PhD School on Agriculture, Environment and Bioenergy

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(XXXIV cycle, 2018-20)

Project draft

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

2.Project title
Root aerenchyma development and mineral nutrient acquisition in rice

3.Tutor
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4.Relevance of the topic and state of the art:
Plants grown on flooded soils may experience hypoxia at the root level because gas diffusion is 10^{-4}-fold slower in solution than in air and O_2 is rapidly removed from soil via respiratory processes. The O_2 depletion in soil causes shifts from aerobic to anaerobic microbial processes, leading to reduction of oxidized nutrients and accumulation of potentially phytotoxic ions such as NH_4^+, Mn^{2+}, Fe^{2+}, and S^{2-}.
Crops growing in submerged soils need to balance tolerance to low O_2 and toxic ions with adequate rates of nutrient uptake in order to optimize the yield. Moreover, under reducing conditions N and S can be lost from soil by volatilization (NO_X, NH_3, H_2S), depending on the rate of microbial transformations, especially in the rhizosphere.
Rice (*Oryza sativa* L.) generally respond to O_2 deficiency in soil by forming adventitious roots containing aerenchyma, a tissue comprising a high proportion of interconnected gas-filled spaces that facilitate internal O_2 transport from aerial to submerged plant organs thus enabling root tissue respiration and growth. The release of part of the transported O_2 contributes to maintain aerobic conditions in the rhizosphere and then the oxidation of Fe^{2+}, NH_4^+ or S^{2-}, by the rhizobacteria. Therefore, the interface between the rice oxygenated rhizosphere and the anoxic bulk soil is a hot spot of complex reactions controlling nutrient availability and environmental fate in rice crops.
Generally, the main source of S for plants is the sulfate (SO_4^{2-}) ion in the rhizosphere which is taken up by the high-affinity sulfate transporters of the roots. Although atmospheric H_2S is known to act as S source for plant growth, the accumulation of reduced S – as a metabolic end product by prokaryotes that oxidize organic compounds using SO_4^{2-} as a terminal electron acceptor – in waterlogged soils often results toxic for plant growth. Little is known about the main S source used by rice during anaerobiosis, as well as about the pathways it generally uses to control the potentially toxic effects du to S^{2-} accumulation in the rhizosphere.

5.Layout of the project
In the first phase of the project a collection of germplasm, consisting in 300 temperate rice accessions, selected by CREA-RIS by mean of a genetic diversity analysis will be analyzed in order to produce the first comprehensive description of the variability inside the Italian rice for adaptive traits related to mineral nutrition under variable redox conditions, such as the total porosity of the root system and the radial O_2 loss (ROL). A core collection of 60 rice genotypes will be extracted from the germoplasm collection, using data of a preliminary cluster analysis. The new sub-collection will be representative of the genetic variability existing in the main collection. Each accession will be grown in aerated hydroponic solutions or liquid media mimicking the slow gas

flow occurring in waterlogged soils (stagnant solution). Genotypes will be phenotyped by measuring total root porosity (percentage gas space per unit tissue volume) with a pycnometer, and ROL using the titanium(III) citrate buffer method.

In the second phase of the project rice genotypes selected for extreme (higher and lower) and intermediate capacity to develop root aerenchyma under hypoxia will be analyzed with respect to S nutrition. The activity is specifically aimed at: i) discriminating the effects of root porosity on the capacity of the root to take up SO_4^{2-} and to increase S^{2-} oxidation through ROL; ii) elucidating whether S^{2-}, besides SO_4^{2-}, could directly contribute to S nutrition of waterlogged plants. For these purposes, plants will be grown in a controlled environment on aerated or waterlogged paddy soils contained in plastic pots. At the end of the growing period: i) roots will be washed to remove the soil and to measure total porosity, and ii) both root and shoot tissues will be analyzed for the levels of total S and key S-containing compounds along the S-assimilation pathway (i.e. SO_4^{2-}, non-protein thiols, glutathione); these latter will be assumed as diagnostic indicators of plant S nutritional status. S-containing compounds will be determined by HPLC or colorimetric and enzymatic methods. The expression levels of the main genes involved in SO_4^{2-} uptake (*SULTR1;1* and *SULTR1;2*) will be also determined by real time PCR analysis. Results of the transcriptional analyses will be used as diagnostic indicators of SO_4^{2-} abundance in the rice rhizosphere, since SULTR transcript levels are closely related to SO_4^{2-} availability. Finally, the capacity of the washed roots to absorb SO_4^{2-} and S^{2-} at different external concentrations will be determined using stable (^{34}S) or radioactive (^{35}S) S isotopes as tracers. Kinetic characteristics of SO_4^{2-}, and eventually S^{2-}, uptake will be studied on intact roots of plants grown in aerated or stagnant solutions in the presence of SO^{42-} and different amount of S^{2-}. Results will be related to root porosity and to the expression levels of *SULTR1;1* and *SULTR1;2*.

If rice roots result unable to directly absorb S^{2-}, a set of experiments will be specifically carried out in order to investigate if alternative routes to root absorption may contribute to S nutrition of plants grown under hypoxia. In particular, it might be interesting to assess if the differentiation of aerenchyma may facilitate gaseous H_2S transport to the leaves, besides favoring S^{2-} oxidation in the rhizosphere and subsequent SO_4^{2-} uptake. To investigate this aspect plant will be grown using stagnant solutions containing SO_4^{2-} as sole S source and different amounts of $^{34}S^{2-}$ will be added at different times. At the end of the pulse periods shoots will be separated from the roots and then analyzed for the presence of ^{34}S in the different fractions of S-containing molecules.

GANTT

	First year		Second year		Third year	
Activity	**I-IV**	**VII-XII**	**I-IV**	**VII-XII**	**I-IV**	**VII-XII**
Screening						
Physiological experiment on sulfate uptake						
Characterization of the routes involved in H_2S detoxification						

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