

PhD School on Agriculture, Environment and Bioenergy

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(XXXV cycle, 2019-21)

Project draft

1. Field of interest

07/E – Agricultural chemistry, agricultural genetics and soil science – AGR/13 - Agricultural chemistry

2. Project title

Characterization of proteomic changes in crops during metabolic adaptation to different nitrogen inputs

3. Tutor

Bhakti Prinsi

Researcher (RTD-B) in Agricultural Chemistry at the Department of Agricultural and Environmental Sciences - Production, Landscape, Agroenergy - University of Milan. Her research activity is aimed at studying topics of plant physiology and biochemistry relevant to crop quality and production: physiological and molecular responses to mineral nutrition and abiotic stresses; fruit ripening in woody plants; relations between primary and secondary metabolism in plants; characterization and biological activities of seed storage proteins in legume and cereal crops. These research activities have enabled her to gain a good expertise in the fields of plant proteomics and metabolomics based on mass spectrometry approaches.

Author of 36 "peer-reviewed" articles. *h-index (Scopus): 16; total citations (Scopus): 1052.*

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

The application of nitrogen (N) based fertilizers in agricultural systems is necessary to sustain crop yields, but it has huge economic, social and environmental costs. Plant N use efficiency is very low, therefore the selection of new strategies to manage N inputs is needed to improve the sustainability of agricultural production [1]. In particular, maize (*Zea mays* L.), representing a staple food and an industrial commodity, is a model organism for the study of plant physiology, biochemistry and genetics [2]. Nitrate and ammonium are the main forms of inorganic N used by crops. Their availabilities differently affect growth, morphology, and metabolism of plants and, ultimately, grain production and fruit quality [3]. Although the relevance of organic N in plant nutrition is still under debate, plants can acquire amino acids from the soil, for which several recent studies proposed a nutritional role previously underestimated [4,5]. In addition, the applications of different amino acids exert distinct and broad effects on root growth and branching [6]. Moreover, the use of novel formulations, such as biostimulants based on protein hydrolysates was recently proposed in order to enhance nutrient uptake by plants [7]. Therefore, it could be very useful improving the understanding about the interactions between inorganic and organic N forms as nutrients and signals for plant growth. In the past, proteomics was successfully applied to gain new knowledge on crop metabolic adaptations to different N inputs, highlighting the involvement of different post-translational modifications and enzyme isoforms [8,9,10,11]. However, information about the responses in specific subcellular compartments, such as organelles and membrane systems, are still fragmentary. Subcellular proteomics, namely the study of the proteome in isolated cell compartments, is very powerful to unravel metabolic networks in plant cells [12]. The study of sub-proteomes of plants exposed to different combinations of inorganic and organic N forms could provide new knowledge about localized cellular responses and biochemical interplay among cell compartments. This information could turn out to be very useful to enhance the sustainability of crop production.

5. Layout of the project (draft)

The project will be aimed at studying the roles of proteomes in sensing and adaptation to different N availabilities in model crops, by a "system biology" approach based on physiological, metabolic and proteomic evaluations, combined with complementary validation analyses.

5.1. Materials & Methods:

Plant materials and nutritional treatments: the project will make use of maize (*Zea mays* L.) as the main model system. Moreover, to broaden knowledge and find common or specific responses, the use of other crop species will be evaluated. In details, commercial lines of maize will be grown in hydroponic systems. During vegetative growth, the effects of different N nutritional treatments on plant growth, root morphology and plant metabolism will be tested. The nutritional treatments will be based on different availabilities and proportions of inorganic (*i.e.* nitrate/ammonium) and organic (*i.e.* amino acids) N forms, provided as a single N source or in combination. The possibility of using specific formulations, such as biostimulants, will be also evaluated.

Evaluation of physiological effects: key physiological parameters related to sensing and utilization of N forms, such as biomass accumulation in roots and leaves, root morphology, and foliar photosynthetic performance, will be evaluated by mean of phenotyping tools and biochemical assays.

