies. O. Richard and S. Chay are conducting a **GWAS study on a population of 350 natural accessions of Arabidopsis** to identify the genetic determinants of the **remobilization of Fe stores during germination**. 2 day-old seedlings have been histochemically stained for Fe and scanned using a Hamamatsu digital slide scanner to assign quantitative scores to the signal intensity or area of Fe staining in the main tissues. These traits have been used to feed the GWAPP application. 8 QTLs have been selected and putative candidate genes are being tested by reverse genetics.

II. Mn homeostasis

This second axis of the TSF projects, launched during the previous 5 year-contract, has gained some strength in the last period thanks to 2 ANR projects and 2 postdocs (Loren Castaings and Santiago Alejandro) who have shared the work between the study of NRAMP1 regulation on one hand, and the characterization of NRAMP2 function on the other hand.

1. Identification of NRAMP2 as a key actor of intracellular Mn distribution (ANR PLANTMAN 2012-2015)

We have shown that among the members of the NRAMP metal transporters, NRAMP2 is a trans-Golgi network protein that transports Mn. Knockout mutants of NRAMP2 have decreased activity of the photosystem II owing to a lower level of Mn in chloroplasts. More generally, several organelles of these mutants have a decreased content of Mn. This suggests that **NRAMP2 functions by building up a cytosolic pool of Mn, which is used to feed downstream organelles (Alejandro et al., 2017**).

2. NRAMP1 is post-translationally regulated through membrane trafficking (ANR MANOMICS)

The root high affinity Mn transporter NRAMP1 is little regulated by the availability of its substrate at the transcript or protein levels (**Rodriguez-Celma et al., 2016).** Instead, we uncovered an original regulatory process by Mn-dependent phosphorylation-triggered endocytosis. NRAMP1 has a dual localization in the plasma membrane (PM) and in the endomembrane system (**Agorio et al., 2017**) and cycles between these two membrane compartments according to the availability of Mn (**Castaings et al., in prep**). When Mn is in excess, NRAMP1 is phosphorylated at the PM on serine residues located at the N-terminus of the protein, which in turn triggers its internalization through clathrin-dependent endocytosis. Mutating the Nter serines in alanines abolishes internalization of NRAMP1, whereas a serine to aspartate mutation, which mimics phosphorylation, induces constitutive removal of NRAMP1 from the PM (**Castaings et al., in prep.**).

III. Test of Agropolymers as biostimulating agents (SOLVAY-Agropolis Fondation 2015-16/2019, SOLVAY 2016-18)

In 2015, our team had the opportunity to participate in a small consortium involving the industrial company Solvay-Rhodia and the Agropolis Foundation, which funded in equal parts a research project aimed at assessing the impact of agropolymers (AP) on plant hydro-mineral nutrition. Initially, we chose to perform the study on Arabidopsis (**NUTRIPOL1, 12 months**), with the hope that once the target process will be identified, the numerous tools provided by this model species would help us to quickly pinpoint the molecular processes at play. Elemental analysis via MP-AES measurement of the plant mineral content upon AP treatment failed to reveal a clear impact of the AP on nutrition. Therefore, in the frame of a second contract (**NUTRIPOL2, 18 months**), financially supported by Solvay alone this time, we investigated the impact of the AP on the transcriptome of Arabidopsis. We found that the AP modifies the expression of genes of the photosynthetic machinery and genes involved in cell wall synthesis. This result has served as ground work to design transcriptomic studies of the response of maize to the AP, which will be developed through a third program co-funded again by Solvay and the Agropolis Foundation during the year 2019 (**AGROMATRIX, 6 months**).

Key events

1. The SAME platform of elemental analysis

The platform SAME (https://www1.montpellier.inra.fr/wp-inra/bpmp/en/platform/multi-elemental-analyses-service/) was created in July 2013 and is managed by the CNRS. The MP-AES apparatus was financed by the lab funds as well as by the INRA BV department contract ASAEfflux (2013) of our team, and upgraded through the TSF ANR project ISISTOR. Sandrine Chay, TRSup of the team, dedicates 40% of her time to run the platform. This common service is open to the research teams of the laboratory but also to all academic laboratories and private companies, through a common procedure that includes the initial dialog with the users, the emission of a quotation (3 formulas are proposed according to the level of technical support requested) and the production of a final report with the quantification of the requested elements. In practice, the platform realizes an average of 15,000 analyses per year, of samples from plant origin (roots, shoots, fruits, seeds, fluids) or soils. Beside BPMP users, the platform was also used by several academic laboratories (IATE, DIADE, LSTM, LEPSE) and one private company (Fertil'Inov). The general procedure for the quantification of elements from plant material was developed and published as an application note (https://www.agilent.com/cs/library/applications/4210_MP-AES-5991-7937EN-plants-app.pdf). Upon special requests, the platform can develop specific procedures (for instance, we developed a procedure to mineralize and quantify silicon from Sorgho samples at the request of JL Verdeil (DIADE, CIRAD, Montpellier).

2. Interaction with an Industrial partner

Over the last period, our team has managed to attract 3 successive contracts (for a total of 300 k€) with an industrial partner, Solvay, a world leading company in chemistry.

FIVE-YEAR PROJECT AND STRATEGY

1- SWOT analysis

- its strong points;

- 6 researchers/Assistant Professor with complementary expertise on cell biology, genetics, (bio)chemistry, molecular biology
- Transversal collaborations with biophysicians and analytical chemists
- Well recognized in the iron community (see iron meeting invitations)
- Partnership with private company

- needs for improvements; cultivated plants

- Increase PhD funding and students
- Extend collaborations
- Add cultivated plants in the studies
- Increase interactions with the local environment

- possibilities brought in by the context / surrounding environment;

- SOLVAY-LABEX projects NUTRIPOL and AGROMATRIX
- Flagship LABEX project CalClim (including local partners: LGDP, Eco&Sols, AGAP…)

- risks linked to the context / environment.

- Our research on model plants is not properly funded by local funding agency (LABEX)
- Competition might arise from overlapping research projects between TSF and FeROS teams
- Deficit of HDR in the team (2/6)

2- Structure, workforce and scientific orientations

The two main axes of research of the team, which are the studies of iron and manganese homeostasis, will be pursued in the next 5 year-contract. In addition, through local projects (MUSE and LABEX), our team has initiated a transition towards climate change (CO_2 and high temperature) and its impact on plant nutrition.

I. Iron homeostasis

1. Forward genetic screens to identify a low affinity Fe transporter - TC Xiong

Under Fe limitation, Fe enters the root through the IRT1 transporter (Vert et al., 2002). However the absence of IRT1 can be bypassed by supplying plants with an excess of Fe, owing to the activity of a low affinity transport system, the identity of which is unknown. TC Xiong will try and identify a low affinity Fe transporter through two different genetic screens.

a. Deregulation of IRT1::LUC in the *irt1* ko mutant

Under the *IRT1* promoter, the Luciferase (LUC) reporter gene is turned on when the plant experiences Fe deficiency. Consequently, LUC is on when expressed in the *irt1* knockout mutant but can be turned off if plants are supplied with an excess of Fe. TC Xiong will screen an EMS-mutagenized population of *irt1/IRT1::LUC* plants on excess Fe conditions. Plants showing LUC activity will represent candidate mutants in a low affinity transport system. The population of EMS plants is already available.

b. amiRNA transporter library screening in the *irt1* ko mutant

The same experiment will be designed only this time by using the PHANTOM sublibrary of amiRNAs that target specifically the 1777 putative transporters of Arabidopsis. This library has been sent to us by Eilon Shani (Tel Aviv, Israel) and will be transformed into the *irt1* mutant. Plants that fail to grow on high Fe will be selected.

2. Fe homeostasis in the seed - S. Mari (ANR ISISTOR 2017-2020)

a. New actors in the regulation of the redox status of Fe - D. Melo-Almeida (Postdoc ANR)

Based on previous results obtained by the team on the chemical forms of Fe that is translocated in seeds (Grillet et al, 2014; Flis et al, 2016), the objective is to identify new actors involved in the process of Fe transport. We are focusing on transporters of organic acids (citrate and malate, Fe chelators) and ascorbate (Fe reduction), through a candidate gene approach. Genes belonging to the ALMT and MATE families that are specifically expressed in seed tissues will be picked for further analyses.

b. Fe unloading from seeds: a GWAS study- Odile Richard, S. Chay

The first campaign of Perls-DAB iron staining of plantlets from 350 natural accessions of Arabidopsis has been realized. The images obtained were analyzed and the corresponding GWAS identified 4 significant QTLs and numerous minor but reproducible and significant QTLs. **We are currently focusing our investigation on 8 QTLs**. knockout mutants, gene expression as well as local LD studies are being carried out for the genes belonging to a region of approx. 10 kb surrounding each of these 8 QTLs. This analysis, together with a fine kinetics of the iron remobilization process on extreme accessions, should provide a better understanding of the processes of iron remobilization from iron sources in the embryo to iron sinks in the developing plant.

3. Fe speciation

a. Implementation of redox-specific probes to image Fe^{2+} and Fe^{3+} - TC Xiong, C. Alcon

Following up on the powerful Perls-DAB method to stain Fe in tissues which our team implemented in the previous period, we are now dedicating much effort to **develop redox-specific probes for Fe that will allow to distinguish real-life fluxes of Fe^{2+} and Fe^{3+} *in planta***. Tou-Cheu Xiong, CR1 INRA, joined us in May 2017 to develop cellular biology approaches using his past expertise on genetically-encoded calcium probes. Together with Carine Alcon, IE CNRS in the team, they are implementing fluorescent dyes for **Fe^{2+} or Fe^{3+}**, which they can apply simultaneously on plantlets to observe the relative distribution of the two Fe redox forms in wild type as well as in Fe homeostasis mutants (Xiong, Alcon et al., unpublished). In parallel, Tou-Cheu is attempting to generate genetically-encoded Fe biosensors based on characterized Fe-binding proteins. These probes will represent valuable tools in assigning a function to putative Fe transporters and Fe oxidases/reductases.

b. New actors in the regulation of the redox status of Fe – S. Mari, A. Brun (PhD)

The balance between Fe^{2+} and Fe^{3+} is key to regulate processes such as transmembrane transport, complexation or storage. Historically, the reduction processes have received most of the attention, leaving the ferroxidation side almost uncharacterized. We have recently identified three proteins belonging to the MultiCopper Oxidase family (MCO) that could potentially act as ferroxidases. Preliminary results indicate that these proteins might be involved in preventing excess Fe to enter cells by keeping Fe oxidized in the apoplast. The biochemical, physiological and molecular characterization of these new genes is being pursued by Alexis Brun during his PhD.

c. Fe ligation: role of nicotianamine in *P. patens* – L. Dirick

In addition to continuing the characterization of the *Ppnas* mutant, we will use a **philogenetic approach** to help understand the role of NA. The finding of a NA synthase gene in the descendant of early land plants, the moss *Physcomitrella patens*, raises the question of its putative role, long before the development of the vascular tissues of Trachaeophytes. We have initiated and will pursue the analysis of *NAS* origin and distribution during Bryophyte and Trachaeophyte evolution, and correlate it with changes (ecological, developmental, cellular, etc…) in those groups. For example, preliminary data indicate that NAS arose from **two horizontal gene transfers** during the evolution. When possible, the predictions arising from those evolutionary scenarios will be tested in the laboratory on the descendant of those early land plants, the moss *P. patens*.

II. Manganese homeostasis- C. Curie, L. Castaings

To support the Mn project that follows, an ANR proposal (DEFIMAN) has been submitted and is under evaluation.

1. Mn-dependent post-translational regulation of NRAMP1

We hope to decipher the post-translational regulation of NRAMP1 and thus grasp the strategy by which plants adapt Mn uptake to the plant needs. We will focus mainly on **finding the protein partners of NRAMP1 that**

mediate post-translational modifications. We have shown the role of NRAMP1 phosphorylation in Mn-triggered endocytosis of NRAMP1 and we have obtained preliminary results, via a collaboration with Gregory Vert (LRSV, Toulouse) indicating that ubiquitination could also be involved. i) A transcript profiling of the early response to Mn exposure will be realized; ii) Post-translational modifications of NRAMP1 (phosphorylations, ubiquitinations) will be mapped by mass spectrometry in the MSPP platform at BPMP; iii) Candidate partners will be searched by split-LUC screening and reverse genetics among members of E3 ubiquitin ligases, CIPK and CPK families of kinases and among candidates released from the membrane-based interactome database Associomics; iv) A PHANTOM sublibrary of amiRNAs targeting phosphatases and kinases will be screened in order to identify kinases from multigenic families with overlapping function.

2. Mn allocation to the seed

We have observed that Mn translocation from old leaves to the seed is particularly inefficient compared to other metals. We hope to decipher the molecular bases for this translocation through a **GWA strategy** (see § III below) and through a **candidate gene approach**. A number of protein families that mediate intracellular Mn transport show expression in the seed. These include NRAMP (6), CAX (2), ECA (2), MTP (2), ZIP (1). We will use reverse genetics and combinations of mutant, that have been in part generated in the team, to investigate the role of these transporters in Mn supply and homeostasis in the seed.

This objective, together with the knowledge of the regulation of Mn acquisition by NRAMP1, will serve to design **strategies for the optimization of Mn content in seeds**.

3. Implementation of Mn biosensors – TC Xiong

To investigate Mn distribution in tissues and especially in the seed, we plan to have two complementary approaches. i) We will collaborate with Marie-Pierre Isaure (IPREM, Pau) to have access to synchrotron-based X-ray microtomography. Our team has had successful interactions with this team in the past to identify Fe pools in the embryo (Roschzttardtz et al, 2011); ii) TC Xiong hopes to be able to implement new methods of imaging of Mn in tissues: through the adaptation of Mn-specific fluorescent dyes on one hand, and by trying to produce a genetically-encoded biosensor derived from known Mn-interacting proteins on the other hand. These tools will help understand the function of newly identified actors of Mn homeostasis.

III. QTL analysis to identify genetic determinants of Fe and Mn allocation into seeds – O. Richard, S. Chay

In parallel to the GWA study of Fe remobilization during germination, Odile Richard and Sandrine Chay are focusing on the process of **metal loading in the seed** with an emphasis on Fe (**ANR ISISTOR**) and Mn. They have scored the **fraction of metal allocated to seeds**, a proxy for the metal harvest index, in a **population of 164 RILs lines**, using samples generated and kindly provided by the lab of Celine Masclaux (IJPB, Versailles). This quantitative trait showed a significant variability for Fe and Mn as well as a strong heritability (around 0.9). QTL identification/mapping is under way and the role of the candidate QTL genes will be confirmed by classical reverse genetics analysis.

Comment: Introduction of new approaches with transversal benefits for the group. In the last 3 years, several colleagues have joined the group, enriching it with complementary skills, which should help remove bottlenecks in our projects. Odile Richard is managing an original quantitative genetics approach, transversal in the team, to discover novel genetic determinants of Fe and Mn loading in the seed and Fe remobilization during germination, and has already obtained promising preliminary results. Moreover, Tou-Cheu Xiong brings his expertise in cell biology and ion imaging to differentially visualize Fe^{2+} and Fe^{3+} ions in plant tissues. His investment, if successful, will equip the community with a means to assess the role of these redox forms of Fe in redox cycling processes and assist in characterizing candidates of Fe reductases and oxidases. In the future, Tou-Cheu will design similar strategies to implement specific Mn biosensors.

IV. Climate change

The climate changes are predicted to have a massive impact on agriculture, due to the direct and induced effects of increasing temperatures and elevated CO2. The TSF team is thriving to contribute to this socio-economic demand, through its implication in a MUSE project (eCO2 THREATS) involving 4 research units of Montpellier and by leading a flagship project proposal to the Agropolis Foundation (CalClim) involving BPMP and the LGDP.

1. MUSE project eCO_2 THREAT – S. Mari

The aim of this project is to evaluate the impact of elevated CO2 on the physiology, yield and nutritional value of the plants. TSF will participate by quantifying the ionome of plant organs in response to high CO_2 and by running a GWAS approach on the metal content in seeds of the model plant Arabidopsis.

2. Agropolis Foundation Flagship project CalClim – S. Mari

The objective of the CalClim project is to characterize the responses of plants (Arabidopsis, Medicago, Durum wheat) to multiple stresses by the combination of a complex nutritional constraint (calcareous soils) and high temperatures. This project proposal is co-elaborated by BPMP (S. Mari) and the LGDP (JP. Reichheld) and will also involve the AGAP and Eco&Sols units. The TSF team will participate in the 4 work packages of the project (phenotypical characterization of the plants' responses to the calcareous and high temperature stress, transcriptomics/epigenetics, GWAS on growth and ionomics, systems biology).

The SWOT analysis pointed out **too few interactions with local environment and little interest of the team for cultivated species.** These weaknesses should be improved through the MUSE and LABEX Flagship projects.

References:

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Alejandro, S., Cailliatte, R., Alcon, C., Dirick, L., Domergue, F., Correia, D., Castaings, L., Briat, J.F., Mari, S. and Curie, C. (2017) Intracellular Distribution of Manganese by the Trans-Golgi Network Transporter NRAMP2 Is Critical for Photosynthesis and Cellular Redox Homeostasis. *Plant Cell*, **29**, 3068-3084.

Barberon, M., Zelazny, E., Robert, S., Conejero, G., Curie, C., Friml, J. and Vert, G. (2011) Monoubiquitin-dependent endocytosis of the IRON-REGULATED TRANSPORTER 1 (IRT1) transporter controls iron uptake in plants. *Proceedings of the National Academy of Sciences of the United States of America*, **108**, E450-458.

Belgaroui, N., Zaidi, I., Farhat, A., Chouayekh, H., Bouain, N., Chay, S., Curie, C., Mari, S., Masmoudi, K., Davidian, J.-C., Berthomieu, P., Rouached, H. and Hanin, M. (2014) Overexpression of the bacterial phytase US417 in Arabidopsis reduces the concentration of phytic acid and reveals its involvement in the regulation of sulfate and phosphate homeostasis and signaling. *Plant and Cell Physiology*, **55**, 1912-1924.

Cailliatte, R., Schikora, A., Briat, J.F., Mari, S. and Curie, C. (2010) High-affinity manganese uptake by the metal transporter NRAMP1 is essential for Arabidopsis growth in low manganese conditions. *The Plant cell*, **22**, 904-917.

Castaings, L., Caquot, A., Loubet, S. and Curie, C. (2016) The high-affinity metal Transporters NRAMP1 and IRT1 Team up to Take up Iron under Sufficient Metal Provision. *Sci Rep*, **6**, 37222.

Castaings, L., Alcon, C., Curie, C. Activity of the NRAMP1 manganese transporter is controlled by its substrate availability via phosphorylation-mediated endocytosis. ***In prep.***

Chay, S., Chaumont, N., Kurita, Y., Alcon, C., Isaure, M.-P., Trcera, N., Mimura, T., Bailly, C., Curie, C. and Mari, S. Iron remobilization in Arabidopsis seeds during germination requires the hydrolysis of phytate complexes by the purple acid phosphatases PAP15 and PAP17. Scientific Reports (in prep.)

Divol, F., Couch, D., Conejero, G., Roschzttardtz, H., Mari, S. and Curie, C. (2013) The Arabidopsis YELLOW STRIPE LIKE4 and 6 transporters control iron release from the chloroplast. *The Plant cell*, **25**, 1040-1055.

Dubeaux, G., Neveu, J., Zelazny, E. and Vert, G. (2018) Metal Sensing by the IRT1 Transporter-Receptor Orchestrates Its Own Degradation and Plant Metal Nutrition. *Mol Cell*, **69**, 953-964 e955.

Flis, P., Ouerdane, L., Grillet, L., Curie, C., Mari, S. and Lobinski, R. (2016) Inventory of metal complexes circulating in plant fluids: a reliable method based on HPLC coupled with dual elemental and high-resolution molecular mass spectrometric detection. *The New phytologist*, **211**, 1129-1141.

Grillet, L., Mari, S. and Schmidt, W. (2014) Iron in seeds - loading pathways and subcellular localization. *Front Plant Sci*, **4**, 535.

Hoang, M., Almeida, D., Chay, S., Alcon, S., Faillie, C., Gibrat, R., Curie, C. and Mari, S. TASC1, a member of the Multidrug And Toxic Compound Extrusion family, is a vacuolar ascorbate transporter that controls intracellular iron cycling in Arabidopsis. Nat Comm (under revision)

Oliveira de Araujo, T., Isaure, M.-P., Trcera, N., Chay, S., Alcon, C., Campos da Silva, L., Curie, C. and Mari, S. *Paspalum urvillei*, an iron tolerant plant with multiple strategies to cope with iron toxicity. Front Plant Sci (under revision).

Rodriguez-Celma, J., Tsai, Y.H., Wen, T.N., Wu, Y.C., Curie, C. and Schmidt, W. (2016) Systems-wide analysis of manganese deficiency-induced changes in gene activity of Arabidopsis roots. *Sci Rep*, **6**, 35846.

Roschzttardtz, H., Conejero, G., Curie, C. and Mari, S. (2009) Identification of the endodermal vacuole as the iron storage compartment in the Arabidopsis embryo. *Plant Physiol*, **151**, 1329-1338.

Annex 4 – Research products and activities

EVALUATION CAMPAIGN 2019-2020

GROUP A

Name of the team concerned by the current contract:	Iron Transport and Signaling
Name of the team concerned by the next contract (if different):	Metal Mobility
Acronym of the current contract:	TSF
Acronym of the next contract (if different):	MeMo
Team leader for the current contract:	Catherine Curie
Team leader for the next contract:	Catherine Curie

I - PRODUCTION OF KNOWLEDGE AND ACTIVITIES CONTRIBUTING TO THE INFLUENCE AND SCIENTIFIC ATTRACTIVENESS OF THE TEAM

1- Articles

Scientific articles

1. **Grillet L., Ouerdane L., Flis P., Hoang M.T.T., Isaure M.P., Lobinski R., Curie C. ,Mari S. (2014) Ascorbate efflux as a new strategy for iron reduction and transport in plants. J. Biol. Chem. 289(5): 2515-2525.(doct, fr)**

2. **Castaings L., Caquot A., Loubet S., Curie C. (2016) The high-affinity metal Transporters NRAMP1 and IRT1 Team up to Take up Iron under Sufficient Metal Provision. Sci. Rep. (nov 16). 6 37222.**

3. **Alejandro S., Cailliatte R., Alcon C., Dirick L., Domergue F., Correia D., Castaings L., Briat J.F., Mari S., Curie, C. (2017) Intracellular manganese distribution by the Trans-Golgi Network NRAMP2 transporter is critical for photosynthesis and cellular redox homeostasis. Plant Cell, 29(12): 3068-3084.**

2014

4. **Belgaroui N., Zaidi I., Farhat A., Chouayekh H., Bouain N., Chay S., Curie C., Mari S., Masmoudi K., Davidian J.C., Berthomieu P., Rouached H., Hanin M. (2014) Over-expression of the Bacterial Phytase US417 in Arabidopsis Reduces the Concentration of Phytic Acid and Reveals Its Involvement in the Regulation of Sulfate and Phosphate Homeostasis and Signaling. Plant Cell Physiol 55 (11): 1912-24.**

2016

5. Flis P., Ouerdane L., Grillet L., Curie C., Mari S., Lobinski R. (2016) Inventory of metal complexes circulating in plant fluids: a reliable method based on HPLC coupled with dual elemental and high resolution molecular mass spectrometric detection. New Phytol. 211 (3): 1129-1141.

6. Rodriguez-Celma J., Tsai Y. H., Wen T. N., Wu Y. C., Curie C., Schmidt W. (2016) Systems-wide analysis of manganese deficiency-induced changes in gene activity of Arabidopsis roots. Scientific reports. 6: 35846.

