PhD School on Agriculture, Environment and Bioenergy

(http://sites.unimi.it/dottorato_aab/)

(XL cycle, 2024-27)

Project draft

1. Field of interest: *AGR/13 – Chimica Agraria*

2. Project title: *Stable isotope approaches for studying mineral nutrition in higher plants*

3. Tutor: *Prof. Fabio Francesco Nocito*; **co-tutor:** *Prof. Luca Espen*

4. Relevance of the topic and state of the art

Plants need 17 essential elements to complete their life cycle. Fourteen of these elements, referred to as mineral nutrients, are usually acquired directly from the soil through the roots, which in turn adjust biochemical, physiological, and morphological processes in response to the availability of soil nutrients and changes in nutritional requirements [1]. In other words, plants can sense the levels of all mineral nutrients in the soil and maintain their intracellular concentrations within optimal ranges, a process commonly referred to as mineral nutrient homeostasis [2]. Understanding how plants regulate mineral nutrient homeostasis is crucial not only for advancing our basic knowledge of how plants meet their metabolic needs and mitigate the adverse effects of mineral nutrient excesses or deficiencies in the environment, but also for agriculture to fulfill crop nutritional requirements while minimizing fertilizer input and its associated environmental impacts. In recent decades, most studies have mainly focused on plant adaptation to changes in the supply of individual nutrients, offering valuable insights into the molecular mechanisms involved in maintaining optimal nutrient levels within cells. These investigations have led to the identification of specific regulatory pathways for each nutrient, where metabolic demand often governs nutrient uptake and systemic allocation. Nevertheless, for effective growth and development, plants require a balanced supply of essential mineral nutrients. Studies have revealed intricate interactions among these nutrients, suggesting that current regulatory models where each nutrient level is controlled by its own mechanisms and signaling pathways need to be integrated into a comprehensive model that considers plant mineral nutrition holistically [3,4]. Indeed, deficiencies or excesses in one nutrient often trigger changes in the demand for or accumulation of other available nutrients [2,5]. Furthermore, comparative transcriptome analyses of nutrient-deprived plants have unveiled common responses to nutrient availability [6,7]. Additionally, plant tissues may contain various trace elements, such as non-essential heavy metals or metalloids, which can influence plant metabolism and mineral nutrient homeostasis [8-9].

5. Layout of the project (draft)

5.1. Materials & Methods

The project activities will be carried out using model plants, with the objective of developing isotopic methods that are valuable for studying the assimilation and systemic distribution of mineral nutrients, along with their interaction with other essential or non-essential elements. The project will primarily focus on two macronutrients – nitrogen (N) and sulfur (S) – by examining the interaction between nitrate and ammonium as nitrogen

sources, as well as the systemic correlation among sulfate uptake, sulfur partitioning, and cadmium accumulation.
The studies on N nutrition will involve growing plants in hydroponic solutions containing either nitrate or ammonium as the sole source of N, or different combinations of the two ions. Nitrate ions will be labeled through enrichments with the stable isotope ^{15}N to determine how the two nitrogen sources contribute to nitrogen nutrition and assimilation. In the same experimental conditions, the effects of the different nutritional conditions on the ability of plants to maintain the anionic balance will be assessed.
The effects of Cd exposure and accumulation on S partitioning will be investigated using natural abundance S stable isotope analysis techniques. In contrast to carbon and nitrogen, natural abundance S stable isotope analysis has been underutilized in studying S allocation and metabolism in plants. This is primarily due to limited understanding of the $^{32}S/^{34}S$ isotope effects potentially occurring during S metabolism and partitioning among different organs. Recent results indicate that the steady-state S isotope composition of the different S metabolic pools of rice mainly results from substantial S isotope fractionations occurring during sulfate assimilation and mixing effects due to the overall S isotope circulation inside the whole plant [10]. Sulfate uptake and allocation in the whole plant involve a family of sulfate transporter proteins whose activities are closely regulated and coordinated with those of the assimilation pathways to control plant S homeostasis.
A few pioneering studies indicated that a little S isotope discrimination occurs during sulfate uptake since the isotope composition measured for plant total S is typically depleted in ^{34}S by 1-2 ‰ with respect to that measured for the sulfate source feeding the plants [10]. This project also aims to develop methods for determining the isotope effects associated to various plant sulfate transporters. To these purposes, yeast mutants defective in sulfate uptake will be utilized to express individual sulfate transporters cloned from model plants. Understanding the isotopic effects linked to specific transporters is a crucial prerequisite for comprehending and modeling systemic sulfate fluxes throughout the entire plant.
All isotopic analyses concerning total nitrogen, total sulfur, and sulfate ion will be conducted using a Flash 2000 HT elemental analyzer coupled, via a ConFLo IV Interface, with a Delta V Advantage IRMS and interconnected to the software Isodat 3.0 (Thermo).

5.2. Schedule and major steps (3 years)

Activity	**Year**		
	I	**II**	**III**
I. Comprehensive description of the contribution of nitrate and ammonium to nitrogen nutrition and assimilation.	x	x	x
II. Cloning and heterologous expression in yeast of genes encoding high-affinity sulfate transporter.	x		
III. Comprehensive description of the effects Cd exposure and accumulation on S partitioning.		x	x
IV. Thesis writing			x

6. Available funds (to support research): Approximately 100,000 euros between European and PNRR funds

7. Co-Financing (to support the bourse): No

8. Literature:

1. Marschner P (2012). Marschner's mineral nutrition of higher plants. London: Academic Press.

2. Rouached H, Rhee SY (2017) System-level understanding of plant mineral nutrition in the big data era. *Current Opinion in Systems Biology* 4: 71–77
3. Baxter I (2009) Ionomics: studying the social network of mineral nutrients. *Current Opinion in Plant Biology* 12: 381–386
4. Williams L, Salt DE (2009). The plant ionome coming into focus. *Current Opinion in Plant Biology* 12: 247–249
5. Maillard A, Sorin E, Etienne P, Diquélou S, Koprivova A, Kopriva S, Arkoun M, Gallardo K, Turner M, Cruz F, Yvin J-C, Ourry A (2016) Non-Specific root transport of nutrient gives access to an early nutritional indicator: the case of sulfate and molybdate. *PLoS One* 11:e0166910
6. Watanabe M, Hubbertenb H-M, Saito K,and Hoefgen R (2010) General regulatory patterns of plant mineral nutrient depletion as revealed by *serat* quadruple mutants disturbed in cysteine synthesis. *Molecular Plant* 3: 438–466
7. Forieri I, Wirtz M, Hell R. 2013. Toward new perspectives on the interaction of iron and sulfur metabolism in plants. *Frontiers in Plant Science* 4: 357
8. Nocito FF, Lancilli C, Crema B, Fourcroy P, Davidian J-C, Sacchi GA (2006) Heavy metal stress and sulfate uptake in maize roots. *Plant Physiology* 141: 1138–1148
9. Rizzardo C, Tomasi N, Monte R, Varanini Z, Nocito FF, Cesco S, Pinton R (2012) Cadmium inhibits the induction of high-affinity nitrate uptake in maize (*Zea mays* L.) roots. *Planta* 236: 1701–1712
10. Cavallaro V, Maghrebi M, Caschetto M, Sacchi GA, Nocito FF (2022) Sulfur stable isotope discrimination in rice: a sulfur isotope mass balance study. *Frontiers in Plant Science* 13:837517.