Evaluation of biochemical responses: western-blot and enzymatic analyses will be performed in order to evaluate the abundance and activities of key enzymes involved in N acquisition (*i.e.* Nitrate Reductase, Glutamine Synthetase) in roots and leaves. In order to describe metabolic adaptations, the levels of key metabolites, such as sugars and amino acids, will be evaluated by colorimetric and/or mass spectrometry analyses.

Characterization of proteomic profiles in different organs: on the basis of the previous analyses the most interesting combinations of N inputs and/or exposure timings will be selected. On the basis of the physiological and biochemical responses observed in the selected conditions, the research will be focused on different organs and/or specific fractions of the proteomes. The sub-compartments will be isolated by means of different biochemical techniques (*i.e.* phase-partitioning, separation by density gradients), and the purity grades of the extracts will be evaluated by biochemical markers. The protein fraction will be characterized by means of large-scale proteomic approaches based on gel-electrophoresis and/or liquid chromatography tandem mass spectrometry (LC-ESI-MS/MS), assuring simultaneously the identification and quantification of hundreds of protein forms. The proteomic profiles will be compared among different experimental conditions and related to the physiological and biochemical evaluations in order to individuate possible molecular determinants involved in N sensing and adaptation to N inputs in plants.

Validation of the results: the proteomic results will be validated through independent and alternative approaches, focusing the analyses on the most interesting proteins forms. Subcellular localization, changes in transcript abundances, and possible post-translational modifications will be validated by means of bioinformatics tools, enzymatic assays, western blot and fluorescence targeted analyses.

5.2. Schedule and major steps (3 years):

First year:

The activities of the first year will be mainly devoted to study inorganic N nutrition

- Study of the scientific literature related to plant N nutrition and proteomics.
- Definition of the nutritional treatments (N inputs, timings) useful to investigate sensing and adaptation to nitrate and ammonium availabilities in maize and, eventually, in other model crops.
- Optimization of the protocols to analyse subcellular proteomes (organelles and membrane systems) in maize.

Second year:

The activities of the second year will be aimed at studying and comparing inorganic and organic N nutrition

(a) Inorganic N nutrition:

- Insights on plant physiological and metabolic responses to the experimental conditions defined in the previous steps.

- Large-scale analysis of proteomes and sub-proteomes in plants exposed to the experimental conditions defined in the previous steps.

(b) Organic N nutrition:

- Definition of the nutritional treatments (N inputs, timings) useful to investigate sensing and adaptation to amino acid availabilities in maize and, eventually, in other model crops.

- Insights on plant physiological and metabolic responses to the experimental conditions defined in the previous step.

- Large-scale analysis of proteomes and sub-proteomes in plants exposed to the experimental conditions defined in the previous steps.

Third year:

Interpretation and validation of the results

- Interpretation and comparison of the proteomics results in relation to the plant physiological and metabolic responses to different N inputs.

- Individuation of molecular determinants related to sensing and adaptation to different N inputs in plants.

- Validation of the results by means of complementary techniques, bioinformatic tools, and targeted analyses.

The training and dissemination activities (schools, congresses, scientific publications) and research periods abroad will be planned during the three years of the PhD school.

6. Available funds (source and amount)

The project will be funded with a grant of 20.000,00 € (FFABR – Individual Annual Funding of Basic Research Activities, Fondazione CARIPLO Project: *Field to field: valorisation of biomolecules from soybean drink by-products as defence products and biostimulants for an improved sustainability of crops cultivation*, and analytical service profit).

7. Literature:

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7. du Jardin P. Plant biostimulants: definition, concept, main categories and regulation. *Sci Horti* 2015, 196: 3-14. DOI: 10.1016/j.scienta.2015.09.021.

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12. Haynes PA, Roberts TH. Subcellular shotgun proteomics in plants: looking beyond the usual suspects. *Proteomics* 2007, 7: 2963-2975. DOI: 10.1002/pmic.200700216.