2017

7. Agorio A., Giraudat J., Bianchi M. W., Marion J., Espagne C., Castaings L., Lelievre F., Curie C., Thomine S., Merlot S. (2017) Phosphatidylinositol 3-phosphate-binding protein AtPH1 controls the localization of the metal transporter NRAMP1 in Arabidopsis. Proc Natl Acad Sci U S A. 114 (16): E3354-E3363.

2019

8. Carrió-Seguí, A., Romero, P., Curie, C., Mari, S. Penarrubia, L. (2019) Copper transporter COPT5 participates in the crosstalk between vacuolar copper and iron pools mobilisation. Sci. Rep. 9:4648.

9. Dader B., Macia JL, Alcon C., Curie C., Gargani D., Assaillit C., Urbach S., Zhou J., Ng J., Brault V., Drucker M. (2019). Split GFP as a tool to study infection with the plant viru *Cauliflower mosaic*. PLOS ONE, *in press.*

Review articles

2014

1. **Grillet L., Mari S., Schmidt W. (2014) Iron in seeds - loading pathways and subcellular localization. Front Plant Sci. 4: 539.**

2017

2. **Curie C., Mari S. (2017) New routes for plant iron mining. New Phytol. 214 (2): 521-525.**

Other articles (professional journals, etc.) (total number)

2015

1. Grillet L., Ouerdane L., Flis P., Hoang M., Isaure M.P., Lobinski R., Curie C., Mari S. (2015). Ascorbate is the key to unlock Fe transport in seeds. Synchrotron Soleil-Highlights

2017

2. Chay S., Mari S. (2017). High Throughput Multi-Elemental Profiling of Plant Samples with the 4200 MP-AES. (Agilent Technologies Eds.)

2- Books

Monographs, critical editions, translations (total number)

Management and coordination of scientific books / Scientific book edition

Book chapters (total number)

Book chapters in English or another foreign language

Edited theses

3- Production in conferences / congresses and research seminars

Articles published in conference proceedings / congress

Other products presented in symposia / congress and research seminars

Invited oral presentations in international symposia/congress

2014

1. Curie C., Castaings L., Alejandro-Martinez S., Cailliatte R., Alcon C. Manganese homeostasis: role of NRAMP transporters and Fe/Mn interplay (July 2014) XVII Iron Symposium on Iron Nutrition and Interactions in Plants. Gatersleben (Germany)

2. Mari S., Grillet L., Hoang M., Ouerdane L., Flis P.,Isaure M.P.,Chay S., Alcon C., Ravet K., Briat J.F., Curie C. The dynamics of iron pools between organelles and within organs. (July 2014) XVII Iron Symposium on Iron Nutrition and Interactions in Plants. Gatersleben (Germany)

2015

3. Curie C. Iron movement in Plants: new routes for novel functions (October 6-10, 2015) 1st trilateral INUPRAG meeting (Nancy, France)

2016

4. Curie C, Hoang M., Castaings L., Chay S., Alcon C., Mari S. (2016) Identification of key players of Fe speciation and transport machineries and regulation of Mn uptake by endocytosis (2016, May 30-June 3) XVIIth International Symposium on Iron Nutrition and Interactions in Plants. Madrid (Spain)

2017

5. Curie C., Castaings L., Alessandro S., Cailliatte R., Alcon C. Manganese transport: some of its actors and their regulation (2017). International Plant Nutrition Colloquium, 19-24 August 2017 (Copenhagen, Denmark)

6. Mari S, Chay S, Richard O, Alcon C, Isaure MP, Lobinski R, Curie C (2017) Iron speciation in seeds of the model plant Arabidopsis thaliana and dynamics of remobilization during germination (27-29 september) Franco-Spanish Workshop on speciation. Huelva (Spain).

2018

7. Curie C., Xiong T.C., Castaings, L., Hiang M., Chay S., Alcon C., Mari S. (2018) Cellular homeostasis of iron and manganese in plants. International Symposium on Root Development and Nutrients/Water absorption, November 26-30, 2017 (Hangzhou, China)

8. Curie C., Castaings L., Alejandro S., Ciailliatte R., Correia D., Dirick L., Mari S. (2018) Mn homeostasis: the NRAMPs at the top of the bill (9-13 July) International Symposium on Iron Nutrition and Interaction in Plants. Taipei (Taiwan)

Invited oral presentations in national symposia/congress

2014

1. Curie C. (2014) MANOMICS - Manganese uptake in Arabidopsis: Genomic analysis and regulatory network. (2-3 octobre 2014) ANR-MOST joint Workshop. Paris (France).

2015

2. Mari S. (2015) Accumulation du fer dans les cellules végétales au niveau des nucléoles, Ligne LUCIA. Deciphering Iron Loading in Seeds and Discovery of a New Pool of Iron in the Nucleolus with Synchrotron Radiation X-ray Fluorescence (21 janvier 2015) INRA-SOLEIL: The Synchrotron Approach in Agriculture, Food and Environment Sciences. Synchrotron SOLEIL, Saint-Aubin (France).

3. Mari S (2015) Deciphering the mechanisms of iron (Fe) transport, storage and remobilization in seeds. (27-29 october) Colloque Graines. Clermont-Ferrand (France)

2016

4. Alcon C. (2016) Molecular dynamic of AtCLC2 a component of clathrin mediated endocytosis in Arabidopsis. (1-2 décembre 2016) Journées 2016 du Groupe de travail Imagerie de la Plante GDR- MIV - Thématique : Super-résolution sur échantillons végétaux. Bordeaux. (France)

2017

5. Mari S., Hoang M, Chay S, Alcon C, Richard O, Curie C (2017) Deciphering iron transport, storage and remobilization in seeds (23-25 October) GDRI, Plant Biology Network Program. Lyon (France)

Selected oral presentations in international symposia/congress

2014

1. Wei L, Pontvianne F, Wu H, Aarts M, Saez-Vazquez J, Curie C, Mari S (2014) Role of nicotianamine in the distribution of iron in the chloroplast and the nucleus (6-10 July) International Symposium on Iron Nutrition In Plants. Gatersleben (Germany)

2015

2. Castaings L., Alcon C., Curie C. (2015) 26th International Conference on Arabidopsis Research, Paris, France (selected for a **poster Highliht**) Abiotic stress session "Regulation of Mn uptake and interplay with Fe transport in *Arabidopsis thaliana*"

2018

3. Xiong T.C., Bal M., Chay S., Alcon C., Curie C., (2018) Iron live imaging in plant (15-17 november) International meeting on optical biosensors. Ghent (Belgium).
4. Mari S., Chay S., Richard O., Alcon C., Isaure M.P., Curie C. (2018) Iron speciation in seeds of Arabidopsis thaliana and dynamics of vacuolar remobilization during germination (9-13 July) International Symposium on Iron Nutrition In Plants. Taipei (Taiwan)

Selected oral presentations in national symposia/congress

2016

1. Alcon C. Molecular dynamic of AtCLC2 in sptPALM" (Décember 2016) Journées du réseau GDR plante. Bordeaux (France)

2017

2. Chay S, Richard O, Alcon C, Isaure MP, Oliveira de Araujo T, Curie C, Mari S (2017) Molecular and biochemical dissection of Fe remobilization process during seed germination (17-19 octobre) Colloque Graines. Montpellier (France).

2018

3. Alcon C. (February 2018) "Projet BioCORP : Couplage entre biosenseurs luminescents et analyse cinématique de la croissance racinaire des plantes " Imagerie des plantes en Occitanie-Pyrénées-Méditerrané. Toulouse (France)
4. Mari S., Chay S. (2018) high throughput multi-elementary analyses for the genetic screen of plants with MP-AES 4200 (5-8 june) Spectr'atom conference. Pau (France)

2019

5. Mari S., Chay S., Richard O., Bierla K., Curie C. (2019) The use of plasma-assisted techniques to unravel the genetics of metal storage in seeds r(3-8 February) European Winter Conference on Plasma Spectrochemistry. Pau (France)

Posters in international symposia/congress

2015

1. Castaings, C., Alcon C., Curie C. (2015) Regulation of Mn uptake and interplay with Fe transport in *Arabidopsis thaliana.* 26th International Conference on Arabidopsis Research. Paris (France)

2016

2. Dirick L., Ahier C., Storti M., Alborei A., Curie C. (2016) Role of the iron chelator Nicotianamine in iron nutrition in *Physcomitrella patens* (5 September) MOSS 2016. University of Leeds (U.K.)
3. Mari S., Hoang M, Chay S, Alcon C, Curie C (2016) TASC1, a tonoplastic transporter of the MATE family, regulates Fe transport from vacuoles during germination (5-10 June) IWPMB, Annapolis (USA)

2017

4. Kwiatkowski P, Berail S, Barre J, Baltrons O, Dirick L, Donard OFX, Mari S, Szpunar J (2017) Determination of Fe natural isotopic composition in yeast and plants by MC-ICP-MS – focus on sample preparation (14-17 August) Metallomics, Vienna (Austria)

2018

5. Alcon C., Chay S., Perrin S., Bal M., De Rossi S., Curie C., Xiong T.C. (2018) Iron live imaging in plant (15-17 november) International meeting on optical biosensors. Ghent (Belgium).

6. Oliveira de Araujo T, Isaure MP, Trcera N, Alcon C, Campos da Silva L, Curie C, Mari S (2018) Paspalum urvillei, an iron-hypertolerant plant with multiple strategies to cope with iron toxicity (9-13 July) International Symposium on Iron Nutrition In Plants. Taipei (Taiwan)
7. Chay S, Richard O, Alcon C, Curie C, Mari S (2018) Can machine learning help to analyze the iron distribution pattern in Arabidopsis thaliana seedlings ? (10-14 September) Plant Systems Biology Conference. Roscoff (France).

Posters in national symposia/congress

2014

1. Isaure M.P., Huguet S., Grillet L., Mari S., Trcera N. LUCIA To Decipher Metal Homeostasis And Toxicity In Plants. (12-13 mai 2014) ENVIronnemEnt @ SOLEIL 2014 : Résultats et nouveaux défis. Saint-Aubin (France) (doct, fr)

2018

2. Le Minh H., Curie C., Xiong T.C. (2018) Early signaling of iron deficiency in Arabidopsis thaliana primary root (5-10 August) 12th Congress of the International Plant Molecular Biology. Montpellier (France)

Invited seminars in foreign institutions

2014

1. Curie C. Iron in plants: acquisition and mobility (December 2014) Laboratoire de Génomique Fonctyionnelle et Imagerie Moléculaire Végétale. Liège University (Belgium).

2016

2. Curie C. Iron movement in plants: new routes for novel functions (March 2016) Department of Biology, University of Padoue (Italy)

2017

3. Curie C. The whereabouts of iron and manganese in plant cells (November 2017) John Innnes Center, Norwich (U.K.)

Invited seminars in French institutions

2014

1. Curie C. Iron in plant : acquisition and mobility (February 2014). CEA Cadarache (France)

2. Mari S. Présentation des travaux de l’équipe sur les approches de type GWAS. (26/11/2014) Au cours des journées scientifiques organisées par BPMP sur le thème de la génétique d’association. (France)

3. Mari S. Présentation de l’utilisation du spectroscope MP-AES. (25/11/2014) Dans le cadre d’une journée de présentation de matériel organisée par Agilent à B&PMP Montpellier (France).

4. Curie C. Distribution et mobilité du fer dans la plante. (13 février 2014.) CEA Cadarache (France).

2015

5. Curie C. Iron in plant : acquisition and mobility (January 2015) BGPI, Montpellier (France)
6. Curie C. Iron in plants : acquisition and mobility (January 2015) RDP, Lyon (France)
7. Mari S. Deciphering the mechanisms of iron tranport, storage and remobilization in seed (March 2015) Agro-Ecology laboratory, Dijon (France)

2016

8. Mari S. Deciphering the mechanisms of iron tranport, storage and remobilization in seed (December 2016) IJPB, Versaille (France)

2018

9. Richard O. Seed Fe Natural Variation in Arabidopsis thaliana (9 October) INRA Orléans (France)

4- Electronic tools and products

Softwares

Databases

5- Instruments and methodology

The multi-elemental analysis platform SAME (https://www1.montpellier.inra.fr/wp-inra/bpmp/en/platform/multi-elemental-analyses-service/) created in july 2016 is devoted to the atomic quantification of plant samples

Prototypes

Platforms and observatories

6- Other products

Theorised artistic creations, staging, movies

7- Editorial activities

Participation in editorial committees (books, collections, etc.)

C. Curie: Associate Editor for Frontiers in Plant Nutrition

Collection and series management

8- Reviewing activities

Reviewing of articles

The TSF members review an average of 12 papers each year

Grant evaluation (public or charities)

An average of 2 per year for the team (BAP INRA, DFG, BBSRC, Austrian Science Foundation, Region Nouvelle-Aquitaine, ISF, NSF, USDA)

Reviewing of research institutes

Participation in institutional committees and juries (CNRS, Inserm, etc.)

Catherine Curie (22015-2019) and Stéphane Mari (2012-2019): members of the evaluation committee of INRA researchers (CSS EGBIP)

Carine Alcon: jury concours technician CIRAD 2017

Jurys de thèses et d’HDR:

Total of 12 PhD thesis and 2 HDR
S. Mari: 6 PhD thesis (3 as a reviewer) and 1 HDR; C. Curie: 6 PhD thesis (5 as reviewer) and 1 HDR

9- Academic research grants

European (ERC, H2020, etc.) and international (NSF, JSPS, NIH, World Bank, FAO, etc.) grants – coordination

European (ERC, H2020, etc.) and international (NSF, JSPS, NIH, World Bank, FAO, etc.) grants - partnership

Other European grants - coordination

Other European grants - partnership

National public grants (ANR, PHRC, FUI, INCA, etc.) - coordination

2012 – 2015 ; **MANOMICS (ANR PRC)** ; Prélèvement du manganèse chez Arabidopsis : analyse génomique et voies de régulation ; CURIE Catherine

2013 – 2017 ; **SUBCELIF (ANR PRC)** ; Distribution intra-cellulaire du fer chez les plantes, spéciation chimique et nouvelles fonctions ; MARI Stéphane

2016 – 2017 ; **IRONSEED (Department BAP INRA)** ; Contrôle du contenu en fer de la graine - transfert de connaissances depuis Arabidopsis vers les légumineuses ; MARI Stéphane

2016 – 2021 ; **ISISTOR (ANR PRC)** ; Amélioration du contenu en fer de la graine ; MARI Stephane

National public grants (ANR, PHRC, FUI, INCA, etc.) - partnership

2012 – 2014 ; **PLANTMAN (ANR PRC)** ; Nutrition en manganèse pour l'optimisation de la photosynthèse et de la croissance des plantes ; CURIE Catherine

Local grants (collectivités territoriales) - coordination

Local grants (collectivités territoriales) - partnership

PIA (labex, equipex etc.) grants - coordination

2019 : **AGROMATRIX (Consortium Agropolis Foundation-SOLVAY)** ; Impact d'Agropolymères Solvay sur le transcriptome du maïs ; CURIE Catherine

PIA (labex, equipex etc.) grants - partnership

2018 – 2021 ; **eCO2THREATS (iSITE Montpellier)** ; Alteration of plant nutrient contents by elevated CO2 : consequences for crop quality, human health and environmental protection ; MARI Stéphane

Grants from foundations and charities (ARC, FMR, FRM, etc.) - coordination

Grants from foundations and charities (ARC, FMR, FRM, etc.) - partnership

10- Visiting senior scientists and post-doc

Post-docs (total number)

Since 2014: 4

Foreign post-docs

Since 2014: 3

Family Name	**First name**	**Contract type & Employer**	**Nationality**	**Recruitment period**	
Alejandro Martinez	Santiago	CDD CNRS ANR	ESPAGNOL	01/03/2013	28/02/2015
Melo Almeida	Diego	CDD INRA ANR	PORTUGUAIS / BRESILIEN	01/05/2017	30/04/2020
Wei	Lili	CDD CNRS ANR	CHINOISE	15/03/2013	15/03/2015

Visiting scientists (total number)

Since 2014 : 5

Foreign visiting scientists

Since 2014 : 4

Family Name	**First name**	**Financing source**	**Nationality**	**Date of arrival**	**Date of departure**	**Status**	**University of origin**
Carrio	Angela	Bourse	Espagnol	15/03/2015	19/09/2015	Doctorant invité	Université Valence
Kumar	Shailender	Bourse	Indien	03/01/2017	03/02/2017	Doctorant invité	Centre for Computational Biology and Bioinformatics
Oliveira dos Santos	Jacqueline	Bourse	Bresilienne	01/04/2017	31/10/2017	Doctorant invité	Universidade Federal de Lavras Brazil
Saito	Akihiro	Salaire	Japonnais	10/05/2019	03/04/2020	Assistant professeur	Tokyo University of Agriculture

11- Scientific recognition

Prizes and/or distinctions

IUF members

Chair of learned and scientific societies

Organisations of meetings and symposia (out of France)

Invitations to meetings and symposia (out of France)

Stéphane Mari : XVIIth Iron Symposium on Iron Nutrition and Interactions in Plants. « The dynamics of iron pool between organelles and within organs». **Gatersleben (Germany).** July 6-10 2014

Catherine Curie : XVIIth Iron Symposium on Iron Nutrition and Interactions in Plants. « Manganese homeostasis: role of NRAMP transporters and Fe/Mn interplay ». **Gatersleben (Germany).** July 6-10 2014.

Catherine Curie : XVIIth International Symposium on Iron Nutrition and Interactions in Plants. "Identification of key players of Fe speciation and transport machineries and regulation of Mn uptake by endocytosis". **Madrid (Spain).** May 30-June 3 2016.

Catherine Curie : International Plant Nutrition Colloquium 2017. “Manganese transport: some of its actors and their regulation”. **Copenhagen**, 19-24 August 2017

Catherine Curie : International Symposium on Root Development and Nutrients/Water absorption, “Cellular homeostasis of iron and manganese in plants”. **Hangzhou (China).** November 26-30, 2018

Members' long-term visits abroad

Odile Richard took a 3 year-leave from September 2013 to September 2016

II - INTERACTION OF THE TEAM WITH THE NON-ACADEMIC WORLD, IMPACTS ON ECONOMY, SOCIETY, CULTURE OR HEALTH

1- Socio-economic interactions / Patents

Invention disclosures

Filed patents

Accepted patents

Licenced patents

2- Socio-economic interactions

Industrial and R&D contracts

2015 – 2016 ; **NUTRIPOL 1 (Rhodia Operations - Labex Agro, Research Contract)** ; Analyse de la nutrition hydrominérale des plantes en présence d'Agropolymères, et de leur comportement en situation de carence ; Coordinator: CURIE Catherine

2016 – 2018 ; **NUTRIPOL 2 (Rhodia Operations - Labex Agro, Research Contract)** Identification des réponses moléculaires de la plante aux polymères dérivés de gomme de guar ; Coordinator: CURIE Catherine

2019 ; **AGROMATRIX (Consortium Agropolis Foundation-SOLVAY)** ; impact d'Agropolymères Solvay sur le transcriptome du maïs ; Coordinator: Catherine CURIE

Cifre fellowships

Creation of labs with private-public partnerships

Networks and mixed units

Start-ups

3- Expertise

Consulting

Participation in expert committees (ANSES etc.)

Legal expertise

Expert and standardization reports

4- Public outreach

Radio broadcasts, TV shows, magazines and newspaper

Journal articles, interviews, book edition, videos, other popularization outputs, debates on science and society, etc.

C. Alcon: participation in the “fête de la science” organized at BPMP

L. Dirick: has always been very much dedicated to the interaction between lab and the Society. Among his actions towards the public, we can cite the following.

Schools :

- In primary schools :
  - Every year : Involvement in ASTEP program (Accompagnement en Sciences et Technologie en école Primaire). Partnesrship between University of Montpellier and Public primary schools of Montpellier and surroundings. Supervision of L3 university students involved in the ASTEP program
  - 1-2 times every year: Punctual interventions in primary schools in SVT programs: biology teaching and practical courses

- In "collèges"/secondary schools:
  - Every year: Teaching/conferences on cell biology and genetics, during the "fête de la science" (yearly event organized by the CNRS)
  - 2017 and 2018 : "Curieux de sciences", collège de Bédarieux . « Au cœur du vivant, la cellule » . Invited speaker, Cell biology conference.

- Others :
  - June 2017: "Bar des Sciences", invited speaker , "Microbes: amis ou ennemis?"
    https://www.umontpellier.fr/articles/bar-des-sciences-microbes-amis-ou-ennemis
  - Mai 2017 : « Café Video du CNRS » (DR13, CNRS) Invited Speaker, « La nuit du vivant »
  - Nov : 2018 : Festival du film scientifique (Montpellier) :Invited speaker, « La boîte noire »
    https://www.umontpellier.fr/articles/1er-festival-du-film-scientifique-sud-de-sciences
  - Video with a master II group of students of the University of Montpellier
    https://www.youtube.com/channel/UCIINwurIIDJDvUd17DKqCwQ/videos

- At BPMP :
  - Yearly: participation at the « Fête de la science » program organized at BPMP
  - From 2016 on: Member of the "BPMP communication group"

III - INVOLVEMENT OF THE TEAM IN TRAINING THROUGH RESEARCH

1- Educational outputs

Books

E-learning, MOOCs, multimedia lessons, etc.

2- Scientific productions (articles, books, etc.) from theses

Scientific productions (articles, books, etc.) from theses

Thesis of Minh Hoang: 1 paper under revision (Hoang et al.)

Thesis of Angela Carrio, who spent 1 year in the team on a Spanish PhD fellowship: 1 paper (Carrio et al., 2019 Scientific Reports)

Thesis of Talita Araujo de Oliveira who spent 1 year in the team on a Brasilian PhD fellowship: 1 paper under revision (Araujo de Oliveira et al.)

Scientific articles

Grillet L., Ouerdane L., Flis P., Hoang M.T.T., Isaure M.P., Lobinski R., Curie C. ,Mari S. (2014) Ascorbate efflux as a new strategy for iron reduction and transport in plants. J. Biol. Chem. 289(5): 2515-2525.(

Flis P., Ouerdane L., Grillet L., Curie C., Mari S., Lobinski R. (2016) Inventory of metal complexes circulating in plant fluids: a reliable method based on HPLC coupled with dual elemental and high resolution molecular mass spectrometric detection. New Phytol. 211 (3): 1129-1141.

Alejandro S., Cailliatte R., Alcon C., Dirick L., Domergue F., Correia D., Castaings L., Briat J.F., Mari S., Curie, C. (2017) Intracellular manganese distribution by the Trans-Golgi Network NRAMP2 transporter is critical for photosynthesis and cellular redox homeostasis. Plant Cell, 29(12): 3068-3084.

Other articles (professional journals, etc.) (total number)

Grillet L., Ouerdane L., Flis P., Hoang M., Isaure M.P., Lobinski R., Curie C., Mari S. (2015). Ascorbate is the key to unlock Fe transport in seeds. Synchrotron Soleil-Highlights

Mean number of publications per student (Biology & Science and technology only)

3- Training

Habilitated (HDR) scientists

2

Family Name	First name	Position	Employer	Date of habilitation
CURIE	Catherine	chercheur	CNRS	2002
MARI	Stéphane	chercheur	INRA MONTPELLIER	2014

HDR obtained during the period

1

Family Name	First name	Position	Employer	Date of habilitation
MARI	Stéphane	chercheur	INRA MONTPELLIER	2014

PhD students (total number)

3

Family Name	First name	Starting date	Defense	Duration (months)	Funding
BRUN	Alexis	01/10/2018		36	CDE
HOANG THI THANH	Minh	10/12/2012	15/12/2015	38	ETR
LE	Minh Hai	01/04/2017	-	17	ETR

PhD students benefiting from a specific doctoral contract

Defended PhDs

1

Family Name	First Name	Date of defense
HOANG THI THANH	Minh	2015

Mean PhD duration

38 months

Internships (M1, M2)

Master 1: 2
Master 2: 3

Family Name	**First Name**	**University Degree**	Period	
Ahier	Célia	Master 1	01/03/2016	30/06/2016
Leveau	Stéphane	Master 1	01/03/2016	31/07/2016
Smokvarska	Marija	Master 2	03/01/2017	30/06/2017
Brun	Alexis	Master 2	08/01/2018	08/07/2018
Kosuth	Thibault	Master 2	07/01/2019	06/07/2019

People in charge for a mention or a master's degree course (total number)

People in charge for a mention or a master's degree course with international certification (Erasmus mundus)

KaliPHruit team
Self-assessment document

Name of the team concerned by the current contract: KaliPHruit
Name of the team concerned by the next contract (if different):
Acronym of the current contract: KaliPHruit
Acronym of the next contract (if different):
Team leader for the current contract: Isabelle Gaillard
Team leader for the next contract: Isabelle Gaillard

RESULTS.

