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Proteomic insight into the mitigation of wheat root drought stress by arbuscular mycorrhizae

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ABSTRACT

Arbuscular mycorrhizal fungi (AMF) are plant growth promoters that ameliorate plant-water relations and the nutrient uptake of wheat. In this work, two cultivars of *Triticum* spp., a bread and a durum wheat, grown under drought stress and inoculated or not by AMF, are evaluated through a shotgun proteomic approach. The AMF association had beneficial effects as compared to non-mycorrhizal roots, in both bread and durum wheat. The beneficial symbiosis was confirmed by measuring morphological and physiological traits. In our work, we identified 50 statistically differential proteins in the bread wheat cultivar and 66 differential proteins in the durum wheat cultivar. The findings highlighted a modulation of proteins related to sugar metabolism, cell wall rearrangement, cytoskeletal organization and sulphur-containing proteins, as well as proteins related to plant stress responses. Among differentially expressed proteins both cultivars evidenced a decrease in sucrose:fructan 6-fructosyltransferas. In durum wheat oxylipin signalling pathway was involved with two proteins: increased 12-oxo-phytodienoic acid reductase and decreased jasmonate-induced protein, both related to the biosynthesis of jasmonic acid. Interactome analysis highlighted the possible involvement of ubiquitin although not evidenced among differentially expressed proteins. The AMF association helps wheat roots reducing the osmotic stress and maintaining cellular integrity.

Biological significance: Drought is one of the major constraints that plants must face in some areas of the world, associated to climate change, negatively affecting the worldwide plant productivity. The adoption of innovative agronomic protocols may represent a winning strategy in facing this challenge. The arbuscular mycorrhizal fungi (AMF) inoculation may represent a natural and sustainable way to mitigate the negative effects due to drought in several crop, ameliorating plant growth and development. Studies on the proteomic responses specific to AMF in drought-stressed plants will help clarify how mycorrhization elicits plant growth, nutrient uptake, and stress-tolerance responses. Such studies also offer the potential to find biological markers and genetic targets to be used during breeding for new drought-resistant varieties.

1. Introduction

Wheat represents one of the most important crops for food, feed, and industrial raw materials, worldwide. According to FAO, wheat production was 749 million tonnes in 2016 (data of December 2016) [http://www.fao.org/worldfoodsituation/csdb/en/]. Wheat productivity can be threatened by abiotic stresses. In particular, drought events, that are expected to occur more frequently associated with the ongoing climate change, are among the principal constraints to global crop productivity [1]. Several strategies are underway to mitigate this stress, ranging from the breeding for more resilient varieties to the adoption of innovative agronomic protocols. In relation to the latter point, literature reports that arbuscular mycorrhizal fungi (AMF) root colonization can mitigate the negative effects of drought [2,3] in several crop species (legumes, strawberry, lettuce, soybean, as reviewed by Augé [4]). In addition, AMF, obligate biotrophs that engage root interactions with most of the land plants, are known to ameliorate plant growth and development, thus contributing to soil structure stabilization [5,6]. These fungi provide phosphorus (P) and nitrogen (N) from the soil to plants in exchange with photosynthetic products [7]. AMF have even the ability to modulate the plant-water relations, determining several physiological effects, ranging from changes in stomatal conductance and transpiration, to the increased efficiency in soil water absorption rate [8]. The overall result is an increased drought tolerance, likely arising from

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complex interactions among multiple mechanisms [9]. Some studies have been conducted, both in open field and in microcosm, to evaluate the responsiveness of wheat to AMF colonization in relation to drought stress. Field studies conducted to evaluate the effects of AMF inoculation on winter wheat grown under well-watered and water-stressed conditions, showed that biomass and grain yields were higher in mycorrhizal than non-mycorrhizal plots, irrespective of soil moisture [10]. In "semi-field" conditions, Zhou et al. [11] found an improvement in both growth and yield as result of AMF-mediated increases in photosynthesis during drought stress. These authors suggested that the mitigating effect of AMF is dependent on the wheat genotype. Durum wheat inoculated with the mycorrhizal fungus *Glomus mosseae* exhibited an increase in biomass, growth and grain yield, as well as higher uptake of nutrients [12].

More detailed knowledge of the molecular processes, that underpin the positive AMF-plant interaction, may enable the breeding for improved varieties, with higher yields or improved stress tolerance. From a molecular point of view, the application of high-throughput technologies to analyse the transcriptomes, proteomes and metabolomes of mycorrhizal plants under various environmental conditions are rapidly developing but still quite limited (reviewed in [13]). In this respect, proteomic techniques represent a cutting-edge technology to study the molecular events regulating agriculturally relevant traits in crop plants.

