PhD School on Agriculture, Environment and Bioenergy

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

(XXXIV cycle, 2018-20)

Project draft

1.Field of interest
Chimica Agraria (AGR/13)

2.Project title
Sulfur nutrition and partitioning in rice under different stress conditions

3.Tutor
Prof. Gian Attilio Sacchi

4.Relevance of the topic and state of the art:
Since 1865 sulfur (S) has been recognized as an essential element for plant growth. In plants, S is found in the amino acids cysteine and methionine, short peptides, vitamins and co-factors, and secondary compounds. Although different forms of S can support plant growth, sulfate (SO_4^{2-}) ions in the rhizosphere can be considered as the major source of S for plants. They are taken up by roots, allocated to the sink tissues, to then be stored in the cell vacuoles or assimilated into different organic compounds. To accomplish the assimilation of sulfur into biomolecules, sulfate ions are first activated by ATP sulfurylase (ATPS) to adenosine-5'-phosphosulfate (APS), which is then channeled toward reduction or sulfation. Most of the APS enters the reductive pathway along which it is transformed to sulfite and this into sulfide, in two sequential reactions catalyzed by APS reductase (APR) and sulfite reductase, respectively. Sulfide is then incorporated into *O*-acetylserine (OAS) to form cysteine (Cys) in a reaction catalyzed by OAS(thiol)lyase. In the sulfation pathway the APS is first phosphorylated by APS kinase to form 3´-phosphoadenosine 5-phosphosulfate (PAPS), the donor of sulfate groups for a variety of sulfation reactions catalyzed by sulfotransferases.
Unlike what has happened with carbon and nitrogen, natural abundance S stable isotope analysis has scarcely been employed to study S allocation and metabolism in plants, mainly because of the lack of knowledge about the $^{32}S/^{34}S$ isotope effects potentially occurring during sulfur metabolism and partitioning among the different organs. Most of the irreversible reactions involving S discriminate between ^{32}S and ^{34}S by favoring the light isotope (^{32}S), thus enriching in ^{34}S the residual substrate molecules left behind. That is to say, that irreversible reactions that do not consume all the substrate fractionate S isotopes, providing useful information for the understanding of the S metabolic fluxes inside the plants.
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 in order 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 sulfur is typically depleted in ^{34}S by 1-2 ‰ with respect to that measured for the sulfate source feeding the plants. On the other hand, little is known about the S isotope composition of the sulfate ions in various plant tissues, which should reflect the metabolic activities in which sulfate is involved inside the cells. Since metabolic sulfate reduction involves changes in the covalent bounding of the S atoms, it is likely to suppose that reductive sulfate assimilation fractionates against ^{34}S.

5.Layout of the project

Four stable isotopes of sulfur (S) exist (^{32}S, ^{33}S, ^{34}S, ^{36}S) whose natural isotopic percentage abundances are 0.94499, 0.0075, 0.0425 and 0.0001 atom fraction, respectively. The most abundant isotopes – ^{32}S and ^{34}S – are now commonly measured using elemental analyzers coupled with isotope ratio mass spectrometers (EA-IRMS). Such an approach is based on the complete transformation of total S to SO_2, which is subsequently analyzed by the mass spectrometer with regards to masses 64 ($^{32}S^{16}O_2$) and 66 ($^{35}S^{16}O_2$ or $^{32}S^{16}O^{18}O$) atomic mass units.
S stable isotopes have been used to trace the movements of the related compounds in plants, in testing S flux models, and in identifying and determining the impact of natural and anthropogenic S sources on the environment. However, the isotope technique applied for S metabolism investigations, as well as for sulfate transport and allocation within the plants, is limited by our current knowledge of the potential $^{32}S/^{34}S$ isotope discrimination that may occur during both S metabolism and sulfate transport.
The relative ^{34}S abundance is traditionally quantified using the δ value: $\delta^{34}S = (R_{sample} - R_{standard})/R_{standard}$, were R is $^{34}S/^{32}S$ isotope ratio.
The project will approach the topic of $^{32}S/^{34}S$ fractionation in plants by investigating the hypothesis that the $\delta^{34}S$-tot of the whole plant could be determined by the activities of the sulfate transporters involved in sulfate uptake (i.e. sulfate transporters discriminate between ^{32}S and ^{34}S). The experimental approaches will be developed in close systems in which a limited amount of sulfate is continuously removed from the solution - by the activity of the sulfate transporters of the roots - and converted in a final product (i.e., S-SO_4^{2-}, S-tot, S-org of the plant). In such systems all reagents and products will be analyzed for their quantitative levels and isotope signatures. The main data will be related to the expression (transcript level) of the main genes involved in sulfate transport and metabolism. Different growing condition – known to modulate sulfate uptake and/or translocation (Cd^{2+} stress and/or Na^+ stress) – will be also analyzed in order to obtain a comprehensive picture of the $^{32}S/^{34}S$ isotope effects occurring during sulfate distribution within the plant.

6. Available funds: the project will be supported by European and national funds (DISAA plant physiology group)