1- Presentation of the team

Introduction

In July 2015, I. Gaillard, I. Chérel, N. Paris and R. Gibrat left their former group (Ion Channel and UBISTRESS) to found the KaliPHruit team. The research report on "KaliPHruit group" activity covers the period from its official start on July 1st 2015 and the team setting period of 1 year and a half before.

Team's workforce and means

*Permanents members: Chérel Isabelle (DR INRA), Gaillard Isabelle (DR INRA), Paris Nadine (CR CNRS), and Gibrat Rémy (DR INRA - retired in 02/2017).

*Non-permanent fellows: Bidzinski Przemyslaw post-doct (03/2015-12/2016), Moreau Hortense (PhD student 2018-2021), Villette Jérémy (PhD student 2016-2019)and 18 undergraduate students (2 to 6 months period each).

**Research grants : SweetKaliGrape ANR (2014-2019), APLIM(2016-2019), PHABABerry (2017-2018), PalmElit(2018-2021), PROTEOMICS (2018-2021). [Please see appendix 4 for details]*

Scientific policy

The aim of the KaliPHruit team is to characterize the functional and molecular gene combinatory involved in the control of grape berry acidity at harvest in relation with the Potassium (K^+) accumulation in the context of the climate change.

Potassium is the most abundant inorganic cation in the cytoplasm of the living cells where it is involved in the regulation of ionic strength, osmotic potential, membrane polarization and neutralization of organic acids. At whole plant level, this ion is involved in osmotically driven functions such as cell movements, regulation of stomatal aperture, cell expansion in growing tissues, or long distance phloem transport. As such, this ion is essential for nutrient and water loading during fruit ripening.

All over the world, French wines are well known for their aromas and their typicity. Grape acidity at harvest is a key factor for wine quality. When the acidity level of berries is too low, wine is of poor sensory quality and has decreased ageing potential. Grape acidity at harvest depends on the ratio between free organic acid (mainly tartaric and malic acids) and organic acids neutralized by potassium (K^+) ion that accumulates during ripening, starting at veraison. Grape berry being a strong sink for this major cellular counter-ion (Mpelasoka et al. 2003, Cuéllar et al. 2013), it is strongly involved in the control of the pH and the acid-base balance of flesh cells (Mpelasoka et al., 2003, Kodur et al., 2011). The climate changes are now significantly impacting this fine acid -base balance at harvest, due to an increase of both K^+ accumulation and malic acid degradation during berry ripening (Kodur et al. 2011, Duchêne et al. 2014, Sweetman et al. 2014). In addition, an excessive accumulation of K^+ in a low-acidic must context leads to the formation of insoluble K^+ bitartrate during winemaking process that not only amplifies the loss of acidity but also gives rise to unstable wines with poor organoleptic properties.

Using a translational biology strategy, the group is focusing on the molecular basis of K^+ accumulation throughout grape berry development. Our objectives are to identify and characterize the major molecular actors involved in K^+ uptake. The KaliPHruit team works simultaneously on two species: the model plant Arabidopsis thaliana and grapevine. The group develops fundamental research strategies to provide the best background of knowledge with the long-term goal to contribute to varietal selection of vines adapted to our future environment. To this end, the team combines multidisciplinary approaches extending from molecular biology, modern transcriptomic methods, biochemical studies, electrophysiology, live cell imaging using pH-sensors, whole plant physiology with a continuum of scales from gene to whole plant.

We are involved in collaboration with several BPMP teams, LEPSE (Montpellier), Cirad (Montpellier), EGFV (Bordeaux), SVQV (Colmar), IJPB Versailles, UC Davis (USA)…

2- Research products and activities for the unit (team / theme)

Scientific track record

1. Implementation and development of methodological approaches

Unpublished baseline experiments on berry acidity, pH, K^+ and organic acid accumulations: *I Gaillard*, N Paris *J. Villette (PhD), T. Simonneau (LEPSE), G Krouk (Honude BPMP), Alexandre Martinière (Aqua, BPMP). [part of SweetKaliGrape ANR]*

*We challenged Syrah grapevine with four climatic stress scenarios (control, heat, water stress and their combination) within the phenotyping platform Phenodyn (http://bioweb.supagro.inra.fr/phenodyn/) and we collected berries at different stages. For each condition, we followed respirational carbon losses and water content and we measured berry pH, its fresh weight, its osmotic pressure and K^+, sugar, organic acid contents. **The analysis of these collected data clearly showed that the high temperature condition is the climatic parameter that disturbs the most the berry composition at harvest.** A RNA-SEQ experiment is currently undergoing on 15 time points along berry development, covering a period from 3 days before veraison to full ripening. Obtained data will be analyzed with a systems biology approach.

*Also, in order to map the pH at subcellular level in grape berry during maturation especially to study the effect of global warming, we designed genetically encoded sensors adapted to monitor pH close to plasma membrane and/or adapted in the acidic range.

2. Potassium Shaker channels in Arabidopsis thaliana and Grapevine:

In plants, K^+ accumulation is controlled by multigenic families that encode a broad spectrum of potassium transporters and channels (Sharma et al. 2013, Véry et al. 2014). During the last five year period, the group focused its research on the Shaker K^+ channel family because they dominate plasma membrane conductance to K^+ in most cell types and thereby they play major roles in the physiology of the plant. Shaker channels are tetrameric proteins associating 4 subunits located at the plasma membrane and can drive inward and/or outward K^+ fluxes.

Translational biology: the Shaker channel family in grapevine *(I. Gaillard, J. Villette, N. Paris, I. Chérel, T. Cuéllar (CIRAD), J.-L. Verdeil (CIRAD), S. Zimmermann (ELSA BPMP)) [SweetKaliGrape ANR and APLIM (Agropolis foundation)*

In continuity with our previous work, (Cuéllar et al, 2010 and 2013) we proceeded to the characterization of grapevine shakers VvK3.1 (an AKT2-like channel, Nieves-Cordones *et al.* 2019) and VvK5.1 (an outward shaker - publication in preparation). Some of these channels have acquired particular functional features or tissue specific expression conferring to them new properties perfectly adapted to the needs of grapevine. In contrast, we found that interacting regulatory networks between CBLs, CIPKs and Shaker channels are strongly conserved between grapevine and *Arabidopsis* (Cuellar *et al.* 2013 and Nieves-Cordones *et al.* 2019).

Regulation of Shaker channel activity – The role of C-Linker in localization and channel activity *(I. Gaillard, I.Chérel, S. Zimmermann (ELSA,BPMP) A. Chavanieu (Institut des Biomolécules Max Mousseron)).*

The intracellular C-terminal region of each shaker subunit contains several regulatory elements separated from the last transmembrane domain by a C-linker region. Using a sequence swapping strategy we identified two contiguous point mutations, V381F and S382P and nine amino acids located in the N-terminal part of this C-linker that are essential for channel localization and activity, respectively (Nieves-Cordones et al., 2014, Nieves-Cordones and Gaillard 2014).

Regulation of Shaker channel activity - protein-protein interaction. *I. Chérel, I. Gaillard [SweetKaliGrape ANR, PHABABerry (INRA Grant)]*

In grapevine, abscisic acid (ABA) is involved in berry ripening and probably also berry metabolism under heat/drought stress. The clade A protein phosphatases 2C are central mediator in ABA signaling. In Arabidopsis.

we found that AtPP2CA inhibits the activity of GORK, a K+ shaker channel mediating K+ efflux in roots and guard cells, *via* an unusual mechanism that would serve as a retro-control of ABA-induced, GORK-mediated stomatal closure (Lefoulon et al., 2016). In grapevine, VvK3.1 K+ shaker is involved in K+ loading into the berry flesh cells from the phloem. Our results indicate that VvPP2CA, whose expression rises under drought stress, strongly interacts with VvK3.1 and its CIPK/CBL partners (Nieves-Cordones et al., 2019) and inhibits the activity of VVK3.1 by suppressing the activating effect of the CIPK/CBL couples on this channel. These regulation processes and others were detailed in a review article on abiotic stress-related post-translational modifications of K+ channels (Chérel and Gaillard, 2019).

Regulation of Shaker channel activity – Plasma membrane associated pH *(N. Paris, I. Gaillard, R. Gibrat, A. Martinière (AquaBPMP) Eduardo Blumwald (UCLA-USA).[SweetKaliGrape ANR]*

SInce we have shown by electrophysiology that the activity of Shaker channels is generally regulated by the pH (Cuéllar et al 2010, 2013, Nieves-Cordones et al., 2019), we developed genetically-encoded fluorescent pH sensors to further investigate the role of pH in Shaker activity. As shown for an the endosomal K+/H+ antiporter NHX5/6 for vacuolar targeting of soluble proteins, such pH sensors can efficiently monitor fine cellular mechanisms in living cells (Martinière et al 2013, Reguera et al 2015). More interestingly, a fluorescent sensor anchored in the plasma membrane with the pH sensing moiety facing the outside of the cell was successfully used in Arabidopsis root (1) to report net proton excretion at root tip when co-cultivated with nitrogen fixing rhizobia (Poitout et al 2017) and (2) to monitor radial proton homeostasis in the mature part of the root with fully elongated root hair (Martinière 2018). Surprisingly, the pH at the vicinity of the membrane surface up to 8nm was found far more alkaline than in the rest of the apoplastic space and does not fully equilibrate with the bulk pH unless a potassium ionophore is provided, indicating a complex homeostasis of proton in the apoplasm in close link to the ionic exchange activity of the plasma membrane. Our preliminary data indicate that this PM-anchored pH sensor can be expressed in grape berries and allows pH measurement at the cellular level in living cells.

Key events

1)The grapevine VvK3.1 Shaker channel is a weakly rectifying channel, mediating both inward and outward K+ currents. We showed that VvK3.1 is highly expressed in the berry phloem. From the onset of fruit ripening, all data support the role of the VvK3.1 channel in the massive K+ efflux from the phloem cell cytosol to the berry apoplast. Switching to its non-rectifying mode, the VvK3.1 drives the K+ efflux that allows K+ ions to move down their transmembrane concentration gradient. This is a major concern for grapevine, as the fruit is energetically limited due to stomata disappearance after veraison. The additional energy stored in the transmembrane K+ gradient also allows sucrose retrieval in energy-limited conditions (Nieves-Cordones et al. 2019. New Phytol. 222(1):286-300). This concept of berry loading during its maturing has contributed to setting up our group project for the next contract.

2) The use of efficient ratiometric sensors to shed light on pH homeostasis in vivo at both sides of plant plasma membrane is an indispensable tool for the future work of the team (see project). A first set of sensors has been validated in the model plant Arabidopsis (Martinière et al. 2018 PNAS 115(25):6488-6493). In mature root cells, we demonstrated that despite a direct contact with the soil, the apoplastic pH close to the plasma membrane was maintained at values ranging from 6.0 to 6.4, whereas the overall pH in the apoplastic space is far more acidic. Furthermore, we found that the cell wall plays a role in proton homeostasis in mature root.

FIVE-YEAR PROJECT AND STRATEGY

1- SWOT analysis

- strong points; The members of the group have complementary expertise to conduct their research objectives. The group has a long standing experience (1) in molecular biology and (electro)-physiological studies on K^+ transport in plants and (2) in protein trafficking to develop functional imaging strategies to monitor pH in living cells. The group is working on two plant models: Arabidopsis thaliana and grapevine

- needs for improvements; Grapevine is a perennial with a long reproductive cycle that is difficult to transform and no mutant collection is available. The group had to find alternative solutions to perform in planta validations.

- possibilities brought in by the context / surrounding environment; Grapevine is a highly productive water stress-adapted plant and a major crop, based on cultivated hectares and economical value. Grape and wine producers have urgent needs for solutions to adapt their culture to climate change

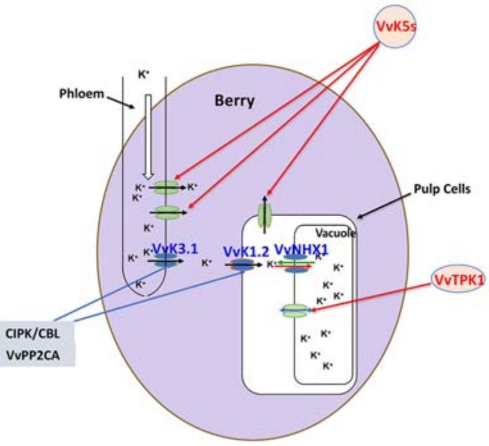
- risks linked to the context / environment. Low possibility to obtain local funding via inter-profession and ANR grants are more and more difficult to obtain

2- Structure, workforce and scientific orientations

In line with our current programs, we aim to continue to decipher the K^+ transport systems and their molecular regulators during berry ripening, more specifically to further understand how berry acidity at harvest is impacted by warmer climate. We want to evolve towards more integrated analysis of berry growth processes, and more precisely flesh cell expansion, that is ensured by a directional flow of water, sugar and nutrients driven by massive K^+ fluxes. Our results support the concept that the non-rectifying mode of VvK3.1 contributes to K^+ secretion into the grape berry apoplast, in addition to switching on the transmembrane K^+ gradient implementation named K^+ battery, which then, allows the sucrose retrieval in energy-limited conditions (Nieves-Cordones et al., 2019). At the same time, the inwardly rectifying VvK1.2 channel, localized in perivascular and flesh cells and strongly regulated by apoplastic pH (Cuellar et al., 2013), contributes in keeping the apoplastic K^+ concentration at low levels therefore allowing berry loading mechanisms. Thus, the phloem stream flux towards the sink would be triggered but also retained as long as the transmembrane gradient of K^+ is maintained at the phloem cell plasma membrane. In our model, many questions remain opened: what are the fine regulators of this loading flow? is the intensity of the flow flexible? can it increase under heat stress? by which mechanisms? how is this K^+ flow conciliates with the acid growth theory necessary for flesh cells expansion since, in grape, cell division is stopped at veraison? is there a channel involved in repolarizing the phloem membrane when necessary? Is VvK3.1 the only one channel driving the K^+ flux from the phloem? How do the flesh cells make it to accumulate all the ions, water and solutes within their vacuole? Thanks to genetically encoded sensors measuring the pH in the vicinity of the plasma membrane (Martinière 2018), we can monitor cell specific pH in the berry in correlation with K^+ fluxes and flesh cell expansion. With a continue interplay, two complementary research axes will be performed: K^+ transport system characterization with their molecular regulators and pH mapping in relation to cell expansion and K^+ transports in berry.

WorkForce : All the members of the KaliPHruit team (I. Chérel, I. Gaillard, N. Paris and two (or more) PhD students). Collaborations with G. Krouk (BPMP EQ Honude), Alexis De Angelis (BPMP EQ INFLUX), Alexandre Martinière (BPMP EQ Aqua), Herman Höfte (IJPB Versailles) and D and F Lecourieux (EGFV Bordeaux).
Financial means are dependent of ANR second round results.

K+ Transport System characterization



***K^+ transport into grape berry after veraison**: Up to now three main transport systems are involved in loading and redistributing of the K^+ into grape berries during the ripening stage. The VvK3.1 channel drives massive K^+ efflux from the phloem to the berry apoplast. VvK1.2 channel plays a major role in K^+ uptake in flesh cells. The vacuolar cation$^+$/H^+ antiporter VvNHX1 may be responsible for vacuolar K^+ accumulation. In red are represented three outward Shaker channels and one vacuolar two pore K^+ channel to be further characterized.*

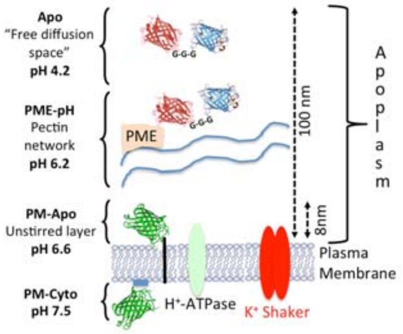
To confirm and precise our current model, especially in the context of the warmer climate, we plan to functionally characterize 3 other outward Shaker channels and one two pore K^+ channel (VvTPK1) that are known to be expressed in berries during its development (data from grape efp browser), suggesting that they could play a role in berry loading, and so in the control of K^+ accumulation and pH set up. Concerning VvTPK1, it will be interesting to know if this channel is involved in the accumulation of K^+ in the flesh cell vacuoles. Our RNA-SEQ results will be analyzed to identify the impact of the high temperature exposure on the expression level of all genes of interest. We will also select new genes with a special attention on those acting in the interplay of gene networks involved in potassium and/or organic acid transports in relation with berry acidity.

In addition to a further characterization of the well-known molecular regulators of the studied channels, (CIPK/CBL, CDPKs and VvPP2CA), we will also address the role of newly identified partners of Shaker channels in grapevine. In particular, in Xenopus oocytes we observed a strong and specific inhibition of VvK3.1 Shaker by a receptor-like kinase. Moreover, in two-hybrid system, we have shown that this receptor-like kinase interacts with VvPP2CA, indicative of some kind of regulation loop for VvK3.1 activity.

The functions of selected grapevine genes encoding channels and their regulators will be validated by over-expression of grapevine genes in *Arabidopsis thaliana*.

Finally, in collaboration with Alexis de Angelis (EQ INFLUX BPMP), the team would like to question organic acid transport in berry because warmer climate induces an increase of malic acid consumption by respiration, therefore affecting free organic acid content of berry at harvest. Members of the ALMT family are ideal candidates to start this work because they mediate the transport of malate and tartrate across the cell membranes in grape berries (De Angeli *et al.* 2013)

pH mapping during grape ripening



***Presentation of apoplastic pH sensors and the respective pH.** The genetically encoded sensors are localized on either side of the plasma membrane (PM-Apo and PM-Cyto, Martinière 2018) or in different subdomains of the apoplast (unpublished). The chosen sensor depends on the range of pH to be monitored, pHluorin or tandem of fluorescent proteins with low pKa. PME = pectin methyl esterase.*

The PM-anchored pH sensor used to monitor pH in close vicinity to the plasma membrane in Arabidopsis root was introduced in berry by agro-infiltration. It is efficiently expressed and allowed cell specific pH measurement during the entire phase of fruit maturation, starting from veraison (unpublished). To further extend pH measurement to acidic environment, we designed sensors based on tandem with two fluorescent proteins of low pKa. The two resulting sensors tested so far allow detection down to either pH 3 or 4. Thanks to fusion with various targeting sequences, the pH sensors allowed identification of three subdomains of the apoplast with distinct pH values in tobacco epidermal cells (publication in preparation). In the coming contract, we will further study pH homeostasis in the apoplast, especially close to plasma membrane as it is the pH sensed by membrane transport proteins such as most potassium shaker are modulated by pH. Our published results suggest a key role of Ca^{++} sequestration by pectin through its methylation status on membrane bound pH. We will therefore focus on cell elongation processes in two model plants, Arabidopsis and grapevine. In this perspective we submitted a research project to ANR 2019, Homeowall coordinated by Herman Höfte in Versailles

Conclusions : Altogether, our future studies are expecting to greatly extend our knowledge on berry loading so that we can reach an integrated view of the mechanisms of K^+ transport and its regulations in correlation with the huge expansion of the flesh cells, the final receptacle of ions, water and solutes stored in their vacuoles. Together with what we already know on this system, we are confident that we will be able to underpin the fine-tuning of the processes that are taking place under warmer temperature. As the berry composition and consequently wine quality is mainly determined by flesh cell vacuolar content (more than 90% of the cell volume), we believe that this work could provide the necessary background to select original and new markers able to contribute to grapevine selection and vineyard management to better adapt to global warming in the future.

References

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Very AA, Nieves-Cordones M, Daly M, Khan I, Fizames C, Sentenac H. 2014. Molecular biology of K+ transport across the plant cell membrane: what do we learn from comparison between plant species? *J Plant Physiol* 171(9): 748-769.

Annex 4 – Research products and activities

EVALUATION CAMPAIGN 2019-2020

GROUP A

KalipHruit Team:	**KaliPHruit**
Acronym:	**KaliPHruit**
Theme leader for the current contract:	**GAILLARD Isabelle**
Theme leader for the next contract:	**GAILLARD Isabelle**

I - PRODUCTION OF KNOWLEDGE AND ACTIVITIES CONTRIBUTING TO THE INFLUENCE AND SCIENTIFIC ATTRACTIVENESS OF THE TEAM

1- Articles

Scientific articles

Most significant articles (20%)

1. **Nieves-Cordones M., Andrianteranagna M., Cuéllar T., Chérel I., Gibrat R., Boeglin M., Moreau B., Paris N., Verdeil J.L., Zimmermann S.D., Gaillard I. (2019). Characterization of the grapevine Shaker K$^+$ channel VvK3.1 supports its function in massive potassium fluxes necessary for berry potassium loading and pulvinus-actuated leaf movements. New Phytol. 222(1):286-300. doi: 10.1111/nph.15604.**

2. **Martinière A., Gibrat R., Sentenac H., Dumont X., Gaillard I., Paris N. (2018) Shedding light on pH at both sides of the root plasma membrane interface using non-invasive imaging. Proc. Natl. Acad. Sci. USA.115(25): 6488-6493.**

3. **Lefoulon C., Boeglin M., Moreau B., Véry A.A., Szponarski W., Dauzat M., Michard E., Gaillard I., Chérel I. (2016) The Arabidopsis AtPP2CA protein phosphatase inhibits the GORK K+ efflux channel and exerts a dominant suppressive effect on phosphomimetic activating mutations.** *J. Biol. Chem.* **291(12): 6521-6533**

2014

4. **Nieves-Cordones M., Chavanieu A., Jeanguenin L., Alcon C., Szponarski W., Estaran S., Chérel I., Zimmermann S., Sentenac H. ,Gaillard I. (2014) Distinct amino acids in the C-linker domain of the plant K+ channel KAT2 determine its subcellular localization and activity at the plasma membrane. Plant physiol. 164(3):1415-1429.**

2015

5. Reguera M., Bassil E., Tajima H., Wimmer M., Chanoca A., Otegui M. S., Paris N., Blumwald E. (2015) pH Regulation by NHX-Type Antiporters Is Required for Receptor-Mediated Protein Trafficking to the Vacuole in Arabidopsis. Plant cell. 27(4):1200-1217.

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2016

7. **Boeglin M., Fuglsang A.T., Luu D.T., Sentenac H., Gaillard I., Chérel I. (2016) Reduced expression of AtNUP62 nucleoporin gene affects auxin response in Arabidopsis. BMC Plant Biol. 16: 2.**

8. Damiani I., Drain A., Guichard M., Balzergue S., Boscari A., Boyer J.C., Brunaud V., Cottaz S., Rancurel C., Da Rocha M., Fizames C., Fort S., Gaillard I., Maillol V., Danchin E. G. J., Rouached H., Samain E., Su Y.H., Thouin J., Touraine B., Puppo A., Frachisse J.M., Pauly N., Sentenac H. (2016) Nod Factor Effects on Root Hair-Specific Transcriptome of Medicago truncatula: Focus on Plasma Membrane Transport Systems and Reactive Oxygen Species Networks. Front. Plant Sci. 7: 794.

