

PhD PROPOSAL FOR THE DOCTORAL SCHOOL « Ecologie, Géosciences, Agronomie, ALimentation »

GENERAL INFORMATION

Thesis title: Understanding the isotope fractionation and mechanism of Rubisco, the cornerstone of photosynthesis
Acronym: ISORUBY
Disciplinary field 1: Agronomy Disciplinary field 2: Food sciences
Three keywords: isotope, Rubisco, enzyme mechanism
Research unit : IRHS (INRAE Angers)
Name of the thesis director: Anis Limami Email address of the thesis director : anis.limami@univ-angers.fr Name of the thesis co-supervisor 1 (if applicable): Guillaume TCHERKEZ Email address of the thesis co-supervisor 1 (if applicable): guillaume.tcherkez@anu.edu.au Name of the thesis co-supervisor 2 (if applicable): Email address of the thesis co-supervisor 2 (if applicable):
Thesis grant (funding origin and amount): PhD contract University of Angers
Contact(s) (mailing address and E-mail): guillaume.tcherkez@anu.edu.au
Recruitment process: Recruitment process depends on thesis funding. To select the corresponding recruitment process, please visit the EGAAL website here . This information is needed for proposal publication. <input checked="" type="checkbox"/> Doctoral school contest <input type="checkbox"/> Interview <input type="checkbox"/> Other (indicate) :

All sections must be filled. Once filled, please save the proposal form in pdf format using the following naming: Supervisor Name_Unit_Subject Acronym_EN.pdf

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SCIENTIFIC DESCRIPTION OF THE PhD PROJECT

Socio-economic and scientific context : (10 lines)

There is currently a strong interest in the application of stable isotopes to dig up agronomical biomarkers associated with plant performance. The probably most famous example (now implemented worldwide) is the use of the ^{13}C natural abundance ($\delta^{13}\text{C}$) to get a relative measurement of water use efficiency and thus obtain a quantitative trait to perform variety selection. In practice, many water-efficient varieties of major crops have been selected using $\delta^{13}\text{C}$ values, like wheat, sunflower, chickpea, peanut, etc. and current agronomical research (in particular in USA and Australia) is trying to appreciate the potential of ^{13}C for selecting C_4 plants. Locally, a grant has been awarded to G. Tcherkez (Connect Talent Ioseed, at IRHS, INRAE Angers) to find isotopic biomarkers (including ^{13}C) of seed quality, from germination to seedling establishment. However, a major conundrum in the use of $\delta^{13}\text{C}$ is the potential variation in the most important isotope discriminating step, CO_2 fixation by ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco), which determines the ^{13}C signature of both seed reserves and seedling photosynthesis .

Assumptions and questions (8 lines)

In fact, it is generally assumed that this enzyme discriminates between CO_2 isotopologues with a fixed value of the $^{12}\text{C}/^{13}\text{C}$ fractionation, of 29‰. This value has been obtained originally *in vitro* on the spinach enzyme in 1984, and close values have been obtained *in vitro* on a small number of species (in particular tobacco). However, we know that this value may change (it is more like 27‰ in C_4 grasses) and quite critically, we don't know how the value changes under non-standard conditions (that is, different from classical assays with Mg^{2+} at pH 7.8). In effect, the fractionation could change because the Rubisco mechanism is sensitive to pH, metals, ion strength, etc. while all of these factors are known to change in the chloroplast stroma under various physiological conditions. In addition, to our knowledge, no direct measurement has been carried out in Legumes, although they have a very specific Rubisco (frequent substitution of Ser 281 to Ala) particularly prone to transglutamination (covalent binding to polyamines).

The main steps of the thesis and scientific procedure (10-12 lines)

To answer these scientific issues, an in-depth analysis of Rubisco isotope fractionation is required, and this will relate to key questions about Rubisco's mechanism:

- What is the isotope fractionation of the enzyme from Legumes and how it compares to other crop species, in its polyaminated and non-polyaminated state?
- Can the isotope fractionation vary with biochemical conditions? In particular, is there any change in the fractionation when pH and the metal ion changes, considering that different metals impact on CO_2/O_2 specificity of the enzyme? The question also arises when ribulose 1,5-bisphosphate (RuBP) is replaced to its natural isomer xylulose 1,5-bisphosphate. We know the reaction can proceed at a very low rate, but is the isotope fractionation the same?
- Is there any link with side products of catalysis, in particular pyruvate that comes from β -elimination (non-productive catalysis)?

Methodological and technical approaches considered (4-6 lines)

Here, we will take advantage of IRMS, NMR and LC-MS techniques readily available at both sites of supervision (EA- and Multiflow-IRMS, 500 MHz NMR, LC-Orbitrap in Angers; MI-IRMS, 700 MHz, and LC-Orbitrap in Canberra) and we will be focusing on alfalfa (*Medicago truncatula*) as a Legume plant model to purify Rubisco. We will also develop a new technique based on ^{13}C labelling and NMR to facilitate measurement of specificity (which are usually based on radioactive RuBP utilisation).

