PhD School on Agriculture, Environment and Bioenergy

(XXXIV cycle, 2018-20)

Project draft

1. Field of interest

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

2. Project title

Characterization of maize subcellular proteomes to study changes and roles under different nitrogen availabilities

3. Tutors:

Prof. Luca Espen

Associated Professor in Agricultural Chemistry at the University of Milan. His research activity can be divided in the following topics: a) biochemical and physiological aspects of seed germination; b) heavy metal toxicity; c) biochemical and physiological mechanisms involved in the regulation of dormancy and sprouting of potato tubers d) molecular and biochemical aspects involved in the vascular reconnection of tree plants grafts e) biochemical and physiological processes of the fruit ripening of orchard tree species; f) biochemical aspects involved in cellular pH homeostasis during the uptake of mineral nutrients; g) biochemical and physiological aspects involved in the abiotic stress responses.
Author of 55 "peer-reviewed" articles. *h-index (Scopus): 20; total citations (Scopus): 1154.*

Bhakti Prinsi

Researcher (RTD-A) in Agricultural Chemistry at the University of Milan. Her research activity is aimed to study some biochemical and plant physiology issues relevant to crop quality and production: physiological and molecular responses to stresses and (a)biotic factors (mineral nutrition, plant-water relations, plant-pathogen interactions); fruit ripening in woody plants; flower pigmentation; agamic multiplication in fruit trees; characterization and biological activities of seed storage proteins in legume and cereal crops. These research activities have enabled her to gain a good experience in the fields of plant proteomics and metabolomics based on mass spectrometry approaches.
Author of 25 "peer-reviewed" articles. *h-index (Scopus): 13; total citations (Scopus): 678.*

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. Especially in cereals, plant N use efficiency (NUE) is very low, therefore the selection of new varieties with higher NUE is one of the more promising strategies to improve the sustainability of agricultural production [1]. Nitrate and ammonium are the main forms of inorganic N used by crops and their availabilities deeply affect plant growth. The use of N by plants is sustained by distinct metabolic events which requires a strict functional coordination among cell compartments [2]. The membrane system plays key roles in perception and signaling of N availability as well as in ion transport and storage [2,3]. Activities and metabolic fluxes between chloroplasts and mitochondria determine the balance between production and consumption of energy and C skeletons required for N assimilation [2,4]. Maize (*Zea mays* L.), representing a staple food and an industrial commodity, is a model organism for studying plant biology, physiology and genetics [5]. Proteomics provided new information about N

nutrition in maize, highlighting the involvement of different post-translational modifications and enzyme isoforms [6,7]. Recently, it has become increasingly evident that subcellular proteomics, namely the study of the proteome in isolated cell compartments, is very powerful to unravelling metabolic functions and communication mechanisms among cell organelles [8]. Nowadays, several subcellular proteomics studies were conducted in maize to investigate physiological and developmental aspects, among which the comprehensive characterization of bundle sheath and mesophyll chloroplasts [9, 10]. The use of these new analytical approaches to study maize genotypes with different NUE could successfully help to better understand the roles of cell organelles as well as to propose novel molecular determinants, potentially useful in selection of new cereal varieties.

5. Layout of the project (draft)

The project will be aimed at studying the roles of subcellular compartments in plant adaptation to different nitrogen (N) availabilities, during vegetative growth, in maize inbred lines characterized by different N use efficiency (NUE).

5.1. Materials & Methods:

Plant materials: the project will be developed using two maize inbred lines T250 and Lo5 previously characterized for a lower and higher NUE, respectively.

Plant growth and nutritional treatments: plants will be grown in hydroponic systems until the development of the third-fourth leaf (*i.e.* 2-3 weeks) in a solution without N, in order to allowed the depletion of the seed N reserves. After starvation, the plants will be exposed to different N availabilities, consisting in low/high N inputs and/or different proportions between mineral N forms (*i.e.* nitrate / ammonium).

Evaluation of physiological status of the plants: key physiological parameters related to NUE, such as biomass accumulation in roots and leaves, photosynthetic performance, foliar water exchanges and oxidative stress will be evaluated by fluorometer, CIRAS and biochemical assays during nutritional treatments.

Evaluation of biochemical responses to N availabilities: during nutritional treatments, enzymatic and western-blot analyses will be performed in order to evaluate abundance and activities of the main enzymes involved in N assimilation (*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 the proteomic profiles in cell compartments during N treatments: the project will be addressed to isolate and characterized the proteomic profiles in microsomal fractions, chloroplasts and mitochondria, in roots and/or leafs. The subcellular 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 then characterized by means of large-scale proteomics approaches based on gel-electrophoresis and/or liquid chromatography tandem mass spectrometry (LC-ESI-MS/MS), assuring simultaneously identification and quantification of hundreds protein forms. The profiles of the subcellular(s) compartments will be compared between the two inbred lines and among different N nutritional conditions and/or time-exposures, in order to study plant metabolic adaptations and to individuate possible molecular determinants affecting NUE.

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

5.2. Schedule and major steps (3 years):

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.
- Definition of the best nutritional treatments (N inputs, timings) to examine NUE-related differences in the physiological and biochemical *status* between the two maize lines.
- Optimization of the protocols to analyze subcellular proteomes (microsomal, chloroplast, mitochondria) in maize.

Second year:

- Pilot proteomic analyses to define the most interesting conditions (N treatments, organ, cell compartments) to study the roles of cell organelles in maize NUE.
- Large-scale subcellular proteomic analysis on the experimental condition(s) defined in previous steps.

Third years:

- Interpretation of the proteomics results in relation to physiological and metabolic responses to N availability in the two maize lines. Individuation of molecular determinants related to NUE in maize.
- Validation of the results by means of bioinformatic tools and targeted-analyses.

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 10.000,00 € [FFABR – Individual Annual Funding of Basic Research Activities (6.000 €) and analytical service profit (4.000 €)].

7. Literature

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4. Sweetlove LJ, Fait A, Nunes-Nesi A, Williams T, Fernie AR. The mitochondrion: an integration point of cellular metabolism and signaling. *Crit Rev Plant Sci* 2007, 26: 17-43. DOI:10.1080/07352680601147919.

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7. Prinsi B, Espen L. Mineral nitrogen sources differently affect root glutamine synthetase isoforms and amino acid balance among organs in maize. *BMC Plant Biol* 2015, 15:96. DOI:10.1186/s12870-015-0482-9.

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

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