2017

9. **Poitout A., Martinière A., Kucharczyk B., Queruel N., Silva-Andia J., Mashkoor S., Gamet L., Varoquaux F., Paris N., Sentenac H., Touraine B., Desbrosses G. (2017) Local signalling pathways regulate the Arabidopsis root developmental response to Mesorhizobium loti inoculation. J. Exp. Bot. 68 (5): 1199-1211.**

10. Sklodowski K., Riedelsberger J., Raddatz N., Riadi G., Caballero J., Chérel I., Schulze W., Graf A., Dreyer I. (2017) The receptor-like pseudokinase MRH1 interacts with the voltage-gated potassium channel AKT2. Sci. Rep. 7 44611.

2018

11. **Guerrero-Galán C., Delteil A., Garcia K., Houdinet G., Conéjéro G., Gaillard I., Sentenac H., Zimmermann S. D. (2018) Plant potassium nutrition in ectomycorrhizal symbiosis: properties and roles of the three fungal TOK potassium channels in Hebeloma cylindrosporum. Environ. Microbiol. 20(5): 1873-1887.**

12. Lee HN., Zarza X., Kim J.H., Yoon M.J., Kim S.H., Lee J.H., Paris N., Munnik T., Otegui M.S., Chung T. (2018) Vacuole trafficking protein VPS38 is dispensable for autophagy. Plant Physiol. 176(2): 1559-1572.

Review articles

Most significant review articles

1. **Chérel I., Gaillard I (2019) The Complex Fine-Tuning of K$^+$ Fluxes in Plants in Relation to Osmotic and Ionic Abiotic Stresses. Int. J. Mol. Sci. 20(3):715. Feb 7;20(3). pii: E715. doi: 10.3390/ijms20030715. “rev Biblio”**

2014

2. **Chérel I., Lefoulon C., Boeglin M., Sentenac H. (2014) Molecular mechanisms involved in plant adaptation to low K+ availability. J.Exp.Bot. .65(3):833-848. “rev Biblio”**

3. Nieves-Cordones M., Gaillard I. (2014) Involvement of the S4-S5 Linker and the C-linker Domain Regions to Voltage-gating in Plant Shaker Channels: Comparison with Animal HCN and Kv Channels. Plant Signal Behav 9(10): e972892. “rev Biblio”

2016

4. **Nieves-Cordones M., Ródenas R., Chavanieu A., Rivero R. M., Martinez V., Gaillard I., Rubio F. (2016) Uneven HAK/KUP/KT Protein Diversity Among Angiosperms: Species Distribution and Perspectives. Front. Plant Sci. 7 : 127.rev Biblio”**

Other articles (professional journals, etc.) (total number)

2015

1. **Gaillard I., Cuéllar T. (2015) Contrôler l’accumulation de potassium dans la baie de raisin pour maintenir l’acidité du vin. Les dossiers d'AGROPOLIS INTERNATIONAL : Vigne et Vin. 21 : 13.**

2. **Paris N. (2015) Des sondes fluorescentes ratiométriques pour une mesure non invasive de pH acides dans la baie de raisin. Les dossiers d'AGROPOLIS INTERNATIONAL : Vigne et Vin. 21 : 13**

2- Books

Monographs, critical editions, translations (total number)

Management and coordination of scientific books / Scientific book edition

Book chapters (total number)

Book chapters in English or another foreign language

Edited theses

3- Production in conferences / congresses and research seminars

Articles published in conference proceedings / congress

Other products presented in symposia / congress and research seminars

Invited oral presentations in international symposia/congress

Invited oral presentations in national symposia/congress

2015

1. Nieves-Cordones M., Azeem F., Mouline K., Duby G., Hosy E., Vavasseur A., Véry A.A., Thibaud J.B., Gaillard I., Sentenac H. (2015) Epidermal cell turgor depends on a K^+ channel to regulate stomatal aperture (13-16 September 2015) 26 eme Colloque des Canaux ioniques. Sète (France).

Selected oral presentations in international symposia/congress

2018

1. Paris N., Martinière A., Gibrat R., Sentenac H., Dumont X., Gaillard I. (2018) Non invasive imaging use to shed light on pH at both sides of the plasma membrane interface in living root. (5 - 10 august) 12th Congress of the International Plant Molecular Biology. Montpellier (France).

2019

2. Houdinet G., Guerrero-Galán C., Garcia K., Delteil A., Conéjéro G., Gaillard I., Sentenac H., Touraine B., Zimmermann S.D. (2019) Role(s) of fungal proteins involved in plant potassium nutrition during ectomycorrhizal symbiosis. 4th international Molecular Mycorrhiza Meeting iMMM, 6 - 8 February 2019, Torino (Italy).

Selected oral presentations in national symposia/congress

Posters in international symposia/congress

2014

1. Drain A, Wang L, Véry AA, Fizames C, Pauly N, Gaillard I, and Sentenac H.(2014) Early Steps in Nodulation signaling : Role of ion channels in Medicago Root hairs (6-10 July 2014) XVI international congress on Molecular Plant-Microbe Interactions MPMI. Rhodes (Greece)

2. Martinière A., Gibrat G., Sentenac H., Paris N. (2014) Media acidification and proton gradient in Arabidopsis thaliana roots. (8-11 September 2014) XVII meeting of European Network for Plant Endomembrane Research. Lecce (Italie).

2018

3. Houdinet G., Guerrero-Galan C., Garcia K., Delteil A., Corratgé-Faillie C., Conéjéro G., Gaillard I., Touraine B., Sentenac H., Zimmermann S.D. (2018) Unravelling nutrient exchange in ectomycorrhizal symbiosis contributing to plant potassium nutrition. (5 -10 August) 12th Congress of the International Plant Molecular Biology. Montpellier (France).

4. Lecourieux D., Delrot S., Moreau H., Gaillard I., Gomès E., Lecourieux F. (2018) Functional characterization of a heat-inducible ethylene response factor and its putative role in the control of the sugar/acid balance in grape berries (July 15-20, 2018). XII international conference on grapevine Breeding and Genetics 2018, Bordeaux (France).

2019

5. Houdinet G., Guerrero-Galán C., Garcia K., Delteil A., Conéjéro G., Gaillard I., Sentenac H., Touraine B., Zimmermann S.D. (2019) Role(s) of fungal proteins involved in plant potassium nutrition during ectomycorrhizal symbiosis. (6 - 8 February 2019) 4th international Molecular Mycorrhiza Meeting iMMM, , Torino (Italy).

Posters in national symposia/congress

Invited seminars in foreign institutions

2019

1. Paris N. Use of ratiometric fluorescent pH sensors in plant cells – potential and limitation. (31-01-2019) University of Amsterdam (The Netherlands).

Invited seminars in French institutions

2014

1. Paris N. Use of fluorescent ratiometric sensors and live imaging to study proton-mediated events (16 May 2014) Agropolis foundation, Montpellier (France).
2. Paris N. Shedding light on pH at both sides of the root plasma membrane interface using non-invasive imaging. (7 june 2014) Montpellier (France).

2017

3. Gaillard I, **SweetKaliGrape**: Towards improvement of grape and wine qualities through the control of K^+ and sugar accumulations.(11 April 2017) ANR-REV workshop, Paris (France)
4. Paris N. New fluorescent ratiometric pH sensors to study proton homeostasis in living plants (29-09-2017) Institut jean-Pierre Bourgin, Versailles (France)

4- Electronic tools and products

Softwares

Databases

5- Instruments and methodology

Prototypes

Platforms and observatories

6- Other products

Theorised artistic creations, staging, movies

7- Editorial activities

Participation in editorial committees (books, collections, etc.)

Nadine PARIS - Member of editorial committee of Plant Signaling & Behavior

Collection and series management

8- Reviewing activities

Reviewing of articles

1 x Nature Plant, 2 x Physiol, 1 x Plant J, 1 x Plant Biotech, 2 Molecular cell, 1 Frontiers in Plant Sciences, 1 IJMS, 1 Plant Science, 2 J Exp Bot

Grant evaluation (public or charities)

1 x Israël Research Grant
1X Pakistan Research Grant

Reviewing of research institutes

Participation in institutional committees and juries (CNRS, Inserm, etc.)

2 x Rapporteur de these de doctorat
4 x PhD committee

9- Academic research grants

European (ERC, H2020, etc.) and international (NSF, JSPS, NIH, World Bank, FAO, etc.) grants – coordination

European (ERC, H2020, etc.) and international (NSF, JSPS, NIH, World Bank, FAO, etc.) grants - partnership

Other European grants - coordination

Other European grants - partnership

National public grants (ANR, PHRC, FUI, INCA, etc.) - coordination

2014 – 2019 ; **SWEETKALIGRAPE (ANR PRC)** ; Vers une amélioration de la qualité du raisin et du vin par le contrôle de l'accumulation du potassium et du sucre ; GAILLARD Isabelle

2017 – 2018 ; **PHABABerry (Department BAP INRA)** ; Rôle des partenaires moléculaires régulateurs de l’activité des canaux potassiques de type Shaker dans le maintien de l’acidité de la baie de raisin en relation avec le changement climatique ; CHEREL Isabelle

National public grants (ANR, PHRC, FUI, INCA, etc.) - partnership

Local grants (collectivités territoriales) - coordination

2018 – 2023 ; **PROTOMICS (Région Languedoc Roussillon)** ; Homéostasie des protons dans la baie de raisin en relation avec le changement climatique ; PARIS Nadine

Local grants (collectivités territoriales) - partnership

PIA (labex, equipex etc.) grants - coordination

PIA (labex, equipex etc.) grants - partnership

2016 – 2019 ; **APLIM (Labex Agro)** ; Development of magnetic resonance technologies (NMR,MRI and nanoprobes) to support integrative biology of plant response to abiotic and biotic constraints - Advanced Plant Life Imaging and Metrology ; GAILLARD Isabelle

2018 – 2022 ; Key Initiative Montpellier MUSE Vine&Wine Sciences ; GAILLARD Isabelle

Grants from foundations and charities (ARC, FMR, FRM, etc.) - coordination

Grants from foundations and charities (ARC, FMR, FRM, etc.) - partnership

10- Visiting senior scientists and post-doc

Post-docs (total number)

Since 2014: 1

Foreign post-docs

Since 2014: 1

Last Name	First Name	Status/Employer	Nationality	Périod	
Bidzinski	Przemyslaw	CDD INRA ANR	POLONAIS	01/03/2015	31/12/2016

Visiting scientists (total number)

Since 2014 : 0

Foreign visiting scientists

Since 2014 : 0

11- Scientific recognition

Prizes and/or distinctions

IUF members

Chair of learned and scientific societies

Organisations of meetings and symposia (out of France)

Invitations to meetings and symposia (out of France)

II - INTERACTION OF THE TEAM WITH THE NON-ACADEMIC WORLD, IMPACTS ON ECONOMY, SOCIETY, CULTURE OR HEALTH

1- Socio-economic interactions / Patents

Invention disclosures

Filed patents

Accepted patents

Licenced patents

2- Socio-economic interactions

Industrial and R&D contracts

Cifre fellowships

Creation of labs with private-public partnerships

Networks and mixed units

Start-ups

3- Expertise

Consulting

Participation in expert committees (ANSES etc.)

Legal expertise

Expert and standardization reports

4- Public outreach

Radio broadcasts, TV shows, magazines and newspaper

Journal articles, interviews, book edition, videos, other popularization outputs, debates on science and society, etc.

III - INVOLVEMENT OF THE TEAM IN TRAINING THROUGH RESEARCH

1- Educational outputs

Books

E-learning, MOOCs, multimedia lessons, etc.

2- Scientific productions (articles, books, etc.) from theses

Scientific productions (articles, books, etc.) from theses

Scientific articles

Lefoulon C., Boeglin M., Moreau B., Véry A.A., Szponarski W., Dauzat M., Michard E., Gaillard I., Chérel I. (2016) The Arabidopsis AtPP2CA protein phosphatase inhibits the GORK K+ efflux channel and exerts a dominant suppressive effect on phosphomimetic activating mutations. J. Biol. Chem. 291(12): 6521-6533

Nieves-Cordones M., Andrianteranagna M., Cuéllar T., Chérel I., Gibrat R., Boeglin M., Moreau B., Paris N., Verdeil J.L., Zimmermann S.D., Gaillard I. (2019). Characterization of the grapevine Shaker K^+ channel VvK3.1 supports its function in massive potassium fluxes necessary for berry potassium loading and pulvinus-actuated leaf movements. New Phytol. 222(1):286-300. doi: 10.1111/nph.15604.

Review articles

Chérel I., Lefoulon C., Boeglin M., Sentenac H. (2014) Molecular mechanisms involved in plant adaptation to low K+ availability. J.Exp.Bot. .65(3):833-848

Mean number of publications per student (Biology & Science and technology only)

3- Training

Habilitated (HDR) scientists

Since 2014 : 3

Last name	First name	Status	Employer	Defense Date
CHEREL	Isabelle	chercheur	INRA MONTPELLIER	2007
GAILLARD	Isabelle	chercheur	INRA MONTPELLIER	2010
PARIS	Nadine	chercheur	CNRS	2002

HDR obtained during the period

Since 2014 : 0 HDR

PhD students (total number)

2 PhD students

PhD students benefiting from a specific doctoral contract

Last Name	First Name	Starting date	Defense date	Duration (months)	Funding
MOREAU	Hortense	01/10/2018		36	CDO
VILLETTE	Jeremy	01/10/2016		36	CDO

Defended PhDs

0 defended PhD

Mean PhD duration

Mean duration: 0 months

Internships (M1, M2)

Master 1: 3

Master 2: 3

Last name	First Name	Status	Period	
Martins	Laura	Master 1	03/03/2014	30/06/2014
El Hajjaji	Salima	Master 1	21/03/2017	21/08/2017
Brenière	Manon	Master 1	05/03/2018	29/06/2018
Laboisse	Samuel	Master 2	03/03/2014	29/08/2014
Commovick	Charlie	Master 2	08/01/2018	08/07/2018
Guillemot	Quentin	Master 2	08/01/2018	08/07/2018

People in charge for a mention or a master's degree course (total number)

People in charge for a mention or a master's degree course with international certification (Erasmus mundus)

Metal Toxicity team
Self-assessment document

Name of the team concerned by the current contract: Metal Phytotoxicity
Acronym of the current contract: Metal toxicity
Team leader for the current contract: Pierre Berthomieu

This team will be closed on 31/12/2020. Therefore, no project is presented for the next contract

RESULTS

1- Presentation of the team

Introduction

The « Metal phytotoxicity » team has been created in 1998. Since 2005 it has been lead by P. Berthomieu. Its objectives have been to uncover mechanisms that plants develop to cope with metals, considering both metal tolerance and metal accumulation. In 2016, P. Berthomieu decided to stop leading the team at the end of the on-going contract (initially scheduled to end in December 2019). As no member of the team wanted to succeed him, the team will not apply for its renewal. Consequently, the members of the team started to move to other teams.

Team's workforce and means

At the beginning of the examined period, the permanent staff of the team comprised 6 scientists (P. Berthomieu, PR SupAgro; P. Doumas, CRHC INRA; F. Gosti, CR CNRS; L. Marquès, MC UM; C. Nespoulous, CR INRA; H. Rouached, CR INRA), and 2 technical teaching assistants at 50% of their time (T. Castillo, ADT UM; V. Thiry, AJT SupAgro). After decision was taken not to renew the team for the next contract period, H. Rouached moved to the HoNuDe team in 2016, L. Marquès and P. Doumas to the Plasticity team also in 2016, P. Berthomieu to the TiCer team in 2019 and V. Thiry to the HoNuDe team in 2019 as well. In agreement with A. Gojon, and C. Maurel, in between January 2019 and December 2020, the « Metal phytotoxicity » team will continue to exist as composed of F. Gosti, C. Nespoulous and T. Castillo, with a co-direction by F. Gosti and C. Nespoulous.

Between 2014 and 2019, the team hosted 5 full-time PhD students (M. Alsulaiman; J. Bertrand; M. Kisko; O. Mith; N.N.T. Nguyen), 4 foreign sandwich-PhD students (N. Belgaroui; N. Bouain; V. Miranda; C. Saenchai), one post-doctoral fellow (S. Pal, 2014-15), two Engineers on short-term contracts (J. Bertrand, I. Ribeiro) and 8 M2, ~10 M1 and ~5 L3 undergraduate students.

Our research activity has been financially supported by a regularly renewed grant from the French 'Institut du Tabac' (Imperial Tobacco group) as well as by grants gained from ANR (EVOFUN, 2012-2016) and Labex AGRO (Defensevolution, 2015-2017; IDP-Pro, 2015-2018; CultiVar, 2017-2018). We have also been supported at least once by the 'solidarity fund' of the B&PMP UMR, which should again occur in 2019 and 2020.

Scientific policy

As mentioned earlier, our scientific objectives have been to uncover new mechanisms that plants develop to cope with metals, considering both metal tolerance and metal accumulation. Several models have been studied, including *Arabidopsis thaliana*, metal-hyper-accumulating species such as *Arabidopsis halleri* or *Hirschfeldia incana*, or crops such as lettuce, tobacco, rice or wheat. Depending on the considered model as well as on the topic of interest, we worked on zinc, cadmium, lead or iron. For most of the projects, the different elements were considered independently from each other. However, one of the projects aimed at analyzing the interaction between different minerals, and that particular project considered the interaction between zinc and phosphorous. In addition, another project aimed at analyzing the interaction between water stress and zinc constraint.

A common guideline underlying the different projects was to uncover new mechanisms of interest through the development of forward genetic or open-minded approaches. Thus, most of the projects usually started by a screening step, based on either functional complementation of a wild-type yeast, a transcriptome approach, a transcriptomic-based modeling approach, or QTL or GWAS approaches. Then when worthwhile candidate genes were identified, more specific experiments have been developed to characterize the roles of these genes as well as the mechanism(s) in which they are involved. Considering this general process, the different projects are at different stages of development, some of them focusing on the identification of genes of interest, while other ones focusing on the characterization of the identified genes.

2- Research products and activities for the team

Scientific track record

Analysis of the contribution of defensins to zinc tolerance, and more generally to the response to different constraints. Functional expression in planta

Previously, our team showed that plant defensins could contribute to increase zinc tolerance in wild-type yeast as well as in *A. thaliana* (Mirouze et al., 2006). Since then, we have tried to identify the mechanisms in which defensins are involved, as well as their multiple roles in planta. In this project, we worked both on yeast, and in planta. This project has been very difficult as defensin are involved in a very diverse set of processes. Thus, a single defensin appeared to display antifungal activity (as expected) together with playing a role in zinc tolerance, in providing tolerance to some oxidizing agents and in reducing root growth when brought to the culture medium (Mith et al., 2015). We also showed that defensins could play their role in yeast when their location was restricted to the endoplasmic reticulum, and that they activate the unfolded protein response in yeast. A transcriptomic approach conducted in yeast and confirmed by biochemical analyses showed that in the presence of zinc, defensin overexpression leads to ergosterol overproduction (Berthomieu et al., 2014). Through collaboration, we also analyzed the structure of the protein (Meindre et al., 2014). After having resolved a high yield purification process of the recombinant protein produced in *Pichia pastoris*, the recombinant protein was shown to be able to chelate zinc in its reduced form but not in its oxidized one. The binding affinity constant and stoichiometry were shown to be less than 10^{-4} M^{-1} and 3 Zn ions per molecule of protein, respectively. The challenge now consists in identifying the residues (among which some of the 8 cysteines) involved in the metal coordination and evaluating their redox potential. The zinc chelation properties of defensins might confer them a ligand-centered redox activity or enable the generation of a zinc signal, both being potent effectors of many cellular processes, depending on the environmental context in which the cell is living.

In order to gain insight into the functional complexity of defensin (*PDF*s and *DEFL*s) family in plant response to both abiotic and biotic stresses (zinc excess and fungal pathogen), we undertook a combination of bioinformatics analyses, molecular genetic approach together with functional and physiological analyses on *A. thaliana PDF1* KO and amiRNA transgenic lines together with *PDF1* overexpression lines. This was performed in collaboration with Dr O. Lamotte and Pr D. Wendehenne (AgroEcologie, Dijon), Dr D. Vile (LEPSE, Montpellier), Dr N. Chantret and Pr V. Ranwez (AGAP, Montpellier), Pr O. Franco (UCB, Brésil) and Dr A. Kozevnikova (Moscow, Russia). We showed that: **(i)** an endogenous *AtPDF1* transcript decrease impacts plant response to both biotic and abiotic stresses *via* different routes (a manuscript should be submitted by June 2019). We are currently performing transcriptomic analysis on amiRNA transgenic lines in early response to zinc application (in collaboration with Drs S. Ruffel and G. Krouk, B&PMP); **(ii)** contrasting zinc tolerance phenotype exists between different *A. thaliana* transgenic lines accumulating each high levels of different *AhPDF1* transcripts, suggesting the existence of expression regulation at the transcriptional and / or translational level. Work on this aspect will be completed by tissue zinc quantification and localization studies (a manuscript should be submitted by the end of 2020); **(iii)** the ability of defensins to confer zinc tolerance is not restricted to the initially discovered *PDF1* clade but is also present in the *PDF2* clade (as tested from *A. thaliana* and *A. halleri* families). Experiments are being completed *in planta* and a manuscript should be submitted by the beginning of 2020; **(iv)** the ability of *PDF*s to confer zinc tolerance extends also to *DEFL*s clades and beyond *Brassicaceae* family, since *Vigna ugniculata* (cowpea, Cp) defensins from the *Fabaceae* family, also confer zinc tolerance in yeast. Since Cp defensins also have anti-alpha amylase activity (which protect plants against insect attack), this result also shows that several defensins activities can be combined within the same defensin scaffold. *In planta* validations for zinc tolerance and pathogens attack (fungal and insects) traits are in progress (a manuscript should be submitted in 2020). Overall, we showed (i) that zinc tolerance can be provided by different *PDF*s or *DEFL*s clades over different plant families and can be combined to other defensin activities and (ii) that a decrease in their expression impacts plant response to both biotic and abiotic stresses.

Impact of plant zinc tolerance on plant response to multiple stresses

In collaboration with D. Vile (LEPSE, Montpellier) the impact of the combined application of a water deficit and zinc constraints was analysed in the metal extremophile species *A. halleri* and in the non-metallophyte species *A. thaliana* and *A. lyrata*. In the presence of zinc the growth of *A. halleri* plants was not altered by the water deficit. This response translates into plasticity in the economics of water use and leaf morphology. The publication of the results is written and is circulating between the authors.

Definition of technical pathways for phytostabilization of mining sites – Response of the lead hyper-accumulating species Hirschfeldia incana to lead constraint

The exploitation of metallic mines generates huge amounts of waste rich in toxic metal elements, often abandoned and subject to the specific climatic hazards of the Mediterranean basin. Wind and water erosions are then leading to diffusion of metallic contamination to the environment and populations. It is desirable to fix the pollution by revegetating the polluted areas. A floristic inventory made in an abandoned metal mines (Oued El Himer, Morocco) lead to the identification of the Brassicaceae *Hirschfeldia incana*, a lead hyper-accumlulating plant perfectly adapted to contaminated soils in a semi-arid zone (Smouni et al, 2010). A transcriptomic analysis has revealed the important role of metallothioneins and of the HMA4 Zn-Cd P_{1B}-type ATPase in adaptation to high lead (Auguy et al, 2013; Fahr et al, 2015; Auguy et al., 2016; Hattab et al., 2016).