Scientific and technical skills required by the candidate

The PhD candidate will have some experience in biochemistry such as carrying enzymatic assays and a general understanding of enzyme mechanisms. Basic knowledge in analytical chemistry and isotope would be appreciated. A general knowledge of plant photosynthesis is also required, so as to understand how Rubisco relates to photosynthetic metabolism.

THESIS SUPERVISION¹

Unit name: IRHS	Team name: SMS
Unit director name: JP Renou	Team director name: Béatrice Teulat
Mailing address of the unit director: jean-pierre.renou@inrae.fr	Mailing address of the team director: beatrice.teulat@agrocampus-ouest.fr
Thesis director Surname, first name: LIMAMI, Anis Position: Professor Obtained date of the HDR (Habilitation thesis to supervise research): YES Employer: University of Angers Doctoral school affiliation: EGAAL Rate of thesis supervision in the present project (%): 50 Total rate of thesis supervision in ongoing theses (supervisions and co-supervisions) (%): 150% (includes the present request) Number of current thesis supervisions/co-supervisions: 1	
Thesis co-supervisor 1 (if applicable) Surname, first name: TCHERKEZ, Guillaume Position: Professor Habilitation thesis to supervise research <input checked="" type="checkbox"/> yes <input type="checkbox"/> no If yes, date diploma received: 10/10/2007 Employer: Australian National University, also PEPS staff member of IRHS Doctoral school affiliation: EGAAL via IRHS Rate of thesis supervision in the present project (%): 50%	

¹ In EGAAL Doctoral School, if only one scientist in thesis supervision = 100% of supervision rate; if 2 people involved in thesis supervision = from 50% to 70% of supervision rate for the director; if 3 people involved in thesis supervision = 40% / 30% / 30% of supervision rate distribution among supervisors.

Total rate of thesis supervision in ongoing theses (supervisions and co-supervisions) (%): 100% (includes the present request)

Number of current thesis supervisions/co-supervisions: 2 (1 France, 1 Australia)

Thesis co-supervisor 2 (if applicable)

Surname, first name:

Position:

Habilitation thesis to supervise research yes no If yes, date diploma received:

Employer:

Doctoral school affiliation:

Rate of thesis supervision in the present project (%):

Total rate of thesis supervision in ongoing theses (supervisions and co-supervisions) (%):

Number of current thesis supervisions/co-supervisions:

Private partner (if CIFRE funding, private funding,...)

Surname, first name:

Position:

Employer:

Rate of thesis supervision in the present project (%):

Total rate of thesis supervision in ongoing theses (supervisions and co-supervisions) (%):

Number of current thesis supervisions/co-supervisions:

International partner (if Cotutelle thesis)

Surname, first name:

Position:

Employer:

Rate of thesis supervision in the present project (%):

Total rate of thesis supervision in ongoing theses (supervisions and co-supervisions) (%):

Number of current thesis supervisions/co-supervisions:

Professional status of previous PhD students supervised by both director and co-supervisors (from 5 years)

Please provide the following information for each PhD students supervised

Surname, first name: DOMERGUE, Jean-Bapstiste

Date of PhD beginning and PhD defence: PhD started Dec. 2018

Thesis supervision: A. Limami/G. Tcherkez

Professional status and location: PhD ongoing

Contract profile (post-doc, fixed-term, permanent): n/a

List of publications from the thesis work:

Domergue, J. B., Abadie, C., Limami, A., Way, D., & Tcherkez, G. (2019). Seed quality and carbon primary metabolism. *Plant, Cell & Environment*, 42(10), 2776-2788.

Five main recent publications of the supervisors on thesis subject:

Tcherkez, G., & Limami, A. M. (2019). Net photosynthetic CO₂ assimilation: more than just CO₂ and O₂ reduction cycles. *New Phytologist*, 223(2), 520-529.

Bathellier, C., Tcherkez, G., Lorimer, G. H., & Farquhar, G. D. (2018). Rubisco is not really so bad. *Plant, Cell & Environment*, 41(4), 705-716.

Tcherkez, G. G., Bathellier, C., Farquhar, G. D., & Lorimer, G. H. (2018). Commentary: Directions for Optimization of Photosynthetic Carbon Fixation: RuBisCO's Efficiency May Not Be So Constrained After All. *Frontiers in plant science*, 9, 929.

Tcherkez, G. (2016). The mechanism of Rubisco-catalysed oxygenation. *Plant, Cell & Environment*, 39(5), 983-997.

Tcherkez, G. (2013). Modelling the reaction mechanism of ribulose-1, 5-bisphosphate carboxylase/oxygenase and consequences for kinetic parameters. *Plant, Cell & Environment*, 36(9), 1586-1596.

THESIS FUNDING

Origin(s) of the thesis funding: PhD contract University of Angers

Gross monthly salary: as per payroll scheme

Thesis funding state : Non acquired

Funding beginning date/Funding ending date: academic year 2020-2021

Date: 2020/03/26

Name, signature of unit director: Jean-Pierre Renou



Name, signature of team director: Beatrice Teulat



Name, signature of thesis project director: Anis Limami

A handwritten signature in black ink, consisting of a stylized, cursive script that appears to be the name 'Anis Limami'.