PhD School on Agriculture, Environment and Bioenergy

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

(XXXVII cycle, 2021-24)

Project draft

1. Field of interest

AGR/13 - Agricultural chemistry

Plant Biochemistry and Physiology; responses to abiotic stress; nitrogen metabolism.

2. Project title

Salt responses and nitrogen use efficiency in Tomato (*Solaum Lycopersicum* L.).

3. Tutors:

Tutor: Prof. Luca Espen
Full Professor in Agricultural Chemistry at the University of Milan. His research activity can be divided into the following topics: a) biochemical and physiological aspects of seed germination; b) heavy metal toxicity; c) molecular and biochemical aspects involved in the vascular reconnection of tree plants grafts d) biochemical and physiological processes of the fruit ripening of orchard tree species; e) biochemical and physiological aspects involved in the abiotic stress responses.
Author of 69 "peer-reviewed" articles. *h-index* (Scopus): 23; *total citations* (Scopus): 1619.

Co-tutor: Dr. Fabrizio Araniti
Researcher (RTD-B) in Agricultural Chemistry at the University of Milan. His academic career, which started in 2008, was mainly focused on allelopathy and weed management. His PhD and Post Doc activities were focused on the isolation, characterization and screening of several phytotoxic allelochemicals. He collaborated in several national/international projects, and he was PI of the project SIR-2014 (RBSI14L9CE) focused on the isolation of natural products with herbicidal activity and the identification of their mode of action through an *-omics* approach. Some research activities are addressed to the biochemical and physiological processes involved in the acquisition of mineral nutrients.
Author of 65 "peer-reviewed" articles. *h-index* (Scopus): 18; *total citations* (Scopus): 795.

4. Relevance of the topic and state of the art.

In Mediterranean agro-ecosystems, crop plants are challenged by different abiotic constraints, such as salinity, that have deep implications on crop yield and quality [1]. Salinity affects plant growth by altering the water balance and nutritional status, as well as inducing cytotoxicity due to the accumulation of ions (i.e., Na^+ and Cl^-). The increase of Reactive Oxygen Species (ROS), which potentially generates oxidative stress, is also a common detrimental salt effect [2]. This stress typically induces a two-phase response: 1) a decrease in soil water availability, similar to drought conditions, and 2) a cytotoxic ion concentration in plant tissues [3]. In response to the alterations in water balance, plants reduce transpiration, affecting many physiological and biochemical processes like growth, photosynthesis and ion movements among tissues [4]. Moreover, in plant response, the cell membrane transport system plays a crucial role, being involved in water and ion uptake/extrusion by the roots, ion compartmentation into root and leaf cell (i.e. transport into the vacuole) and ion allocation to the shoot, which mainly depends on xylem transport and foliar accumulation [3-5]. Furthermore, osmoprotectants synthesis, mainly consisting of sugars, amino acids and ammonium derived compounds, represents another typical response to salt stress [6]. All these activities, having together a very high energetic cost, deeply impact plant metabolic and physiological functions [7].

Nitrogen (N), mainly provided as nitrate (NO_3^-) in agricultural soils, is one of the major mineral nutrients that significantly affect plant growth and crop yield [8,9]. The N-limited bio-availability in the pedosphere, with respect to the crops request, has spawned a dramatic increase in N fertilization, which results in high environmental and economic costs [10-12]. A pivotal challenge to increase agricultural sustainability is the selection of crop genotypes with improved Nitrogen Use Efficiency (NUE). NUE is a complex trait determined by the interactions among many physiological, genetic, developmental and environmental factors. It encompasses the plant efficiency to uptake N from the soil (NUpE component), and also the ability to assimilate, transport and reallocate the acquired N, according to the plant needs (NUtE component) [13-14]. Moreover, NO_3^- availability affects photosynthesis, leaf transpiration, ion transport, cell redox status, as well as N and carbon metabolisms [10].

Salt stress deeply influences NO_3^- uptake by roots and its translocation and assimilation [15]. Therefore, N metabolism and salt responses appear closely related, and their relationships play a central role in plant adaptations to salinity.

Tomato (*Solanum Lycopersicon* L.), which is one of the most important Mediterranean crops (http://faostat.fao.org), is cultivated in the geographic area in which salt stress is one of the main factors that could affect its production.