In parallel, P. Doumas constituted and coordinated a multidisciplinary network (geology, geochemistry, hydrology, biology, microbiology, medicine, human and social sciences, history ...) associating 24 teams spread across 5 countries (Algeria, France, Lebanon, Morocco and Tunisia) with ~50 permanent researchers to develop a comparative analysis of different mining sites in the Mediterranean basin (SICMED Min-Med project). The purpose of the project was to identify scientific and technical levers ensuring a sustainable and reasoned management of waste from mining sites (Doumas et al., 2018). This work was continued in France by developing programs for the phytostabilization of mine wastes by metallic tolerant plants associated with their symbiotic microorganisms. With the support of ADEME the feasibility of phytomanagement at the Carnoulès mining site (Saint-Sébastien d'Aigrefeuille, Gard) has been studied. The main challenge was to propose a program to significantly reduce acidic drainage and entrainment of metals and metalloids by runoff. This project is expected to end in 2019.

Analysis of cadmium accumulation in tobacco (Nicotiana tabacum)

Our team has a long-standing partnership (since 2007) with the French 'Institut du Tabac'. The aim of that partnership has been to propose means to reduce the amount of cadmium accumulated in tobacco leaves. Two approaches have been developed. Before 2014, the Company was interested in focusing on the so-called 'HMA4' genes, which had been shown to play a key-role in the root-to-shoot translocation of cadmium in *A. thaliana*. We identified the tobacco orthologues of AtHMA4 and showed that mutating these genes could result in reducing cadmium accumulation in the leaves. However this was linked to a reduction of the zinc accumulation, which unfortunately lead to developmental defects; the low-cadmium variants could not be used for tobacco production (Hermand et al. 2014). In the last 5 years, we developed in parallel a GWAS approach and a QTL approach to exploit the natural variation in cadmium accumulation observed between a large set of commercial and ancient tobacco varieties. This work has been very tricky as a first draft of the tobacco genome sequence has been published no earlier than in 2017, and this draft only covers 60% of the whole genome. We thus developed our own, new markers, and associated them to each other and to variation of the cadmium content from partial sequence information provided by 'Institut du Tabac'. Also, accumulation of cadmium in tobacco leaves is very different in greenhouse and in the field. Nevertheless, we identified several loci controlling cadmium accumulation in tobacco leaves in production condition. For at least two of these loci, we identified candidate genes, the orthologues of which have already been described to be potentially involved in cadmium transport in *A. thaliana*. Validation of these candidates is going on. Two manuscripts are in preparation.

Discovery of new molecular mechanisms that control Pi content in a Zn-dependent manner

Interactions between zinc (Zn) and (Pi) nutrition in plants have long been recognized, but little information is available on their molecular bases and biological significance.

First, the well known PHR1-microRNA399-PHO2 pathway regulating the response to Pi deficiency in *A. thaliana* was shown not to be involved in the regulation of Pi accumulation in response to zinc deprivation. However, PHR1 and PHO1 were shown to participate in the coregulation of Zn and Pi homeostasis. Zn deprivation had a very limited effect on the transcript levels of Pi-starvation-responsive genes or of members of the high-affinity Pi transporter family PHT1. It solely had an impact on the PHO1 homolog PHO1;H3. When grown in Zn-free medium, pho1;h3 mutant plants were shown to over-accumulate Pi in the shoot. PHO1;H3 was shown to restrict root-to-shoot Pi transfer in a PHO1-dependent manner in response to Zn deficiency (Khan et al., 2014).

Second, an algorithm, named TransDetect, has been established to predict TF combinations controlling the expression level of any gene of interest from the *in silico* analysis of transcriptomic data available in databases. TransDetect lead to the identification of TF modules (MYB15, MYB84, bHLH35 and ICE1) regulating the expression of the zinc-responsive phosphate transporter PHO1;H3. Phenotypic and genetic analyses of KO mutants lead to the uncovering of the organization of these four TFs and PHO1;H3 in a new gene regulatory network controlling phosphate accumulation in the shoot in a zinc-dependent manner (Pal et al., 2017).

Third, Zn deficiency signaling causes an over-accumulation of Pi. Analyzing natural variation in *A. thaliana* to study the role of Zn deficiency in regulating root growth revealed the key role of AZI1, a gene previously known to be involved in systemic immunity. AZI1 modulates primary root length depending on the Zn and defense status and this mechanism appeared to be conserved in rice. This result supports the hypothesis that nutrient cues can determine the balance between growth and immune responses in plants (Bouain et al., 2018).

Fourth, a genome-wide association study revealed that the Lyso-PhosphatidylCholineAcylTransferase 1 (LPCAT1) gene was a key determinant of Pi accumulation in the shoot under Zn deficiency. In -Zn condition loss of function of LPCAT1 increases the phospholipid Lyso-PhosphatidylCholine/PhosphatidylCholine ratio and the expression of the Pi transporter PHT1;1. Regulation of LPCAT1 expression is the cause of the phenotypic variation.

It was shown that this regulation involves a bZIP23 transcription factor, for which a new binding site sequence has been identified (Kisko et al., 2018).

Key events

Altogether, within the 2014-2019 time period, the different projects lead to the publication of 28 articles in indexed international peer-reviewed journals. Seven PhD theses (among which 2 sandwich-PhD theses) were defended. All the projects were developed in collaboration with around 15-20 different labs all over the world.

Annex 4 – Research products and activities

EVALUATION CAMPAIGN 2019-2020

GROUP A

Name of the theme: **Metal Phytotoxicity**
Acronym: **METAUX**

Team leader for the current contract: **Pierre Berthomieu**

I - PRODUCTION OF KNOWLEDGE AND ACTIVITIES CONTRIBUTING TO THE INFLUENCE AND SCIENTIFIC ATTRACTIVENESS OF THE TEAM

1- Articles

Scientific articles

Most significant articles (20%)

1. Belgaroui N., Zaidi I., Farhat A., Chouayekh H., Bouain N., Chay S., Curie C., Mari S., Masmoudi K., Davidian J.C., Berthomieu P., Rouached H., Hanin M. (2014) Over-expression of the Bacterial Phytase US417 in Arabidopsis Reduces the Concentration of Phytic Acid and Reveals Its Involvement in the Regulation of Sulfate and Phosphate Homeostasis and Signaling. Plant Cell Physiol. 55 (11): 1912-24.

2. Khan G.A., Bouraine S., Wege S., Li Y., De Carbonnel M., Berthomieu P., Poirier Y., Rouached H. (2014) Coordination between zinc and phosphate homeostasis involves the transcription factor PHR1, the phosphate exporter PHO1, and its homologue PHO1;H3 in Arabidopsis. J. Exp. Bot. 65(3):871-884.

3. Nguyen N.N.T., Ranwez V., Ville D., Soulie M.C., Dellagi A., Expert D., Gosti F. (2014) Evolutionary Tinkering of the Expression of PDF1s Suggests their Joint Effect on Zinc Tolerance and the Response To Pathogen Attack. Front. Plant Sci. 5: 70.

4. Belgaroui N., Berthomieu P., Rouached A., Hanin M. (2016) The secretion of the bacterial phytase PHY - US417 by Arabidopsis roots reveals its potential for increasing phosphate acquisition and biomass production during cogrowth. Plant Biotech. J. 14(9): 1914-1924.

5. Pal S., Kisko M., Dubos C., Lacombe B., Berthomieu P., Krouk G., Rouached H. (2017) TransDetect identifies a new regulatory module controlling phosphate accumulation. Plant Physiol. 175(2):916-926.

6. Bouain N., Satbhai S. B., Korte A., Saenchai C., Desbrosses G., Berthomieu P., Busch W., Rouached H. (2018) Natural allelic variation of the AZI1 gene controls root growth under zinc-limiting condition. PLoS Genet. 14 (4): e1007304.

2014

7. Bouain N., Kisko M., Rouached A., Dauzat M., Lacombe B., Belgaroui N., Ghnaya T., Davidian J.C., Berthomieu P., Abdelly C., Rouached H. (2014) Phosphate/Zinc Interaction Analysis in Two Lettuce Varieties Reveals Contrasting Effects on Biomass, Photosynthesis, and Dynamics of Pi Transport. BioMed Res Int. 2014: 548254.

8. Hermand V., Julio E., Dorlhac De Borne F., Punshon T., Richachenevsky F.K., Bellec A., Gosti F., Berthomieu P. (2014) Inactivation of two newly identified tobacco heavy metal ATPases leads to reduced Zn and Cd accumulation in shoots and reduced pollen germination Metallomics 6(8):1427-1440.

9. Meindre F., Lelièvre D., Loth K., Mith O., Aucagne V., Berthomieu P., Marques L., Delmas A. F., Landon C., Paquet F. (2014) NMR solution structure of the synthetic AhPDF1.1b plant defensin evidences structural feature within the gamma-motif. Biochem. 53: 7745–7754.

2015

10. Briat J. F., Rouached H., Tissot N., Gaymard F., Dubos C. (2015) Integration of P, S, Fe and Zn nutrition signals in Arabidopsis thaliana: potential involvement of PHOSPHATE STARVATION RESPONSE 1 (PHR1). Front. Plant Sci. 6: 290.

11. Fahr M., Laplaze L., Mzibri M.E., Doumas P., Bendaou N., Hocher V., Bogusz D., Smouni A. (2015) Assessment of lead tolerance and accumulation in metallicolous and non-metallicolous populations of Hirschfeldia incana. Environ. Exp Bot. 109: 186-192.

12. Krouk G., Carré C., Fizames C., Gojon A., Ruffel S., Lacombe B. (2015) GeneCloud reveals semantic enrichment in lists of gene descriptions. Mol Plant. 8(6): 971-973.

13. **Mith O., Benhamdi A., Castillo T., Bergé M., MacDiamid C.W., Steffen J., Eide D.J., Perrier V., Subileau M., Gosti F., Berthomieu P., Marquès L. (2015) The antifungal plant defensin AhPDF1.1b is a beneficial factor involved in adaptive response to zinc overload when it is expressed in yeast cells. Microbiol. Open. 4(3): 409-422.**

14. **Shahzad Z.,Marquès L., Berthomieu P., Gosti F. (2015) An Assay to Test the Capacity of Arabidopsis Plant Defensin Type1 Protein to Induce Cellular Zinc (Zn) Tolerance in Yeast. Bio-protocol. 5 (22): e1653.**

2016

15. **Auguy F., Fahr M., Moulin P., El Mzibri M., Smouni A., Filali-Maltouf A., Béna G., Doumas P. (2016) Transcriptome Changes in Hirschfeldia incana in Response to Lead Exposure. Front. Plant. Sci.6 : 1231.**

16. **Damiani I., Drain A., Guichard M., Balzergue S., Boscari A., Boyer J.C., Brunaud V., Cottaz S., Rancurel C., Da Rocha M., Fizames C., Fort S., Gaillard I., Maillol V., Danchin E. G. J., Rouached H., Samain E., Su Y.H., Thouin J., Touraine B., Puppo A., Frachisse J.M., Pauly N., Sentenac H. (2016) Nod Factor Effects on Root Hair-Specific Transcriptome of Medicago truncatula: Focus on Plasma Membrane Transport Systems and Reactive Oxygen Species Networks. Front Plant Sci. 7 : 794.**

17. Hattab S., Hattab S., Flores-Casseres M.L., Boussetta H., Doumas P., Hernandez L.E., Banni M. (2016) Characterisation of lead-induced stress molecular biomarkers in Medicago sativa plants. Env. Exp. Bot. 123 : 1-12.

18. Prom-u-thai C., Jamrus S., Jaksomsak P., Rouached H., Rerkasem B. (2016) Iron, Zinc and Total Antioxidant Capacity in Different Layers of Rice Grain among Different Varieties. Int. J. Agric. Biol. 18 (06): 1131-1136.

19. **Saenchai C., Bouain N., Kisko M., Prom-u-thai C., Doumas P., Rouached H. (2016) The involvement of OsPHO1;1 in the regulation of iron transport through integration of phosphate and zinc deficiency signalling. Front. Plant Sci. 7: 396.**

2017

20. Kozhevnikova, A.D., Seregin I. V., Gosti F., Schat H. (2016) Zinc accumulation and distribution over tissues in Noccaea caerulescens in nature and in hydroponics: a comparison. Plant Soil. 411(1): 5-16.

2018

21. **Doumas P., Munoz M., Banni M., Becerra S., Bruneel O., Casiot C., Cleyet-Marel J.C., Gardon J., Noack Y., Sappin-Didier V. (2016) Polymetallic pollution from abandoned mines in Mediterranean regions: a multidisciplinary approach to environmental risks. Reg. Environ. Change. 18(3): 677-692.**

2019

22. **Zorrig W., Cornu J.-Y., Maisonneuve B, Rouached A., Sarrobert C., Shahzad Z., Abdelly C., Davidian J.-C., Berthomieu P. (2019) Genetic analysis of cadmium accumulation in lettuce (*Lactuca sativa*). Plant Physiol. Biochem. 136: 67-75.**

Review articles

Most significant articles (20%)

1. **Shahzad Z., Rouached H., Rakha A. (2014) Combating Mineral Malnutrition through Iron and Zinc Biofortification of Cereals. Compr Rev Food Sci F. 13(3):329-346.**

2014

2. Bouain N., Shahzad Z., Rouached A., Khan G. A., Berthomieu P., Abdelly C., Poirier Y., Rouached H. (2014) Phosphate and Zinc Transport and Signalling in Plants: 1 Toward a Better Understanding Of Their Homeostasis Interaction. . J. Exp. Bot. 65(20): 5725-5741.

2015

3. Kisko M., Bouain N., Rouached A., Choudhary S. P., Rouached H. (2015) Molecular mechanisms of phosphate and zinc signaling crosstalk in plants: Phosphate and zinc loading into root xylem in Arabidopsis. Environ.Exp.Bot. 114: 57-64.

4. Rouached H., Pal S., Rachmilevitch S., Libault M., Phan Tran L.S. (2015) Plants Coping Abiotic and Biotic Stresses: A Tale of Diligent Management. BioMed Res Int. vol. 2015, Article ID 754754.

5. Rouached H., Tran L.S. (2015) Regulation of Plant Mineral Nutrition: Transport, Sensing and Signaling. Int. J. Mol. Sci. 16 (12): 29717-29719.

2016

6. Bouain N., Doumas P., Rouached H. (2016) Recent advances in understanding the molecular mechanisms regulating the root system response to phosphate deficiency in Arabidopsis. Curr. Genomics 17(4): 308-314.

Other articles (professional journals, etc.) (total number)

2- Books

Monographs, critical editions, translations (total number)

Management and coordination of scientific books / Scientific book edition

Book chapters (total number)

Book chapters in English or another foreign language

Edited theses

3- Production in conferences / congresses and research seminars

Articles published in conference proceedings / congress

Other products presented in symposia / congress and research seminars

Invited oral presentations in international symposia/congress

2014

1. Berthomieu P. De la tentative de compréhension des mécanismes de tolérance des plantes aux métaux à la mise au point d'un procédé biotechnologique de production des stérols (20-24/12/2014) Journées Internationales de Biotechnologie de l'Association Tunisienne de Biotechnologie. Hammamet (Tunisie).

Invited oral presentations in national symposia/congress

2014

1. Marquès L. A plant defensin that confers cellular zinc tolerance (27-28/10/2014) 1ères journées du GDR 'Multifonctions des PAMs'. Dourdan (France).

2016

2. **Nguyen N.N.T., Shahzad Z., Mai T.D., Alsulaiman M., Berthomieu P., Gosti F. (2016) Evolutionary Insight on Plant Defensin type 1 (PDF1). (02-08 Juin) AMP2016 international symposium on antimicrobial peptides. Montpellier (France).**

Selected oral presentations in international symposia/congress

2014

1. **Zorrig W., Rouached A., Abdelly C., Berthomieu P. Identification de gènes impliqués dans l'accumulation des métaux lourds chez la laitue (Lactuca sativa) (24-27 avril 2014) 1er congrès International de Technologies Alimentaires et Contrôle Qualité des Aliments. Djerba (Tunisia).**

Selected oral presentations in national symposia/congress

2015

1. **Gosti F. (2015) Functional expression of Defensins for plant zinc tolerance. (30 Juin-02Juillet 2015) GDRI LocoMet (Transport, Localisation and Complexation of metals in hyperaccumulating plants). Lille (France).**
2. **Nguyen N.N.T., Shahzad Z., Mai T.D., Berthomieu P., Gosti F. (2015) Plant Defensin type 1 (PDF1) from an evolutionary perspective". (26-27 Octobre 2015) GDR MUFOPAM (Multi-Fonction des Peptides Anti-Microbiens). Orléans, France.**

2016

3. **Gosti F. (2016) Zinc tolerance within the Plant Defensin Family. (06-08 Juillet 2016) GDRI LocoMet (Transport, Localisation and Complexation of metals in hyperaccumulating plants). Lille (France).**

Posters in international symposia/congress

2015

1. **Miranda V., Berthomieu P., Nolasco D., Franco O., Gosti F. (2015) Engineering ideotype plants from Defensin Protein Promiscuity. (26-27 Octobre 2015) GDR MUFOPAM (Multi-Fonction des Peptides Anti-Microbiens) Orléans (France).**
2. **Nguyen N.N.T., Shahzad Z., Mai T.D., Alsulaiman M., Alcon C., Berthomieu P., Gosti F.(2015) Plant response to zinc excess: insight on Plant Defensin type 1 (PDF1) expression regulation. (10-11 Juillet 2015) Post-transcriptional Gene Regulation in Plants Meeting. Paris (France).**

2018

3. **Beye A., Thiry V., Roumet P., David J., Berthomieu P. (2018) Towards the positional cloning of genes controlling zinc and iron content in durum wheat grain. (19-21 mars 2018) EUCARPIA cereal section. Clermont-Ferrand (France).**

Invited seminars in foreign institutions

2016

1. Nguyen N. N.T., Shahzad Z., Mai T. D., Berthomieu P., Gosti F. Plant Defensin type 1 (PDF1) from an evolutionary perspective. (26 Fevrier 2016) Universidade Catolica Dom Bosco, Campo Grande (Brésil).
2. Nguyen N. N.T., Shahzad Z., Mai T. D., Berthomieu P., Gosti F. Plant Defensin type 1 (PDF1) from an evolutionary perspective. (19 Fevrier 2016) EMBRAPA (Empresa Brasileira de Pesquisa Agropecuária, Brazilian Agricultural Research Corporation) Brasilia (Brésil).
3. Nguyen N. N.T., Shahzad Z., Mai T. D., Berthomieu P., Gosti F. Plant Defensin type 1 (PDF1) from an evolutionary perspective. (16 Fevrier 2016) Universidade Catolica de Brasilia, Brasilia, (Brésil).

4- Electronic tools and products

Softwares

Databases

5- Instruments and methodology

Prototypes

Platforms and observatories

6- Other products

Theorised artistic creations, staging, movies

7- Editorial activities

Participation in editorial committees (books, collections, etc.)

Collection and series management

8- Reviewing activities

Reviewing of articles

All the scientific members of the group reviewed submitted manuscript. Depending on the person considered, this activity ranged between 1-5 manuscripts / year.

Grant evaluation (public or charities)

All the scientific members of the group reviewed submitted grants. Depending on the person considered, this activity ranged between 1-2 grant / year

Reviewing of research institutes

Participation in institutional committees and juries (CNRS, Inserm, etc.)

2014-2017: P. Berthomieu has been President of the fifth section of the Commission Nationale des Enseignants-Chercheurs relevant du Ministère de l'Agriculture (CNECA).

9- Academic research grants

European (ERC, H2020, etc.) and international (NSF, JSPS, NIH, World Bank, FAO, etc.) grants – coordination

European (ERC, H2020, etc.) and international (NSF, JSPS, NIH, World Bank, FAO, etc.) grants - partnership

Other European grants - coordination

Other European grants - partnership

National public grants (ANR, PHRC, FUI, INCA, etc.) - coordination

2012 – 2016 ; **EVOFUN (ANR)** ; Analyses évolutive et fonctionnelle du rôle de la famille multigénique MTP1 dans l'acquisition de la tolérance au zinc chez Arabidopsis halleri ; BERTHOMIEU Pierre

National public grants (ANR, PHRC, FUI, INCA, etc.) - partnership

Local grants (collectivités territoriales) - coordination

Local grants (collectivités territoriales) - partnership

PIA (labex, equipex etc.) grants - coordination

2015 – 2016 ; **DEFENS EVOLUTION (Labex AGRO)** ; Tackling the DEFENSIN family Evolution within the extremophile species Arabidopsis halleri ; GOSTI Françoise

2015 – 2018 ; **IDP PRO (Labex AGRO)** ; Construction de plantes idéotype sur la base du caractère promiscuitaire des protéines défensines ; GOSTI Françoise

PIA (labex, equipex etc.) grants - partnership

2017 ; **(Projet Cultivar - Labex AGRO)** ; intitulé du projet pour Abdoulaye Beye à ajouter ; BERTHOMIEU Pierre

2017 ; **(Projet Cultivar - Labex AGRO)** ; intitulé du projet pour DUC TAM MAI à ajouter ; GOSTI Françoise

2018 ; **(Projet Cultivar - Labex AGRO)** ; Carthographie fine de loci contrôlant l'accumulation de zinc, de fer et de phytate dans le grain de blé dur ; BERTHOMIEU Pierre

Grants from foundations and charities (ARC, FMR, FRM, etc.) - coordination

Grants from foundations and charities (ARC, FMR, FRM, etc.) - partnership

10- Visiting senior scientists and post-doc

Post-docs (total number)

Depuis 2014: 1

Foreign post-docs

Depuis 2014: 1

Name	First Name	Contract type & Employer	Nationality	Périod	
Pal	Sikander	CDD INRA Agreenskills	INDIEN	20/01/2014	19/01/2016

Visiting scientists (total number)

Depuis 2014 : 16

Foreign visiting scientists

Since 2014 : 16

Name	First name	Financing source	Nationality	Date of arrival	Date of departure	Status	University of origin
Asif	Muhammad	Bourse	Turc	15/07/2015	30/09/2015	Doctorant invité	Université de Sabanci Istanbul Turquie
Chaiwong	Nantana	Bourse	Thailandais	04/07/2016	15/10/2016	Doctorant invité	Fac Chiang Mai bourse université
Gafsaoui	Rabiaa	Bourse	Tunisien	09/11/2015	11/12/2015	Doctorant invité	Institut National des Recherches en Génie Rural, Eau, Forêts Tunisie
Gomez Gallego	Tamara Maria	Bourse	Espagnol	12/09/2017	15/12/2017	Doctorant invité	Estacion experimental del zaidin Espagne
Grami	Rihab	Bourse	Tunisien	23/10/2015	23/11/2015	Doctorant invité	Fac des Sciences Tunis
Hattab	Sara	Bourse	Tunisien	14/04/2014	30/06/2014	Doctorant invité	ISA Chott-Mariem Tunisie
Kouas	Saber	Salaire	Tunisien	01/07/2014	30/09/2014	Maitre assistant invité	Fac des sciences de Gabes
Kozhevnikova	Anna	Salaire	Russe	17/08/2015	17/10/2015	Chercheur invitée	Université de Russie

Le Thi	Van Ahn	Bourse	Vietnamien	01/11/2015	20/11/2015	Chercheur invitée	Université des Sciences et Technologies d'Hanoï Vietnam
Lehout	Amel	Bourse	Algerien	01/10/2014	31/03/2016	Doctorant invité	Centre de recherche Biotechnologie Constantine Algérie
Meghnous	Ouissem	Bourse	Algerien	04/01/2016	04/04/2016	Doctorant invité	Fac des freres Mentouri Algerie
Miranda	Vivian	Bourse	Bresilienne	06/04/2015	27/11/2015	Doctorant invité	Université du Brezil
Mongon	Jenjira	Bourse	Thailandais	01/05/2016	31/10/2016	Post-doc invité	Bodhivijjalaya College akharinwirot University
Nsibi	Khaoula	Bourse	Tunisien	15/09/2016	15/12/2016	Doctorant invité	Fac Tunis El Manar
Saenchai	Chorpet	Bourse campus France	Thailandais	01/07/2015	31/12/2015	Doctorant invité	Faculté d'agriculture Chiang May Thaïlande
Seregin	Llya	Salaire	Russe	17/08/2015	17/10/2015	Chercheur invitée	Université de Russie

11- Scientific recognition

Prizes and/or distinctions

IUF members

Chair of learned and scientific societies

Organisations of meetings and symposia (out of France)

Invitations to meetings and symposia (out of France)

II - INTERACTION OF THE TEAM WITH THE NON-ACADEMIC WORLD, IMPACTS ON ECONOMY, SOCIETY, CULTURE OR HEALTH

1- Socio-economic interactions / Patents

Invention disclosures

Filed patents

1- Berthomieu P, Mith O, Marquès-Bastide L, Perrier V, Dubreucq E (2014) Procédé de production d'un composé de la voie de biosynthèse des stérols chez un organisme eucaryote. FR1458763 - PCT/FR2015/052485

2- World extension of the previous filed patent : Berthomieu P, Mith O, Marquès-Bastide L, Perrier V, Dubreucq E (2016) Procédé de production d'un composé de la voie de biosynthèse des stérols chez un organisme eucaryote. WO 2016/042267

Since attempts to develop the TRL3 step were not successful and no licence could be expected, INRA cancelled the application in 2017.