Proteomics has been extensively used to investigate plant response under abiotic stress [14–18]. Some research has been done on plant proteome responses to AMF inoculation. In major details, Bestel-Corre et al. [19] reviewed the first, pioneering proteomic studies in the field of plant-microbe symbioses. They reported the identification of about 400 proteins associated with both mycorrhizal and rhizobial symbioses. For example, in the experimental models *M. truncatula* - *G. mosseae* and *Daucus carota* - *G. intraradices* 73 newly induced protein spots were detected, among which proteins involved in signalisation and gene regulation processes. Additionally, putative amino acid sequences were obtained for nine proteins of *G. intraradices* extraradical mycelium by Dumas-Gaudot and collegues [20], six of them with homologies, including enzymes from central metabolism. For the first time, a systematic proteome analysis during mycorrhization has opened up the possibility of functional identification taking place in this symbiotic process. Mathesius [21] and De-la-Pena and Loyola-Vargas, [22] reviewed a wide range of proteomic approaches to studying the biotic interactions within the rhizosphere. The majority of proteome characterization has been done in the model legumes that interact with symbiotic rhizobia and mycorrhizal fungi. In legume roots infected with AMF, changes in proteins involved in redox- or defence responses (peroxidases and glutathione-S-transferases), respiration, cell wall, nutrient and water transport. Interestingly, findings on the signalling pathways utilized by rhizobia and mycorrhizal fungi to invade legume roots has been helped on the hypothesis that the more ancient AM symbiosis was the precursor for the more recent *Rhizobium*-legume symbiosis. Specific compounds of the rhizosphere, secreted by roots and microbes, are important players in the underground habitat and can affect crop productivity. Auxins, glomulins, strigolactones and polygalacturonases are example of metabolites and proteins that have a key role in plant productivity, affecting it either positively or negatively. Valot et al. [23] studied the membrane-associated protein modifications induced by *Glomus intraradices* colonization in *Medicago truncatula* roots, finding 36 spots differentially displayed in response to the fungal colonization. Among the up-regulated proteins the authors found nodulins (present even in the peribacteroid membrane of root nodules) and a putative defence-associated acid phosphatase. A higher number of proteins were found to be down-regulated, including ferritin, DREPPS, lipoxygenase, lectins, metallopeptidases. Recorbet et al. [24] characterized the proteome of extra-radical mycelium (ERM) of *Glomus intraradices* developed on carrot root organ cultures. ERM has an important role not only in the ecology of AMF symbiosis, but also in nutrient fluxes through the soil and biogeochemical cycling. Proteins belonging to 11 metabolic pathways were found in ERM, among others enzymes for the use of neutral lipids as the main respiratory substrate, enzymes involved in dark CO_2 fixation, glycolysis/gluconeogenesis, pentose phosphate and glutamine biosynthesis, proteins related to cell redox homeostasis and vesicular trafficking.

These same authors compared the proteomes of *Medicago truncatula* mycorrhizal roots in response to distinct AM fungi, providing evidence for the existence, at the protein level, of a conserved set of expressed functions upon mycorrhization, not only in the host plant, but also in the microsymbiont [25].

However, rather few studies have evaluated the effects of AMF inoculation in plants subjected to abiotic stress, by means of a proteomic approach, but to our knowledge, none evaluated responses to drought. Concerning abiotic stresses, the metal toxicity (cadmium and arsenic) was evaluated through proteomics in mycorrhizal plants [26–27].

In this work, we have investigated the modulation of wheat root proteome induced by AMF inoculation under drought stress, using a bread and a durum wheat cultivar. These results can contribute to the knowledge on the advantages in focusing AMF to improve wheat quality and productivity, to overcome detrimental environmental conditions induced by drought.

2. Materials and methods

2.1. Plant growth and AMF colonization

The fully randomised experimental design comprised two *Triticum* species either inoculated with AMF or not, and subjected to drought. The pot experiment was conducted in a growth chamber using two wheat genotypes, namely Mongibello (durum wheat) known as drought-tolerant wheat and Chinese Spring (bread wheat) known as drought-sensitive [28], and a pure AMF strain of *Glomus mosseae*, obtained from MycAgro Lab (Technopole Agro-Environnement, Bretenière, France). The experiments had two treatments, mycorrhizal and non-mycorrhizal plants were grown in 15 × 15 cm square pots (20 cm depth), filled with a 50:50 mixture of sterile sand and field soil. This soil was collected from the experimental farm of the Italian Council for Agricultural Research and Economics, located in Fiorenzuola d'Arda, in Northern Italy (44°55′19.4″N 9°54′32.3″E), sieved (2 mm) and dry treated at 100 °C × 1 h × 3 times, in three consecutive days [29]. The pasteurized sand-mixed soil resulted alkaline (pH 8.21), with the following characteristics: total carbonate 12.66%; inorganic carbon 15.19 g/kg; total carbon 28.1 g/kg; organic carbon 12.9 g/kg; organic matter 2.23%; total N 0.11%; C:N ratio 11.8; P_2O_5 35.4 mg/kg; cation exchange capacity 7.17 cmol(+)/kg. Before potting, all pots were washed with 0.1% (v/v) HCl and deionized water and filled with 3.5 kg of soil and sand mixture. *Glomus mosseae* was inoculated 2–3 cm below the planting holes at the concentration of 10 propagules g^{-1} soil. Other pots were not inoculated and used as control. Seeds of wheat were surface-sterilized and germinated on wet filter paper in Petri dishes for 5 days in the dark, at 20 °C. Five seedlings were transplanted into each pot, and after 3 days the pots were thinned to three plants each. Plants (three pots per treatment) were cultivated in a growth chamber at 20 °C day/16 °C night with 12 h photoperiod. Two weeks later, seedling were subjected to drought stress for a month by withholding watering; the seedling survived until the end of the experiment because the applied drought stress was moderate. Relative soil water content (RSWC) was controlled gravimetrically be weighing the pots every 2 to 3 days. During the drought treatment, RSWC dropped to 45% on average within 20 days and was kept constant at 45% afterwards. At harvest, some morphological plant traits were measured and wheat roots were carefully washed from soil, immediately frozen in liquid nitrogen, and then stored at −80 °C until analyses.

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