5. Layout of the project.

The project aims to investigate on the biochemical and physiological mechanisms that govern the plant adaptability to saline environments in two-nitrogen use efficiency (NUE)-contrasting tomato genotypes.

5.1 Materials & Methods.

Plant material and experimental conditions

The study will be carried out on two NUE-contrasting tomato genotypes, Regina Ostuni and UC82.

Physiological and biochemical measurements

Relative chlorophyll content, PSII efficiency, chlorophyll *a* fluorescence, and the proton motive force parameters [ECSt (difference in proton motive force from light to dark), vH+ (flow rate of H+ through ATP synthase) and gH+ (permeability of thylakoid membrane)] will be monitored (MultisepQ v. 2.0). Beside, net CO_2 assimilation rate (An, µmol CO_2 m^{-2} s^{-1}) and stomatal conductance (gs, mmol H_2O m^{-2} s^{-1}) will be measured (CIRAS-2, PP Systems) and, the An/gs ratio will be used to calculate the water use efficiency (WUE) under different treatments. The water contents and osmotic potentials ($\Psi\pi$) will be measured (model K-7400, Knauer) and used to define the water balance. To obtain further information on osmotic responses, total sugars, amino acids, proline, and chloride contents will be determined through colorimetric methods. Cation concentrations, such as K^+, Na^+ and other minerals, will be obtained by ionomic analyses (ICP-MS).
The N assimilation pathway will be evaluated through the activities of some key enzymes, such as nitrate reductase (NR), glutamine synthetase (GS) and glutamate synthase (GOGAT). At the more interesting point times, the amino acid composition in root, shoot and xylem sap will be determined (LC-ESI-MS) to investigate the effects of salt stress on the N compounds xylem translocation and the balance between root and shoot N metabolism. The oxidative stress will be evaluated. On both root and shoot, through the evaluation of the membrane stability and lipid peroxidation index, the last estimated by the malondialdehyde levels. Also, O_2^- and H_2O_2 localization will be evaluated through the DAB (3,3'-diaminobenzidine) and NBT (nitroblue tetrazolium) semi-quantitative staining techniques [16].

Proteomic analyses

Proteomic analyses will be performed combining one-dimensional (1D) Gel Liquid Chromatography-Mass Spectrometry (LC-ESI-MS/MS), using an Agilent 6520 Q-TOF mass spectrometer equipped with an HPLC Chip Cube and a 1200 series nano/capillary LC system. Mass spectrum interpretation and quantitation will be performed by Spectrum Mill MS Proteomics workbench, making the best use of the large set of public genetic information.

Metabolomic analyses

Metabolomic analysis will be used to zanalyze primary metabolites, such as sugars, amino acids and organic acids. For this goal, the polar fraction will be extracted and derivatized for the GC-MS analysis. The analyses will be performed through a GC-MS approach using the instrument comprising the gas chromatograph and the single-quadrupole spectrometer (7890, 5975 Agilent Technologies).

5.2 Schedule and major steps (3 years):

Firstly, time-course experiments will be conducted to zanalyze different responses between genotypes and/or treatments as well as to identify the times samplings for further analyses. For each time sampling and treatment, plants will be collected, and the effects of salt stress will be clarified, as well as their relations with nitrogen availability, through a physiological and biochemical approach. Water balance, photosynthetic performances, N-metabolism and use efficiency and oxidative stress responses will be evaluated.

Proteomic and metabolomic analyses will be performed on root and shoot, sampled at point times corresponding to the most interesting biochemical and physiological differences among treatments and/or genotypes.

The schedule of the project can be summarized in the achievement of intermediate objectives during the three years of research activity:

First year:

- Critical review of the scientific literature concerning plant N nutrition and proteomics.
- Time-course experiments to analyze different responses between genotypes and/or treatments as well as to identify the times samplings for further analyses.

Second year:

- Proteomic analyses
- Metabolomic analyses

Third years:

- Interpretation of the proteomics and metabolomic results in relation to physiological and biochemical responses.
- Validation of the results by means of bioinformatic tools and targeted-analyses.
- Preparation of the final thesis.

Study and dissemination activities (schools, congresses, scientific papers) and a research period abroad will be planned during the three years of the PhD school.

6. Available funds)

The project will be funded with a grant of 15.000,00 € obtained by some projects as well as analytical service profit.

6. Literature

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[14] Xu et al. Annu. Rev. Plant Biol. 2012, 63 (1), 153–182.
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