Accepted patents

Licenced patents

2- Socio-economic interactions

Industrial and R&D contracts

2014 – 2016 ; **ARN3 (ARN - Research contract)** ; Identification de déterminants génétiques contrôlant l'accumulation du cadmium, nickel, plomb et arsenic chez le tabac à partir de l'analyse d'une large collection variétale ; BERTHOMIEU Pierre

2015 – 2017 ; **ERGOSTEROL (SATT)** ; Procédé de production d'un composé de la voie de biosynthèse des stérols chez un organisme eucaryote ; BERTHOMIEU Pierre

2016 – 2018 ; **SEITA (SEITA - Research contract)** ; Identification and validation of genetic determinants controlling cadmium accumulation in tobacco ; BERTHOMIEU PIERRE

Cifre fellowships

Creation of labs with private-public partnerships

Networks and mixed units

Start-ups

3- Expertise

Consulting

Participation in expert committees (ANSES etc.)

Legal expertise

Expert and standardization reports

4- Public outreach

Radio broadcasts, TV shows, magazines and newspaper

Journal articles, interviews, book edition, videos, other popularization outputs, debates on science and society, etc.

III - INVOLVEMENT OF THE TEAM IN TRAINING THROUGH RESEARCH

1- Educational outputs

Books

E-learning, MOOCs, multimedia lessons, etc.

2- Scientific productions (articles, books, etc.) from theses

Scientific productions (articles, books, etc.) from theses

Scientific articles

Belgaroui N., Zaidi I., Farhat A., Chouayekh H., Bouain N., Chay S., Curie C., Mari S., Masmoudi K., Davidian J.C., Berthomieu P., Rouached H., Hanin M. (2014) Over-expression of the Bacterial Phytase US417 in Arabidopsis Reduces the Concentration of Phytic Acid and Reveals Its Involvement in the Regulation of Sulfate and Phosphate Homeostasis and Signaling. Plant Cell Physiol. 55 (11): 1912-24

Bouain N., Kisko M., Rouached A., Dauzat M., Lacombe B., Belgaroui N., Ghnaya T., Davidian J.C., Berthomieu P., Abdelly C., Rouached H. (2014) Phosphate/Zinc Interaction Analysis in Two Lettuce Varieties Reveals Contrasting Effects on Biomass, Photosynthesis, and Dynamics of Pi Transport. BioMed Res Int. 2014: 548254.

Hermand V., Julio E., Dorlhac De Borne F., Punshon T., Richachenevsky F.K., Bellec A., Gosti F., Berthomieu P. (2014) Inactivation of two newly identified tobacco heavy metal ATPases leads to reduced Zn and Cd accumulation in shoots and reduced pollen germination Metallomics 6(8):1427-1440.

Meindre F., Lelièvre D., Loth K., Mith O., Aucagne V., Berthomieu P., Marques L., Delmas A. F., Landon C., Paquet F. (2014) NMR solution structure of the synthetic AhPDF1.1b plant defensin evidences structural feature within the gamma-motif. Biochem. 53: 7745–7754.

Nguyen N.N.T., Ranwez V., Ville D., Soulie M.C., Dellagi A., Expert D., Gosti F. (2014) Evolutionary Tinkering of the Expression of PDF1s Suggests their Joint Effect on Zinc Tolerance and the Response To Pathogen Attack. Front. Plant Sci. 5: 70.

Mith O., Benhamdi A., Castillo T., Bergé M., MacDiamid C.W., Steffen J., Eide D.J., Perrier V., Subileau M., Gosti F., Berthomieu P., Marquès L. (2015) The antifungal plant defensin AhPDF1.1b is a beneficial factor involved in adaptive response to zinc overload when it is expressed in yeast cells. Microbiol. Open. 4(3): 409-422

Auguy F., Fahr M., Moulin P., El Mzibri M., Smouni A., Filali-Maltouf A., Béna G., Doumas P. (2016) Transcriptome Changes in Hirschfeldia incana in Response to Lead Exposure. Front. Plant. Sci.6 : 1231

Belgaroui N., Berthomieu P., Rouached A., Hanin M. (2016) The secretion of the bacterial phytase PHY -US417 by Arabidopsis roots reveals its potential for increasing phosphate acquisition and biomass production during cogrowth. Plant Biotech. J. 14(9): 1914-1924.

Saenchai C., Bouain N., Kisko M., Prom-u-thai C., Doumas P., Rouached H. (2016) The involvement of OsPHO1;1 in the regulation of iron transport through integration of phosphate and zinc deficiency signalling. Front. Plant Sci. 7: 396.

Pal S., Kisko M., Dubos C., Lacombe B., Berthomieu P., Krouk G., Rouached H. (2017) TransDetect identifies a new regulatory module controlling phosphate accumulation. Plant Physiol. 175(2):916-926.

Zorrig W., Cornu J.-Y., Maisonneuve B, Rouached A., Sarrobert C., Shahzad Z., Abdelly C., Davidian J.C., Berthomieu P. (2019) Genetic analysis of cadmium accumulation in lettuce (*Lactuca sativa*). Plant Physiol. Biochem. 136: 67-75.

Review articles

Bouain N., Shahzad Z., Rouached A., Khan G. A., Berthomieu P., Abdelly C., Poirier Y., Rouached H. (2014) Phosphate and Zinc Transport and Signalling in Plants: 1 Toward a Better Understanding Of Their Homeostasis Interaction. . J. Exp. Bot. 65(20): 5725-5741.

Shahzad Z., Rouached H., Rakha A. (2014) Combating Mineral Malnutrition through Iron and Zinc Biofortification of Cereals. Compr Rev Food Sci F. 13(3):329-346.

Kisko M., Bouain N., Rouached A., Choudhary S. P., Rouached H. (2015) Molecular mechanisms of phosphate and zinc signaling crosstalk in plants: Phosphate and zinc loading into root xylem in Arabidopsis. Environ.Exp.Bot. 114: 57-64.

Bouain N., Doumas P., Rouached H. (2016) Recent advances in understanding the molecular mechanisms regulating the root system response to phosphate deficiency in Arabidopsis. Curr. Genomics 17(4): 308-314.

Other articles (professional journals, etc.) (total number)

Book chapters in English or another foreign language

Mean number of publications per student (Biology & Science and technology only)

3- Training

Habilitated (HDR) scientists

Since 2014: 5

Family Name	First name	Position	Employer	Date of habilitation
BERTHOMIEU	Pierre	enseignant-chercheur	MONTPELLIER SUPAGRO	2007
DOUMAS	Patrick	chercheur	INRA MONTPELLIER	2011
GOSTI	Francoise	chercheur	CNRS	2012
MARQUES	Laurence	enseignant-chercheur	UNIVERSITE MONTPELLIER	2015
ROUACHED	Hatem	chercheur	INRA MONTPELLIER	2011

HDR obtained during the period

Since 2014: 1 HDR

Family Name	First name	Position	Employer	Date of habilitation
MARQUES	Laurence	enseignant-chercheur	UNIVERSITE MONTPELLIER	2015

PhD students (total number)

6 PhD students

PhD students benefiting from a specific doctoral contract

Family Name	First name	Starting date	Defense	Duration (months)	Funding
BELGAROUI	Nibras	01/04/2013	17/12/2016	45	ETR
BOUAIN	Nadia	01/04/2014	30/06/2016	27	ETR
MITH	Oriane	03/10/2011	19/09/2014	36	CDO
NGUYEN-THI	Ngoc-Nga	11/04/2011	24/03/2014	35	ETR
ALSULAIMAN	Mohannad	01/10/2014	13/09/2018	48	ETR
BERTRAND	Jérémy	15/10/2016	30/11/2018	25	Contrat industriel

Defended PhDs

6 defended PhDs

Family Name	First name	Date of defense
BELGAROUI	Nibras	2016
BOUAIN	Nadia	2016
MITH	Oriane	2014
NGUYEN-THI	Ngoc-Nga	2014
ALSULAIMAN	Mohannad	2018
BERTRAND	Jérémy	2018

Mean PhD duration

Mean duration: 36 months

Internships (M1, M2)

Master 1: 5

Master 2: 5

Family Name	First Name	University Degree	Period	
Mevizou	Rudy	Master 1	23/02/2015	22/04/2015
Mai	Duc Tam	Master 1	06/03/2017	31/07/2017
Beye	Abdoulaye	Master 1	01/05/2017	31/08/2017
Seck	Fallou	Master 1	07/05/2018	31/07/2018
Ngom	Aïda	Master 1	17/05/2018	31/08/2018
Hammami	Amal	Master 2	06/01/2014	26/06/2014

Fendri	Rania	Master 2	01/02/2014	31/05/2014
Villette	Jeremy	Master 2	05/01/2015	30/06/2015
Lelgouarch	Elsa	Master 2	01/02/2017	01/08/2017
Trinh	Van Giap	Master 2	23/05/2016	23/11/2016

People in charge for a mention or a master's degree course (total number)

L. Marquès: in charge of the ‘Plant Functional Biology’ mention of the License of Biology of University of Montpellier (2014-2018)

People in charge for a mention or a master's degree course with international certification (Erasmus mundus)

FeROS team
Self-assessment document

Name of the team concerned by the current contract: Mineral Nutrition and Oxidative Stress
Name of the team concerned by the next contract (if different):
Acronym of the current contract: FeROS
Acronym of the next contract (if different):
Team leader for the current contract: Frédéric Gaymard (2014/2016) & Christian Dubos (since 2017)

Team leader for the next contract: Christian Dubos

RESULTS

1- Presentation of the team

Introduction

The group currently comprises 6 permanent members, including 4 Researchers and 2 engineers. While being stable in people number, the physiognomy of the group, created by F. Gaymard in 2008, has substantially changed during the 2014-2019 period. As it will be described below (see the Unit's workforce and means paragraph), several movements among the permanent staff have occurred: 4 people left the group, 3 joined the group and 2 made a transient stay. Another important change has been the replacement of the former group leader by C. Dubos who animates the group since January 2017. All these changes have slightly influenced the research organization without, however, modifying the overall scientific strategy of the team that remains focused on the study of the molecular mechanisms that regulate Fe homeostasis in plants (see the scientific policy paragraph for details).

Team's workforce and means

Six permanent staff are present in the group: Christian Dubos (DR2, INRA, Group leader), Frédéric Gaymard (DR1, INRA), Esther Izquierdo-Alegre (CRCN, INRA), Florence Vignols (CRCN, CNRS), Nathalie Berger (IEHC, INRA, 50%) and Brigitte Touraine (IE2, INRA). During the previous period several movements occurred among the permanent staff as follow: **retirements**, JF Briat (DREX, CNRS) & P. Fourcroy (CRCN, CNRS) in 2016; **departures** to other groups within the institute, F. Cellier (CRCN, INRA) in 2016 & J. Boucherez (TREX, INRA) in 2017, **transients stays**: TC Xiong (CRCN, INRA) & F. Sanchez (TR, INRA) between 2015 to 2017, **arrivals**: N. Berger (IEHC) in 2015 and F. Vignols (CRCN, CNRS) & E. Izquierdo-Alegre (CRCN, INRA) in 2016. For the next period, the group will be reinforced by the arrival of C. Nespoulous (CRCN, INRA).

The non-permanent staff present in the group are: Linda Boufeldja (PhD Student, 50%, 2018/2021), Fei Gao (PhD Student, 2017/2021), Kevin Robe (PhD Student, 2018/2020) and Maël Taupin-Broggini (PhD Student, 2018/2021). During the previous period, the group hosted 18 students: 2 BTS, 10 L3, 4 M1 and 2 M2 among which 2 (1 L3 & 1 M1) were supported by ERASMUS programs. The group has also hosted 1 ATER lecturer (M. Bettembourg, 2017/2018), 1 CDD IE (C. Magno, 2013/2015) and 1 postdoc (Marie Sklodowska-Curie fellowship, K. Ravet, 2014).

The funding for the group comes from local, national and international agencies: the **I-SITE MUSE** with the eCO2THREATS project (Alteration of plant nutrients content by elevated CO_2: consequences for crop quality, human health and environmental protection - 2019/2022 - Coord; A. Gojon), the **Labex Agro** with the FACCE project (Using Continuous Frontal Capillary Electrophoresis to characterize interactions between transcription factors and target DNA sequences - 2017/2018 - Coords. C. Dubos & J. Chamieh), the **INRA BAP** department with the BolAFER (Functions of plant BolA proteins in ferric nutrition - 2019/2020 - Coord. F. Vignols), the MULTICSTRESS (Role of TIC in plant responses to environmental stresses - 2017/2018 - Coord. E. Izquierdo-Alegre) & the RACINE projects (Role of the iron / ROS interaction in the control of root architecture - 2015/2016 - Coord. TC. Xiong), the Carnot **Plant2Pro** Institute with the POSITIF project (Promote plant tolerance to abiotic (iron) and biotic (phytopathogenic) stress via biotic interactions in the rhizosphere - 2018/2020 - Coord. P. Lemonceau), the **ANR** PRC with the MOBIFER (Dynamics of coumarin secretion by plant roots into the soil to improve iron nutrition - 2018/2021 - Coord. C. Dubos) & the Fe-S TRAFFIC projects (The cellular trafficking of Fe-S clusters in plants - 2013/2017 - Coord. N. Rouhier) and the **ECOS-FONDACYT** with a project aiming at studying the transcriptional regulation of iron homeostasis in plants (2019/2021 - Coords. C. Dubos & H. Roschzttardtz).

Scientific policy

Iron (Fe) is an essential micronutrient for plant growth and development. Due to its physico-chemical and redox properties, Fe is a main cofactor for many enzymes that catalyze redox reactions and thus participates in many fundamental biological functions (e. g. respiration, photosynthesis, nitrogen and sulphur assimilation). Although Fe is one of the most abundant elements found in soil, it is generally poorly available for plants because it is mainly present in the form of insoluble Fe^{3+}-chelates. This is for instance the case in calcareous soils that represent one-third of the world's cultivated lands. This extremely low availability of free Fe in these soils makes Fe deficiency a common nutritional disorder in plants, affecting plant growth and crop productivity. In human populations, insufficient dietary Fe intake resulting from low Fe concentrations in edible plant parts is the cause of Fe deficiency-induced anemia, affecting about one billion people worldwide. However, Fe is potentially toxic to the plants due to its ability to produce hydroxyl radicals in the presence of oxygen. This Fe toxicity occurs mainly on acidic or anoxic soils, where Fe is present in a reduced state (Fe^{2+}), a form that can be easily assimilated by plants. Similar to Fe deficiency, Fe excess results in a decrease of plant growth and crop yield. Fe is therefore a necessary but toxic element for plants whose homeostasis must be tightly regulated.

The research conducted in our group aims at deciphering the molecular mechanisms that participate to the regulation and to the maintenance of Fe homeostasis. During the 2015-2019 period, our research focused on three main axes:

1- Determining if in plants, like it is the case in mammals, there is an integrated mechanism involving common players that regulates responses to both Fe deficiency and Fe excess.

2- Deciphering the role played by coumarins - Fe-mobilizing phenolic compounds secreted by the roots into the rhizosphere - in plant Fe nutrition.

3- Characterizing the machineries leading to [Fe-S] cluster assembly in chloroplasts and in mitochondria, a mechanism that is crucial for Fe assimilation and for the whole plant metabolism.

It is noteworthy that our research on the study of plant Fe homeostasis is orthogonal - by the approaches and the objectives - but complementary to the one developed on this micronutrient in the group of C. Curie (BPMP). These differences are an asset for answering global questions on plant Fe nutrition and thus the development of ambitious and transversal projects for the laboratory as it is the case with eCO2THREAT.

Actions taken to implement recommendations received during the previous evaluation:

1- Maintain a critical mass of researchers in the team: our policy to attract scientists and engineer to maintain the critical size of the team while gathering skills necessary for the project (e.g. proteomics) has been successful. Overall the number of permanent staff has remained stable.

2- Widen the sources of funding beyond the ANR: We have explored several sources for financial support, in particular at the local level through the Labex Agro or the iSITE MUSE and through specific call from the INRA BAP department.

3- Transfer of knowledge to a species of agronomic interest: we have initiated some studies using tomato, in particular for the above-described axes 1 and 2 (e.g. eCO2TRHEAT project). We benefit from tight connections with M. Causse (GAFL, Genetics and Breeding of Fruit and Vegetables, INRA Avignon) and C. Rothan (BFP, Fruit Biology and Pathology, INRA Bordeaux) who have extended expertise, tools and knowledge on this crop.

2- Research products and activities for the team

Scientific track record

1- Transcriptional control of Fe homeostasis: ILR3 is a central regulator.

For several years, the research developed in the group was focused on the study (i.e. physiological role and regulation) of ferritin genes, the main markers of plant response to Fe excess (Petit et al., 2001; Arnaud et al., 2006; Ravet et al., 2009a, Ravet et al., 2009b; Ravet et al., 2012; Bournier et al., 2013; Reyt et al., 2015). It led to original findings, such as the establishment of an unsuspected connection between Fe homeostasis and the circadian clock, phosphate availability or the DownSTream mRNA decay pathway that is essential for plant fitness (Duc et al., 2009; Ravet et al., 2012; Bournier et al., 2013). However, if these studies showed that ferritins synthesis was transcriptionally activated by Fe, they failed to identify any regulator in common with the well-described transcriptional cascade that regulates plant response to Fe deficiency (Gao et al., 2019). This was surprising since Fe homeostasis in mammals relies on an integrated mechanism that couples the regulation of both Fe deficiency and Fe excess responses.

We have thus developed a strategy to determine if in plants, like it is the case in mammals, there is an integrated mechanism involving common players that regulate both responses. For this purpose, *AtFER1* - the main ferritin isoform present in *Arabidopsis thaliana* - was used as model. We found that ILR3 (IAA LEUCINE RESISTANT 3, bHLH105) - a transcriptional activator of responses to Fe shortage - was a repressor of *AtFER1* expression (Tissot et al., 2019). Further investigations revealed that ILR3 repressed the expression of several structural genes that function in the control of Fe homeostasis and that ILR3 repressive activity was conferred by its dimerization with another bHLH transcription factor named PYE/bHLH47. We also found that important facets of plant growth in response to Fe deficiency or excess rely on ILR3 activity. Altogether, our work demonstrated that ILR3 is at the center of the transcriptional regulatory network that controls Fe homeostasis in Arabidopsis, in which it acts as both transcriptional activator and repressor. Nevertheless, several lines of evidence indicate that additional key transcriptional regulators involved in this pathway are still to be identified.

Therefore, since the identification of ILR3 has been a important step forward in elucidating the regulatory network controlling Fe homeostasis, it appears obvious to us that we have to follow on this path (Tissot et al., 2019). We have chosen to anchor our research on ILR3 by precisely depicting the role of this transcription factor in the control of Fe homeostasis. It implies first identifying all ILR3 targets and protein partners - using omic-driven approaches - prior to reconstructing the whole regulatory network that controls Fe homeostasis. This choice was reinforced by the fact that, to date, none of the groups working in this field has initiated a similar strategy. This topic is conducted in collaboration with M. Benhamed (IPS2, Orsay, France - Omic experiments and analysis),

H. Roschzttardtz (Pontifical Catholic University of Chile, Santiago, Chile - Transcriptional control of Fe homeostasis), W. Schmidt (Academia Sinica, Taipei, Taiwan - Long distance Fe signaling) and the MSPP (Mass Spectrometry Proteomics Platform) at BPMP. We have recently obtained support for people exchange with the groups of H. Roschzttardtz (ECOS-FONDACYT, 2019-2021) and W. Schmidt (Ministry of Science and Technology, Taiwan, 2018).

2- Plant Fe nutrition: a novel role for Coumarins.

In response to Fe shortage, plants have evolved a reduction-based mechanism to mine Fe from the soil (Brumbarova et al., 2015; Curie and Mari, 2017). In Arabidopsis, Fe^{3+} chelates present in the soil are reduced by the FERRIC REDUCTION OXIDASE 2 (FRO2) activity and the generated Fe^{2+} is then transported across the rhizodermis cell membranes through the IRON- REGULATED TRANSPORTER 1 (IRT1). This process is facilitated by the activity of the AHA2 proton-ATPase whose activity leads to rhizosphere acidification. We, and others, have demonstrated that Fe acquisition is facilitated by the synthesis and excretion by the rhizodermis-specific ATP-BINDING CASSETTE G37/PLEIOTROPIC DRUG RESISTANCE 9 (PDR9/ABCG37) transporter of phenolic compounds belonging to the coumarin subfamily (Rodriguez-Celma et al., 2013; Fourcroy et al., 2014; Schmid et al., 2014; Rajniak et al., 2018). Coumarins are secondary metabolites derived from the phenylpropanoid pathway (Fourcroy et al., 2014; Sisó-Terraza et al., 2016; Rajniak et al., 2018). We have also demonstrated that the secreted coumarins are involved in the remobilization of insoluble Fe^{3+} present in the growth medium rendering it available to the FRO2/IRT1 high-affinity root Fe^{2+} transport system (Fourcroy et al., 2016). This later finding highlights the preponderant role played by secreted coumarin compounds in the plant response to Fe shortage by improving their capacity to mine the soil. Because the importance of coumarins for Fe nutrition of non-grass plant species has been underestimated, the mechanisms leading to their secretion into the rhizosphere still need to be investigated as manipulating these pathways potentially represents a novel route for improving plant Fe nutrition.

We are thus pursuing our investigations on coumarin biosynthesis and their involvement in the control of Fe homeostasis. This was an obvious choice since we have been part of the leading teams - at the international level - on this topic and since we are the sole group that has developed an original method allowing coumarins visualization *in planta* (i.e. spectral imaging) and thus the *in vivo* study of coumarin biosynthesis and trafficking. This project is currently supported by the ANR (MOBIFER project) and is conducted in collaboration with S. Thomine (I2BC, Paris-Saclay, France - Coumarin transporter characterization) and N. Rouhier (University of Loraine, Nancy, France - Glutathione S-transferase (GST) characterizations). Locally, this project strongly benefits from PHIV (Histocytology and Plant Cell Imaging platform, Montpellier) and from the expertise of G. Conejero (BPMP). Interestingly, the secretion of such secondary metabolites by plants into the rhizosphere is known to have an impact on the microorganism communities that are present in the soil which in turn influences plant growth, development and health (Philippot et al., 2013; Stringlis et al., 2018). The impact of coumarins secreted by plants in shaping microbiome communities in the vicinity of their roots has increased our interest for the plant biotic interactions. Indeed, following this path necessitates collaborating with groups working on soil sciences and microorganism communities. In this regard, we have been solicited to be part of three initiatives, the POSITIF and eCO2THREAT projects and an ITN action (Marie Sklodowska-Curie Innovative Training Networks) that aims at deciphering belowground chemical communication between plants and microbes (BECOME project, under evaluation, Coordinator: P. Garbeva, NIOO-KNAW, Netherlands).

3- [Fe-S] cluster assembly: deciphering the role played by [Fe-S] carrier proteins

Iron–sulfur ([Fe-S]) clusters are prosthetic groups generally embedded into protein scaffolds via ligation of Fe through cysteine side chains, and bridging sulfur atoms. Iron–sulfur proteins ([Fe-S] proteins) are one of the most abundant and versatile classes of redox proteins. They are near universal in nature, playing important roles in a wide range of biological processes, ranging from electron transfer to catalysis, Fe level regulation and storage and transport of ligands within the cell. They participate in important functions such as respiration, DNA translation and repair, sulfur and nitrogen assimilation or photosynthesis. The [Fe-S] cluster biogenesis machinery is composed of scaffold proteins that build the cluster de novo and of carrier proteins that transfer the [Fe-S] prosthetic groups to their target proteins (also called *apo*-proteins). In plants three different machineries lead to [Fe-S] cluster assembly, namely the mitochondrial ISC (Iron Sulfer Cluster) system, the chloroplastic SUF (SUlFur mobilization) system and the cytosolic CIA (Cytosolic [Fe-S] cluster Assembly) system (Lill and Mühlenhoff, 2008). Our team has been focusing for several years on the chloroplastic SUF system and specifically on the characterization of the three [Fe-S] carrier proteins named NFUs (NiFU-LIKE PROTEINS). For instance, we have shown that NFU2 and NFU3 were involved in photosystem I (PSI) assembly and thus in photosynthesis (Touraine et al., 2004; Touraine et al., 2019). More recently we have found that NFU2 is crucial for proper root development by participating in the maturation of the DHAD (DIHYDROXYACID DEHYDRATASE) protein, an enzyme involved in the synthesis of branched-chain amino acids (i.e. valine, leucine, isoleucine) (Touraine et al., 2019). Interestingly, this work also highlighted that NFU2 participates as a direct partner in the maturation of both [2Fe-2S] and [4Fe-4S] clusters, unlike other prokaryotic and eukaryotic NFU proteins that are specifically involved in the maturation of [4Fe-4S] proteins.

While characterizing the machineries leading to [Fe-S] cluster assembly, we have found that this mechanism is more complex than it was originally thought, in particular because of the several partial redundancies that exist between the protein involved, because of the lack of knowledge for several actors whose loss-of-function mutants display lethal phenotypes and also because the precise biochemical function of some of the actors is not clearly established. How NFUs interact with the other [Fe-S] carrier and associated proteins (i.e. SUFA1, GRXS14, GRXS16, HCF101, BolA) to deliver [Fe-S] cluster to the final acceptors (45 potential targets in Arabidopsis) is still to be determined and is a question that our group aims at answering (BolAFER project). This aspect of the work developed in the group benefits from long standing collaborations, in particular with N. Rouhier (University of Loraine, Nancy, France) and J. Balk (John Innes Center, Norwich UK), with who two previous ANR projects were obtained on this topic (FIRES, 2011-2013 & Fe-S Traffic, 2014-2017).

Key events

- Identification of PDR9 as the transporter allowing the secretion of coumarins into the rhizosphere and the demonstration of the tight connection between coumarins extrusion and the IRT1/FRO2 high affinity Fe uptake machinery (Fourcroy et al., 2014; Fourcroy et al., 2016). Beside the general interest of these findings in the field of plant Fe nutrition, it has opened new perspectives for the team on the study of the interactions between plants and microorganisms.

- The Montpellier international school on ion and water transport in plant (MISTRAL) in 2016 and 2018 (organization, scientific & pedagogical committees, teaching). This is a transversal project for the unit that offers a unique opportunity to exchange on what makes the specificity of BPMP with students from all around the world.

FIVE-YEAR PROJECT AND STRATEGY

3- SWOT analysis

Strong points

- High complementarities (skills and knowledge) between the members of the group.
- Long standing expertise in plant Fe nutrition, redox signaling, transcriptional regulation and secondary metabolism.
- Large array of potential collaborators due to the diverse origins of the group members.
- Strong and lasting collaborations.

Needs for improvements

- Connections with the university of Montpellier - Teaching.
- Integration of abiotic factors in our studies.
- Links with private partners (We and other researchers from BPMP have contributed to the building of a research project within a partnership with the Frayssinet private company, deposited in Mai 2019 in the frame of the LabCom ANR call for research proposals).
- Relates our work to crop species (tomato)

Possibilities brought in by the context / surrounding environment

- Get access to students from the University of Montpellier and SupAgro.
- Get access to financial support from the Labex Agro and the I-SITE MUSE.
- Set up integrated projects (from the soil to the plant to the quality of their derived products) by interacting with other units focusing on complementary topics (e.g. soil sciences, biotic interactions, crop genetics, food quality).

Risks linked to the context / environment.

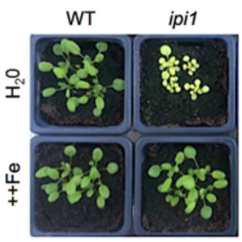
- Local policy favoring studies on tropical/Mediterranean species and agroecology.

4- Structure, workforce and scientific orientations

During the 2015-2019 period, we have obtained significant results on our three research axes. From these results, our project has evolved and gained in maturity modifying the course of our investigations. Importantly, during the same period, we have established (and maintained) several collaborations that will be essential for the success of our project (see the Scientific track record for details). For these reasons we have decided to follow up on our three research axes.

Transcriptional regulation of Fe homeostasis.

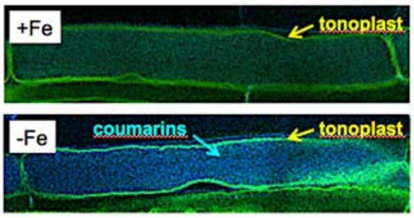
We have recently identified ILR3 as a key player in recalibrating Fe homeostasis. Our objective will be to fully decrypt the molecular mechanisms that are dependent on ILR3 activity. This will necessitate identifying and characterizing ILR3 protein partners and target genes. We have recently initiated preliminary experiment (i.e. IP-MS) at BPMP with the MSPP (Mass Spectrometry Proteomics Platform) which led to the identification of a novel bHLH transcription factor that interacts with ILR3 - we have named IPI1 (ILR3 Protein Interactor 1) - and whose activity is required to maintain Fe homeostasis. This recent finding is a novel step that comforts us in pursuing the study of ILR3. The next objective will be to decrypt, at the whole-genome level, the role of the ILR3-depedent regulatory complexes in reprogramming gene expression in accordance with Fe availability in both roots and aerial tissues. Altogether, in addition to the identification of new actors and molecular mechanisms underlying the control of cellular Fe homeostasis, we will aim at providing new insights into how plants can sense fluctuating concentrations of Fe and trigger systemic signals dictating the root response. **Permanent staff involved:** C. Dubos (PI) E. Izquierdo-Alegre & N. Berger (25%).



ipi1 loss-of-function mutant displays a chlorotic phenotype due to Fe homeostasis defects

Dynamics of coumarins secretion by plant roots into the soil.

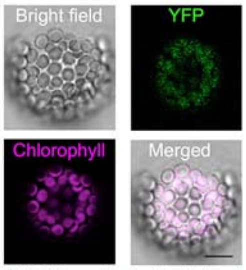
We have recently shown that coumarins secretion into the rhizosphere plays an important role for plant Fe uptake, and thus for the maintenance of plant Fe homeostasis. However, the secretion of coumarins is a mechanism that has a cost for the plant as it necessitates deriving a part of the assimilated carbon through the photosynthesis into compounds that will be likely lost upon secretion. In addition, accumulating phenolic compounds may be toxic to the cell. They are thus compartmentalized, mostly in the extracellular space and the vacuole in order to protect the cellular components towards their deleterious effects. Finally, the secretion of such secondary metabolites by the plant into the rhizosphere is known to have an impact on the microorganism communities that are present in the soil affecting plant growth and health. Remarkably, despite the importance of coumarins secretion into the rhizosphere for plant adaptation to Fe deficiency as well as its interaction with its surrounding belowground environment, very little is known on the mechanisms involved in coumarins transport and storage within the roots. This is precisely this mechanism that we aim at elucidating. It will necessitate to precisely depicting coumarins trafficking within root cells and to identify the molecular actors involved. **Permanent staff involved:** C. Dubos (Co-PI), E. Izquierdo-Alegre (Co-PI) & N. Berger (25%).



Coumarins visualisation by spectral imaging in the vacuole of the root cells of plant grown under Fe deficiency.
Green: TIP2;3:GFP; Bleu: coumarins.

[Fe-S] cluster biogenesis.

Regarding our future research on [Fe-S] assembly machineries, a first task will be to continue our efforts on the functional characterization of [Fe-S] shuttle proteins and on the identification of their related *apo*-receptor proteins in the chloroplast. At first, we will characterize HCF101, a [4Fe-4S] shuttle protein acting downstream of NFU functions and essential for photosynthesis performances. To date, only one HCF101 target has been identified, but several other potential *apo*-receptor proteins remain to be identified and/or functionally validated. A second task will be dedicated to the study of two other protein families, the CGFS-type Glutaredoxins (GRX) and the BolA proteins. Monothiol GRXs and BolA physically interact in several compartments of the cell including the chloroplast. Interestingly, they have also coevolved upon time in most organisms, suggesting important associated functions. In human and in yeast, their key role in Fe homeostasis signaling has notably been demonstrated, but the true function(s) of GRX-BolA complexes remain to be identified in plants. For both tasks, the analysis of hypomorphous transgenic Arabidopsis lines and mechanistic studies on *apo*-receptor candidates and GRX-BolA targets should fulfill important gaps of knowledge in these fields. Finally in a long-term third task, we will address the question of [Fe-S] assembly machineries regulation in plants (redox states at first) in connection with the environmental changes to which the plant is subjected. **Permanent staff involved:** F. Vignols (PI), F. Gaymard, C. Nespoulous & B. Touraine.



BiFC interaction in Arabidopsis chloroplast of HCF101 with a novel partner

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Annex 4 – Research products and activities

EVALUATION CAMPAIGN 2019-2020

GENERAL INFORMATION

Name of the team: Mineral nutrition and oxidative stress
Acronym: FeROS

Project leader for the current contract: F. Gaymard (2014/2016) & C. Dubos (since 2017)
Project leader for the next contract: C. Dubos

I - PRODUCTION OF KNOWLEDGE AND ACTIVITIES CONTRIBUTING TO THE INFLUENCE AND SCIENTIFIC ATTRACTIVENESS OF THE TEAM

1- Articles

Scientific articles

1. **Tissot N, Robe K*, Gao F*, Grant-Grant S, Boucherez J, Bellegarde F, Maghiaoui A, Marcelin R, Izquierdo E, Benhamed M, Martin A, Vignols F, Roschzttardtz H, Gaymard F, Briat J-F, Dubos C✉ (2019) Transcriptional integration of the responses to iron availability in Arabidopsis by the bHLH factor ILR3. New Phytol., (accepted)**

2. **Touraine B, Vignols F*, Przybyla-Toscano J*, Ischebeck T, Dhalleine T, Wu H-C, Magno C, Berger N, Couturier J, Dubos C, Feussner I, Caffarri S, Havaux M, Rouhier N✉, Gaymard F✉ (2019) Iron-sulfur protein NFU2 is required for branched-chain amino acid synthesis in Arabidopsis roots. J. Exp. Bot., 70(6):1875-1889**

3. **Fourcroy P*, Tissot N*, Gaymard F, Briat J-F, Dubos C✉ (2016) Facilitated Fe nutrition by phenolic compounds excreted by the Arabidopsis ABCG37/PDR9 transporter requires the IRT1 / FRO2 high affinity root Fe2+ transport system. Mol. Plant, 9(3):485-488**

2014

1. Couturier J., Wu H.C., Dhalleine T., Pégeot H., Sudre F., Gualberto J., Jacquot J.P., Gaymard F., Vignols F., Rouhier N. (2014) Monothiol glutaredoxin-BolA interactions: redox control of Arabidopsis thaliana BolA2 and SufE1. Mol Plant. 7(1):187-205.

2. **Fourcroy P., Siso-Terraza P., Sudre D., Saviron M., Reyt G., Gaymard F., Abadia A., Abadia J., Alvarez-Fernandez A., Briat J.F. (2014) Involvement of the ABCG37 transporter in secretion of scopoletin and derivatives by Arabidopsis roots in response to iron deficiency. New Phytol. 201(1):155-167.**

2015

3. Knuesting J., Riondet C., Maria C., Kruse I., Bécuwe N., König N., Berndt C., Tourrette S., Guilleminot-Montoya J., Herrero E., Gaymard F., Balk J., Belli G., Scheibe R., Reichheld J.P., Rouhier N., Rey P. (2015) Arabidopsis glutaredoxin S17 and its partner NF-YC11/NC2α contribute to maintenance of the shoot apical meristem under long-day photoperiod. Plant Physiol. 167(4): 1643-1658.

4. **Reyt G., Boudouf S., Boucherez J., Gaymard F., Briat J. F. (2015) Iron and ferritin dependent ROS distribution impact Arabidopsis root system architecture. Mol Plant. 8(3): 439-453.**

2016

5. Siso-Terreza P., Luis-Villarroya A., Fourcroy P., Briat J. F., Abadia A., Gaymard F., Abadia J., Alvarez-Fernandez A. (2016) Accumulation and Secretion of Coumarinolignans and other Coumarins in Arabidopsis thaliana Roots in Response to Iron Deficiency at High pH. Front. Plant Sci. 7: 1711.

2017

6. **Alejandro S, Cailliatte R, Alcon C, Dirick L, Domergue F, Correia D, Castaings L, Briat J.F., Mari S, Curie C (2017) Intracellular distribution of manganese by the trans-golgi network transporter NRAMP2 is critical for photosynthesis and cellular redox homeostasis. Plant Cell 29(12): 3068-3084**

7. **Pal S, Kisko M, Dubos C, Lacombe B, Berthomieu P, Krouk G, Rouached H (2017) TransDetect identifies a new regulatory module controlling phosphate accumulation. Plant Physiol. 175(2):916-926**

Review articles

1. **Briat J-F✉, Dubos C, Gaymard F (2015) Iron nutrition, biomass production and plant product quality. Trends Plant Sci., 20(1):33-40**

2014

2. **Tissot N., Przybyla-Toscano J., Reyt G., Castel B., Duc C., Boucherez J., Gaymard F., Briat J.F., Dubos C. (2014) Iron around the clock Plant Sci. 224: 112-119. (Revue bibilographique)**

2015

3. **Briat J. F., Rouached H., Tissot N., Gaymard F., Dubos C. (2015) Integration of P, S, Fe and Zn nutrition signals in Arabidopsis thaliana : potential involvement of PHOSPHATE STARVATION RESPONSE 1 (PHR1).Front. Plant Sci. 6: 290. (revue bibliographique)**

2016

4. **Vigani G. and Briat J.F. (2016) Impairment of respiratory chain under nutrient deficiencies in plants: does it play a role in the regulation of iron and sulfur responsive genes? Front. Plant Sci.6 : 1185. (revue bibliographique)**

2018

5. Przybyla-Toscano J., Roland M., Gaymard F., Couturier J., Rouhier N. (2018) Roles and maturation of iron-sulfur proteins in plastids. J. Biol. Inorg. Chem. 23(4): 545-566. (Revue bibliographique)

2019

6. **Gao F., Robe K., Gaymard F., Izquierdo E., Dubos C. (2019) The Transcriptional Control of Iron Homeostasis in Plants: A Tale of bHLH Transcription Factors? Front Plant Sci. 10: 6. (Revue bibilographique)**

7. Sylvestre-Gonon E., Law S., Schwartz M., Robe K., Keech O., Didierjean C., Dubos C., Rouhier N., Hecker A. (2019) Functional, structural and biochemical features of plant serinyl-glutathione transferases. . Front Plant Sci. 10: 608. (Revue bibilographique)

8. **Rey P., Taupin-Broggini M., Couturier J., Vignols F., Rouhier N. (2019) Is there a role for glutaredoxins and BOLAs in the perception of the cellular iron status in plants? Front. Plant Sci. 10:712. (review)**

Other articles (professional journals, etc.) (total number)

2015

1. Sanchez F., Xiong T.C. (2015) Outils de gestion des ressources biologiques. Le Cahier des Techniques de l'INRA. 84 (1).

2016

2. Briat J.F. (2016) La vie fixée des plantes et ses contraintes In Encyclopédie de l'environnement (Communauté de l'Université. Grenoble-Alpes eds) http://www.encyclopedie-environnement.org/vivant/vie-fixee-plantes-contraintes/

2018

3. Safi A., Medici A., Szponarski W., Marshall-Colon A., Ruffel S., Gaymard F., Coruzzi G., Lacombe B., Krouk G. (2018) HRS1/HHOs GARP transcription factors and reactive oxygen species are regulators of Arabidopsis nitrogen starvation response. bioRxiv.

2- Books

Monographs, critical editions, translations (total number)

Management and coordination of scientific books / Scientific book edition

2017

1. **Briat J.F., Job D.(2017) Les sols et la vie souterraine : Des enjeux majeurs en agroécologie: Collection Synthèses (QUAE Eds) p. 328 ISBN : 978-2-7592-2651-1.**

Book chapters (total number)

Book chapters in English or another foreign language

2016

1. **Kelemen Z., Przybyla-Toscano J., Tissot N., Lepiniec L., Dubos C. (2016) Fast and Efficient Cloning of Cis-Regulatory Sequences for High-Throughput Yeast One-Hybrid Analyses of Transcription Factors. *In* Methods in Molecular Biology : Plant Synthetic Promoters. J.S. WALKER (ed.), Springer-Verlag GmbH, Heidelberg, Germany. 1482: 139-49.**

2. **Thévenin J., Xu W., Vaisman L., Lepiniec L., Dubreucq B., Dubos C. (2016) The Physcomitrella patens System for Transient Gene Expression Assays. *In* Methods in Molecular Biology : Plant Synthetic Promoters. J.S. WALKER (ed.), Springer-Verlag GmbH, Heidelberg, Germany. 1482: 151-161.**

3. **Xiong TC., Sanchez F., Briat J. F., Gaymard F., Dubos C. (2016) Spatio-Temporal Imaging of Promoter Activity in Intact Plant Tissues. *In* Methods in Molecular Biology : Plant Synthetic Promoters. J.S. WALKER (ed.), Springer-Verlag GmbH, Heidelberg, Germany. 1482: 103-110.**

Edited theses

3- Production in conferences / congresses and research seminars

Articles published in conference proceedings / congress

Other products presented in symposia / congress and research seminars

Invited oral presentations in international symposia/congress

2014

1. Briat J.F., Reyt G., Tissot N., Gaymard F., Dubos C.Twenty five years of ferritin research : what's up now ? » (July 2014) XVII Iron Symposium on Iron Nutrition and Interactions in Plants. Gatersleben (Germany).

2. Couturier J, Roret T, Wu HC, Tsan P, Tiphaine D, Pégeot H, Jacquot JP, Vignols F, Didierjean C, Rouhier N. Biochemical, spectroscopic and structural insights into BolA-glutaredoxin complexes. (20-25 July 2014) Gordon research conference “Thiol-based redox regulation and signalling”, Girona (Spain).

3. Mari S., Grillet L., Hoang M., Ouerdane L., Flis P.,Isaure M.P.,Chay S., Alcon C., Ravet K., Briat J.F., Curie C. The dynamics of iron pools between organelles and within organs. (July 2014) XVII Iron Symposium on Iron Nutrition and Interactions in Plants. Gatersleben (Germany)

2015

4. Przybyla-Toscano J, Touraine B, Couturier J, Gaymard F, Vignols F, Rouhier N. The cellular trafficking of Fe-S clusters: physiological roles of chloroplastic and mitochondrial NFUs. (24-26 June 2015) 7th International Conference on Iron-Sulfur Cluster Biogenesis and Regulation., Bergamo (Italia).

2016

5. Przybyla-Toscano J, Touraine B, Jacquot JP, Gaymard F, Couturier J, Vignols F, Rouhier N. Deciphering the late-steps of FeS cluster assembly in plant organelles: focus on NFUs. (21-24 sept 2016) COST action CA15133 FeSBioNET “The biogenesis of iron-sulfur proteins: from cellular biology to molecular aspects”, Patras (Grece).

2017

6. Tissot N., Boucherez J., Bellegarde F., Maghiaoui A., Marcelin R., Benhamed M., Martin A., Gaymard F., Briat J.F., Dubos C. (2017) Integration of the responses to iron availability fluctuations in Arabidopsis thaliana. (December 4-7) 12e Chilean Society of Plant Biologists Meeting, Villarrica (Chile).

7. Tissot N., Xu W., Kelemen Z., Grain D., Boucherez J., Berger N., Harscoët E., Routaboul J.M., Lepiniec L., Briat J.F., Gaymard F., Dubos C. (2017) Transcriptional regulation of gene expression, from flavonoid biosynthesis to iron homeostasis. (31 November - 01 December) Regulation of plant gene expression workshop, Talca (Chile).

2018

8. Przybyla-Toscano J, McLean A, Azam T, Magno C, Dhalleine T, Couturier J, Gaymard F, Vignols F, Johnson MK, Balk J, Rouhier N. Functional characterization of mitochondrial NFU proteins in plants. (29 may-02 June 2018) 8th International Conference on Iron-Sulfur Cluster Biogenesis and Regulation, Madison (USA).

9. Tissot N, Gao F, Robe K, Grant S, Boucherez J, Bellegarde F, Maghiaoui A, Marcelin R, Berger N, Izquierdo E, Latrasse D, Roschzttardtz H, Benhamed M, Martin A, Gaymard F, Briat JF, Dubos C (2018) Control of iron homeostais in Arabidopsis thaliana. 13e Chilean Society of Plant Biologists Meeting, Puerto Varas, Chile, December 4-6.

Invited oral presentations in national symposia/congress

2015

1. Przybyla-Toscano J, Jacquot JP, Couturier J, Vignols F, Rouhier N. Exploring the partners of late-acting Fe-S transfer proteins belonging to the mitochondrial ISC machinery in Arabidopsis thaliana. (21-22 september 2018) Mitocross 2015: mitochondria at the crossroad. September 2015. Strasbourg (France).

2017

2. Touraine B, Przybyla-Toscano J, Berger N, Couturier J, Gaymard F, Vignols F, Rouhier N. How are Fe-S cofactors and proteins assembled in plant cells? focus on NFUs, involved in the late steps of the maturation process in organelles. (01-02 June 2017) Days of the French society of photosynthesis, (France).

2018

3. Przybyla-Toscano J, McLean A, Azam T, Magno C, Dhalleine T, Couturier J, Gaymard F, Vignols F, Johnson MK, Balk J, Rouhier N. The maturation of Fe-S proteins in chloroplasts and mitochondria. (15-19 october 2018) Jacques Monod conference “Retrograde signalling from endosymbiotic organelles”, Roscoff (France).

Selected oral presentations in international symposia/congress

2016

1. Tissot N, Boucherez J, Maghiaoui A, Marcelin R, Gaymard F, Briat JF, Dubos C. (2016) Identification and characterization of an integrator of the plant responses to iron avalability. XVIII International Symposium on Iron Nutrition and Interactions in Plants, Madrid, Spain, 30 May - 3 June.

2018

2. Tissot N, Gao F, Robe K, Grant S, Boucherez J, Bellegarde F, Maghiaoui A, Marcelin R, Berger N, Izquierdo E, Latrasse D, Roschzttardtz H, Benhamed M, Martin A, Gaymard F, Briat JF, Dubos C (2018) Integration of the responses to iron availability fluctuations in Arabidopsis thaliana. XVIII International Symposium on Iron Nutrition and Interactions in Plants, Taipei, Taiwan, July 9-13.

3. Robe K, Conéjéro G, Tissot N, Fei G, Berger N, Bianchi M, Rouhier N, Fourcroy P, Gaymar F, Thomine S, Hecker A, Izquierdo E, Dubos C (2018) Dynamics of coumarin secretion by plant roots into the soil to improve iron nutrition. XVIII International Symposium on Iron Nutrition and Interactions in Plants, Taipei, Taiwan, July 9-13.

Selected oral presentations in national symposia/congress

2016

1. Tissot N., Boucherez J., Maghiaoui A., Marcelin R., Gaymard F., Briat J.F., Dubos C. (2016) Identification and characterization of a key integrator of the plant responses to iron avalability.(2016, July 6-8) 11^{e} Colloque National de la SFBV, Angers (France).

2. Xiong T.C., Sciallano C., Sanchez F., Briat J.F., Gaymard F. Iron-induced release of intracellular free calcium is involved in growth arrest of Arabidopsis thaliana primary root. (2016, July 6-8) 11th national meeting of the SFBV Angers Angers (France).

Posters in international symposia/congress

2014

1. Couturier J, Roret T, Wu HC, Tsan P, Dhalleine T, Pégeot H, Jacquot JP, Vignols F, Didierjean C, Rouhier N (2014) Biochemical, spectroscopic and structural insights into BolA-glutaredoxin. Gordon Research Conferences on Thiol-Based Redox Regulation & Signaling, Girona (Spain).

2. Reyt G., Boudouf S., Boucherez J., Gaymard F., Briat J.F. (2014) Iron and ferritin dependent ROS distribution impact Arabidopsis root. (July 2014) XVII Iron Symposium on Iron Nutrition and Interactions in Plants. Gatersleben (Germany).

3. Tissot N., Boucherez J., Arnaud N., Gaymard F., Briat J.F., Dubos F. (2014) New prospects on the regulation of AtFer1 expression. (July 2014) XVII Iron Symposium on Iron Nutrition and Interactions in Plants. Gatersleben (Germany).

2015

4. Tissot N., Boucherez J., Marcelin R., Martins L.,Maghiaoui A., Gaymard F., Briat J.F., Dubos C. (2015) New prospects on AtFer1 ferritin gene regulation.(2015, July 5-9) 26th International Conference on Arabidopsis Research. Paris (France).

2016

5. Izquierdo Alegre E., Fourcroy P., Boucherez J., Conéjéro G., Tissot N., Rothan C., Bres C., Mauxion J.P., Causse M., Sisó-Terraza P., Álvarez-Ferández A., Abadía J., Briat J.F., Gaymard F., Dubos C. (2016) Impact of iron availability on tomato fruit quality. (2016, 30 May-3 June) XVIII International Symposium on Iron Nutrition and Interactions in Plants. Madrid (Spain).

6. Przybyla-Toscano J., Uzarska M, Magno C., Mühlenhoff U, Vignols F., Couturier J, Lill R, Gaymard F., Rouhier N.(2016) Mitochondrial Arabidopsis thaliana NFU transfer proteins: cooperation with ISCA proteins to deliver [4Fe-4S] cluster to specific apo-targets. (2016, 30 May - 3 June) XVIII International Symposium on Iron Nutrition and Interactions in Plants. Madrid (Spain).

7. Touraine B., Magno C., Rey P., Wu H.C., Briat J.F., Rouhier N, Gaymard F., Vignols F. Nfu2 and Nfu3 act with HCF101 to transfer 4Fe- 4S clusters to PSI. (2016, 30 May - 3 June) XVIII International Symposium on Iron Nutrition and Interactions in Plants. Madrid (Spain).

2017

8. Sylvestre Gonon E, Girardet J-M, Dubos C, Rouhier N, Hecker A (2017) Biochemical characterization of glutathione transferases potentially involved in the metabolism of coumarins in Arabidopsis thaliana during iron deficiency. (September 17–21) Thiol oxidation in toxicity and signalling, Sant Feliu de Guixols (Spain).

2018

9. Berger N, Touraine B, Rofidal V, Magno C, Demolombe-Liozu V, Hem S, Vignols F, Rouhier N, Santoni V, Gaymard F, Dubos C (2018) Decrypting the chloroplastic [Fe-S] cluster assembly machinery. (July 9-13) XVIII International Symposium on Iron Nutrition and Interactions in Plants, Taipei (Taiwan).

10. Berger N, Touraine B, Magno C, Hem S, Rofidal V, Demolombe-Liozu V, Vignols F, Rouhier N, Feussner K, Ischebeck T, Zienkiewicz K, Feussner I, Santoni V, Gaymard F, Dubos C (2018) Decrypting the chloroplastic [Fe-S] cluster assembly machinery. (August 5-10) 12th International Plant Molecular Biology Meeting. Montpellier (France).

11. Fei G, Robe K, Bonillo P, Tissot N, Gaymard F, Fourcroy P, Izquierdo E, Dubos C (2018) Antagonistic effets of iron and sulfur defciencies on Arabidopsis plants. (August 5-10) 12th International Plant Molecular Biology Meeting. Montpellier (France).

12. Przybyla-Toscano J., McLean AE., Azam T., Magno C., Dhalleine T., Couturier J., Gaymard F., Vignols F., Johnson M., Balk J., Rouhier N. Arabidopsis thaliana NFU proteins participate in the final steps of the maturation of mitochondrial iron-sulfur proteins. (July 15 - 20) Gordon Research Conferences on Thiol-Based Redox Regulation & Signaling, Barga (Italia).

13. Robe K, Conéjéro G, Tissot N, Fei G, Berger N, Bianchi M, Rouhier N, Fourcroy P, Gaymard F, Thomine S, Hecker A, Izquierdo E, Dubos C (2018) Dynamics of coumarin secretion by plant roots into the soil to improve iron nutrition. (July 9-13) XVIII International Symposium on Iron Nutrition and Interactions in Plants, Taipei (Taiwan).

14. Tissot N, Gao F, Robe K, Grant S, Boucherez J, Bellegarde F, Maghiaoui A, Marcelin R, Berger N, Izquierdo E, Latrasse D, Roschzttardtz H, Benhamed M, Martin A, Gaymard F, Briat JF, Dubos C (2018) Integration of the responses to iron availability fluctuations in Arabidopsis thaliana. (July 9-13) XVIII International Symposium on Iron Nutrition and Interactions in Plants, Taipei (Taiwan).

15. Tissot N, Gao F, Robe K, Grant S, Boucherez J, Bellegarde F, Maghiaoui A, Marcelin R, Berger N, Izquierdo E, Latrasse D, Roschzttardtz H, Benhamed M, Martin A, Gaymard F, Briat JF, Dubos C (2018) Integration of the responses to iron availability fluctuations in Arabidopsis thaliana. (August 5-10) 12th International Plant Molecular Biology Meeting. Montpellier (France).

16. Touraine B., Taupin-Broggini M., Wu H.C., Sudre D., Rouhier N., Dubos C., Vignols F. Functional characterization of Arabidopsis BolA proteins in iron-sulfur center transfer pathways and in iron homeostasis. (August 5-10) 12th International Plant Molecular Biology Congress, Montpellier (France).

Posters in national symposia/congress

2016

1. Berger N., Touraine B., Magno C., Hem S., Rofidal V., Vignols F., Santoni V., Gaymard F. (2016). Decrypting the chloroplastic [fe-s] cluster assembly machinery using a label free quantification interactomic strategy (2016, 10-12 october) 33ème congrès national de la SFEAP. Chamberry (France).

2. Izquierdo Alegre E., Fourcroy P., Boucherez J., Conéjéro G., Tissot N., Rothan C., Bres C., Mauxion J.P., Causse M., Sisó-Terraza P., Álvarez-Ferández A., Abadía J., Briat J.F., Gaymard F., Dubos C. (2016) Influence of iron availability on tomato fruit quality.(2016, 6-8 July) 11e Colloque National de la SFBV. Angers (France).

2018

3. Berger N., Touraine B., Magno C., Hem S., Rofidal V., Demolombe-Liozu V., Vignols F., Rouhier N., Feussner K., Ischebeck T., Zienkiewicz K., Feussner I., Santoni V., Gaymard F., Dubos C.(2018) Decrypting the chloroplastic [fe-s] cluster assembly machinery. (5 -10 août) 12th Congress of the International Plant Molecular Biology. Montpellier (France).

4. Gao F., Robe K., Bonillo P., Tissot N., Gaymard F., Fourcroy P., Izquierdo E., Dubos C.(2018) Antagonistic effects of iron and sulfur deficiencies on arabidopsis plants. (5 -10 août) 12th Congress of the International Plant Molecular Biology. Montpellier (France).

Invited seminars in foreign institutions

2014

1. Vignols F. How biochemical and biophysical studies demonstrated the reversible zinc binding and redox-dependent flexibility of the viral suppressor/activator of RNA silencing P1rymv. Action COST, workshop Bucharest (Romania).

2. Vignols F. A Yeast Two Hybrid System detailed : The Gal4-based Y2H system. EMBRAPA (The Brazilian Agricultural Research Corporation), Brasilia (Brasil).

2015

3. Couturier J, Roret T, Wu HC, Tsan P, Tiphaine D, Pégeot H, Jacquot JP, Vignols F, Didierjean C, Rouhier N. Roles of monothiol/class II glutaredoxins in plants: redox signalling vs iron-sulfur cluster maturation. (7th August 2015) University of Freiburg (Germany).

2016

4. Couturier J, Roret T, Wu HC, Tsan P, Tiphaine D, Pégeot H, Jacquot JP, Vignols F, Didierjean C, Rouhier N. How are Fe-S cofactors and proteins assembled in plant cells? (27th june 2016) University of Kaiserslautern, (Germany).

5. Przybyla-Toscano J, Touraine B, Jacquot JP, Gaymard F, Couturier J, Vignols F, Rouhier N. The maturation Fe-S cofactors and proteins in plant cells, is there a role for glutaredoxins? (14th July 2016) University of Göttingen (Germany).

6. Przybyla-Toscano J, Touraine B, Jacquot JP, Gaymard F, Couturier J, Vignols F, Rouhier N. How are Fe-S cofactors and proteins assembled in plant cells? focus on NFUs, involved in the late steps of the maturation process in organelles. (18th July 2016) University of Marburg, (Germany).

Invited seminars in French institutions

2017

1. Dubos C. The role of phenolic compounds in plant iron acquisition. (2 février 2017) Université de Lorraine, Unité Interactions Arbres/Micro-organismes (IAM), Nancy (France).

4- Electronic tools and products

Softwares

Databases

5- Instruments and methodology

Prototypes

Platforms and observatories

6- Other products

Theorised artistic creations, staging, movies

Christian Dubos et Jean-François Briat : Movie: in support of the manuscript « Iron nutrition, biomass production, and plant product quality » published in « Trends in PlantScience » (2015). This movie is vailable on the « Trends in PlantScience » journal web site (http://www.sciencedirect.com/science/article/pii/S1360138514001988) or on WebTv , the TV channel from the University of Montpellier (http://youtu.be/afKcJUIShQg; http://www.webtv.umontpellier.fr/19934/iron-nutrition-biomass-production-plant-product-quality/).

7- Editorial activities

Participation in editorial committees (books, collections, etc.)

Collection and series management

2017

Briat J.F., Job D.(2017) Les sols et la vie souterraine : Des enjeux majeurs en agroécologie: Collection Synthèses (QUAE Eds) p. 328 ISBN : 978-2-7592-2651-1.

8- Reviewing activities

Reviewing of articles

We are reviewing papers for top rank peer reviewed journals such as Planta, Frontiers in Plant Science, Plant Molecular Biology, Plant Cell and Environment, Plant Cell and physiology, Journal of Experimental Botany, Plant Biotechnology Journal, Plant Journal, Plant Physiology, New Phytologist, The Plant Cell, Molecular Plant, Trends in Plant Science & Nature Communication

Grant evaluation (public or charities)

- BARD: The US-Israel Agricultural Research & Development Fund.
- FWO: The Research Foundation Flanders (Belgium).
- DFG: The German Research Foundation.
- COFECUB: The French Committee for Evaluation of Scientific and University Cooperation with Brazil.
- ESF: European Science Foundation (Region Grand-Est Project)
- INRA: Plant Biology and Breeding (BAP) & Science for Food and Bioproduct Engineering Departments (CEPIA).

Reviewing of research institutes

Participation in institutional committees and juries (CNRS, Inserm, etc.)

- Jury member for INRA recruitment committees: 5 (F. Gaymard & N. Berger)

- President of the jury for INRA recruitment committees: 4 (F. Gaymard)

- Jury member at the University of Montpellier for the BFP (Plant Functional Biology) and BPT (Biotechnology of tropical plants) Master degrees (since 2008). (C. Dubos & F. Vignols)

- Jury member at the University of Montpellier for the L3 professional (Plant Experimentation for the Improvement and Protection of Mediterranean and tropical plants) degree (since 2018). (F. Vignols)

- Jury member for PhD thesis defence: 6 in France (University of Montpellier, Paris Sud, Perpignan and Toulouse) and 1 in Spain (University of Grenada). (C. Dubos & F. Vignols)

- Member of PhD supervision committees: 8 in France (University of Montpellier, Nancy, Rouen and Toulouse) and 1 in Chile (University Pontifical of Santiago). (C. Dubos & F. Vignols)

9- Academic research grants

European (ERC, H2020, etc.) and international (NSF, JSPS, NIH, World Bank, FAO, etc.) grants – coordination

2018 – 2020 ; **(ECOS Sud - CONICYT)** ; Transcriptional regulation of iron homeostasis in seeds ; GAYMARD Frédéric, Hannetz Roschzttardtz (Pontificia Universidad Católica de Chile)

European (ERC, H2020, etc.) and international (NSF, JSPS, NIH, World Bank, FAO, etc.) grants - partnership

Other European grants - coordination

Other European grants - partnership

National public grants (ANR, PHRC, FUI, INCA, etc.) - coordination

2014 – 2015 ; **HARSH (Department BAP INRA)** ; Iron homeostasis and water stress ; DUBOS Christian

2015 – 2016 ; **RACINE (Department BAP INRA)** ; Role of the iron / ROS interaction in the control of root architecture ; XIONG Tou-Cheu

2017 – 2018 ; **MulTICstress (Department BAP INRA)** ; Role of TIC in integrating multistress responses ; IZQUIERDO ALEGRE Esther

2017 – 2020 ; **MOBIFER (ANR PRC)** ; Dynamics of coumarin secretion by plant roots into the soil to improve iron nutrition ; DUBOS Christian

2019 – 2020 ; **BolAFER (Department BAP INRA)** ; Functions of plant BolA proteins in ferric nutrition ; VIGNOLS Florence

National public grants (ANR, PHRC, FUI, INCA, etc.) - partnership

2014 – 2017 ; **FES-TRAFFIC (ANR PRC)** ; Cell exchanges of iron-sulfur clusters in plants ; GAYMARD Frédéric

2018 – 2020 ; **POSITIF (Institut Carnot Plant2Pro)** ; Promote pea tolerance to abiotic (iron) and biotic (phytopathogenic) stress via biotic interactions in the rhizosphere ; GAYMARD Frédéric

Local grants (collectivités territoriales) - coordination

Local grants (collectivités territoriales) - partnership

PIA (labex, equipex etc.) grants - coordination

2017 - 2018 ; **FACCE (Labex AGRO)** ; Using continuous frontal capillary electrophoresis for the characterization of the interactions between transcription factors and target DNA sequences ; DUBOS Christian

PIA (labex, equipex etc.) grants - partnership

2018 – 2021 ; **eCO2THREATS (iSITE Montpellier)** ; Alteration of plant nutrient contents by elevated CO2 : consequences for crop quality, human health and environmental protection ; DUBOS Christian

Grants from foundations and charities (ARC, FMR, FRM, etc.) - coordination

Grants from foundations and charities (ARC, FMR, FRM, etc.) - partnership

10- Visiting senior scientists and post-doc

Post-docs (total number)

Depuis 2014: 2

Foreign post-docs

Depuis 2014: 0

Visiting scientists (total number)

Depuis 2014 : 6

Foreign visiting scientists

Depuis 2014 : 3

Family Name	First name	Financing source	Nationality	Date of arrival	Date of departure	Status	University of origin
Mendez Catro	Tamara Yvonne	Grant from Chile	Chilean	13/03/2018	13/06/2018	PhD Invited	University of Talca Chile
Wu	Hui-Chen	Own financial support	Taiwanese	26/08/2016 13/11/2017	16/09/2016 15/11/2017	Assistant professor invited	National University of Tainan Taiwan
Roschzttardtz	Hannetz	Own financial support	Chilean	14/07/2018	04/08/2018	Assistant professor invited	University of Santiago Chile

11- Scientific recognition

Prizes and/or distinctions

IUF members

Chair of learned and scientific societies

Organisations of meetings and symposia (out of France)

Invitations to meetings and symposia (out of France)

II - INTERACTION OF THE TEAM WITH THE NON-ACADEMIC WORLD, IMPACTS ON ECONOMY, SOCIETY, CULTURE OR HEALTH

1- Socio-economic interactions / Patents

Invention disclosures

Filed patents

Accepted patents

Licenced patents

2- Socio-economic interactions

Industrial and R&D contracts

Cifre fellowships

Creation of labs with private-public partnerships

Networks and mixed units

Start-ups

3- Expertise

Consulting

Participation in expert committees (ANSES etc.)

Since 2018, F. Gaymard is an european expert for the HR excellence label (https://euraxess.ec.europa.eu/jobs/hrs4r). Between 1 and 3 assessments per year.

Legal expertise

Expert and standardization reports

4- Public outreach

Radio broadcasts, TV shows, magazines and newspaper

Journal articles, interviews, book edition, videos, other popularization outputs, debates on science and society, etc.

III - INVOLVEMENT OF THE TEAM IN TRAINING THROUGH RESEARCH

1- Educational outputs

Books

E-learning, MOOCs, multimedia lessons, etc.

2- Scientific productions (articles, books, etc.) from theses

Scientific productions (articles, books, etc.) from theses

- 4 articles, 3 reviews & 1 book chapter

Scientific articles

Fourcroy P., Siso-Terraza P., Sudre D., Saviron M., Reyt G., Gaymard F., Abadia A., Abadia J., Alvarez-Fernandez A., Briat J.F. (2014) Involvement of the ABCG37 transporter in secretion of scopoletin and derivatives by Arabidopsis roots in response to iron deficiency. New Phytol. 201(1):155-167.

Reyt G., Boudouf S., Boucherez J., Gaymard F., Briat J. F. (2015) Iron and ferritin dependent ROS distribution impact Arabidopsis root system architecture. Mol Plant. 8(3): 439-453.

Fourcroy P., Tissot N., Gaymard F., Briat J.F., Dubos C. (2016) Facilitated Fe nutrition by phenolic compounds excreted by the Arabidopsis ABCG37/PDR9 transporter requires the IRT1 / FRO2 high affinity root Fe2+ transport system. Mol Plant. 9(3): 485-488.

Tissot N., Robe K., Gao F., Grant-Grant S., Boucherez J., Bellegarde F., Maghiaoui A., Marcelin R., Izquierdo E., Benhamed M., Martin A., Vignols F., Roschzttardtz H., Gaymard F., Briat J. F., Dubos C. (2019) Transcriptional integration of the responses to iron availability in Arabidopsis by the bHLH factor ILR3 New Phytol (in press).

Review articles

Tissot N., Przybyla-Toscano J., Reyt G., Castel B., Duc C., Boucherez J., Gaymard F., Briat J.F., Dubos C. (2014) Iron around the clock Plant Sci. 224: 112-119.

Briat J. F., Rouached H., Tissot N., Gaymard F., Dubos C. (2015) Integration of P, S, Fe and Zn nutrition signals in Arabidopsis thaliana : potential involvement of PHOSPHATE STARVATION RESPONSE 1 (PHR1).Front. Plant Sci. 6: 290.

Gao F., Robe K., Gaymard F., Izquierdo E., Dubos C. (2019) The Transcriptional Control of Iron Homeostasis in Plants: A Tale of bHLH Transcription Factors? Front Plant Sci. 10: 6

Book chapters in English or another foreign language

Kelemen Z., Przybyla-Toscano J., Tissot N., Lepiniec L., Dubos C. (2016) Fast and Efficient Cloning of Cis-Regulatory Sequences for High-Throughput Yeast One-Hybrid Analyses of Transcription Factors. *In* Methods in Molecular Biology : Plant Synthetic Promoters. J.S. WALKER (ed.), Springer-Verlag GmbH, Heidelberg, Germany. 1482: 139-49.

Mean number of publications per student (Biology & Science and technology only)

- 2 articles, 2 reviews & 1 book chapter

3- Training

Habilitated (HDR) scientists

Since 2014 : 4

Family Name	First name	Position	Tutelle d'appartenance	Date de l'HDR
BRIAT	Jean-François	DR	CNRS	1983
DUBOS	Christian	DR	INRA MONTPELLIER	2014
GAYMARD	Frédéric	DR	INRA MONTPELLIER	2001
VIGNOLS	Florence	CR	CNRS	2016

HDR obtained during the period

Since 2014 : 2 HDR

Family Name	First name	Position	Employer	Date of habilitation
DUBOS	Christian	DR	INRA MONTPELLIER	2014
VIGNOLS	Florence		CNRS	2016

PhD students (total number)

5 PhD students

PhD students benefiting from a specific doctoral contract

Family Name	First Name	Starting date	Defense	Duration (months)	Funding
BOUFELDJA	Linda	01/10/2018		36	ETR
GAO	Fei	06/10/2017		36	ETR
ROBE	Kevin	01/01/2018		36	CDO
TAUPIN BROGGINI	Maël	01/10/2018		36	CDE
TISSOT	Nicolas	01/10/2013	06/12/2016	38	CDE

Defended PhDs

1 defended PhDs

Family Name	First Name	Date of defense
TISSOT	Nicolas	2016

Mean PhD duration

Mean duration: 38 months

Internships (M1, M2)

Master 1: 3

Master 2: 4

Family Name	First Name	University Degree	Period	
Abdou-Issa	Chafika	Master 1	01/03/2017	31/07/2017
Boudouf	Soukaina	Master 1	01/03/2014	31/07/2014
Bonillo	Pauline	Master 1	01/03/2016	30/06/2016
Safi	Alaeddine	Master 2	06/01/2014	25/06/2014

Martins	Laura	Master 2	05/01/2015	05/07/2015
Robe	Kevin	Master 2	27/03/2017	27/09/2017
Taupin-Broggini	Maël	Master 2	08/01/2018	08/07/2018

People in charge for a mention or a master's degree course (total number)

- Master 1 BFP course: "Major metabolic functions and mineral nutrition: Transcriptional regulation of iron homeostasis" (C. Dubos)

- Master 2 BFP course: “Interactions and Signaling” (F. Vignols)

People in charge for a mention or a master's degree course with international certification (Erasmus mundus)

- Organisation and scientific comity for the “Montpellier International School on ion and water Transports in Plants” (MISTRAL) summer school (Montpellier, BPMP, 2016 & 2018). (C. Dubos)

- Practice and theory courses given in the frame of the MISTRAL summer school: “Yeast as a versatile heterologous expression system for the characterization of transporter functions and regulations ». 15 participants: French and foreign PhD students. (C. Dubos & F. Vignols)

- Practice and theory courses given in the frame of the “International School on Interactomics” (Brasilia, Brazil, 2015). 50 participants: Brazilian PhD students and researchers. (F. Vignols)