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RESEARCH ARTICLE

Modulation of protein expression in alfalfa (*Medicago sativa* L.) root and leaf tissues by *Fusarium proliferatum*

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Abstract

Alfalfa (*Medicago sativa* L.) is an important forage crop and is also a target of many fungal diseases including *Fusarium* spp. As of today, very little information is available about molecular mechanisms that contribute to pathogenesis and defense responses in alfalfa against *Fusarium* spp. and specifically against *Fusarium proliferatum*, the causal agent of alfalfa root rot. In this study, we used a proteomic approach to identify inducible proteins in alfalfa during a compatible interaction with *F. proliferatum* strain YQC-L1. Samples used for the two-dimensional gel electrophoresis (2-DE) and MALDI-TOF/TOF mass spectrometry were from roots and leaves of alfalfa cultivar AmeriGraze 401+Z and WL656HQ. Plants were grown in hydroponic conditions and at 4 days post inoculation with YQC-L1. Our disease symptom assays indicated that AmeriGraze 401+Z was tolerant to YQC-L1 infection while WL656HQ was highly susceptible. Analysis of differentially expressed proteins found in the 2-DE was further characterized using the MASCOT MS/MS ion search software and associated databases to identify multiple proteins that might be involved in *F. proliferatum* resistance. A total of 66 and 27 differentially expressed proteins were found in the roots and leaves of the plants inoculated with YQC-L1, respectively. These identified proteins were placed in various categories including defense and stress response related metabolism, photosynthesis and protein synthesis. Thirteen identified proteins were validated for their expressions by quantitative reverse transcription (qRT)-PCR. Our results suggested that some of the identified proteins might play important roles in alfalfa resistance against *Fusarium* spp. These finding could facilitate further dissections of molecular mechanisms controlling root rot disease in alfalfa and potentially other legume crops.

Keywords: alfalfa, proteome, 2-DE, mass spectrometry, *Fusarium proliferatum*

1. Introduction

Alfalfa (*Medicago sativa* L.) is one of the most important perennial legume crops worldwide (Zhou *et al.* 2011). It serves as a superior forage crop and contains high protein, minerals and vitamins content as well as a well-balanced amino acid composition (Wang *et al.* 2015). Notably, its nitrogen-fixing ability significantly reduces

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nitrogen application in field, attributing to which soil quality is improved dramatically. Fungal infection is one of the major threats to alfalfa production (Cramer *et al.* 2011). For example, alfalfa root rot disease caused by *Fusarium* species (e.g., *F. proliferatum* and *F. solani*) often cause significant reduction of alfalfa stand density and biomass loss worldwide (Leath and Kendall 1977; Salter *et al.* 1994; Cong *et al.* 2016). Currently, the most common and effective control method to combat this pathogen is to grow disease resistant cultivars. For many years, researchers have engaged in breeding alfalfa cultivars resistant to *Fusarium* spp. infection. For instance, two alfalfa populations created by Richard (1980) were reported to have a durable resistance to *F. roseum* var. *acuminatum*. The authors also showed that there were significant resistance variations to this disease among the polycrossed progenies, indicating that the potential of enhancing root rot disease resistance could be further improved through additional breeding (Richard *et al.* 1980). Over the past few years, several alfalfa genes have been reported to play important roles in resistance against *Fusarium* spp. (Román-Avilés and Kelly 2005; Vitale *et al.* 2014; Chittem *et al.* 2015), even though the detailed functions of these genes were not fully understood. However, on protein level, studies on alfalfa root rot disease resistance are rather rare.

In our previous work, we reported a strain of *F. proliferatum* from diseased alfalfa and designated this strain as YQC-L1 (Cong *et al.* 2016). *F. proliferatum* is a main pathogenic fungus causing root rot disease in many plant species including alfalfa (Cong *et al.* 2016), soybean (Arias *et al.* 2015; Chang *et al.* 2015), garlic (Salvalaggio and Ridao 2013), blueberry (Pérez *et al.* 2016) and asparagus (Elmer 1990). The YQC-L1 strain was used to screen 14 alfalfa cultivars, among them, cultivar AmeriGraze 401+Z was the most tolerant one while cultivar WL656HQ was the most susceptible. In the current study, we compared protein expressions inYQC-L1-challenged AmeriGraze 401+Z and WL656HQ alfalfa cultivars to non-challenged plants using two-dimensional gel electrophoresis (2-DE) and MALDI-TOF/TOF mass spectrometry. Root and leaf tissues used for the analysis were collected 4 days after pathogen infection when root rot disease symptoms began to show in the YQC-L1-inoculated WL656HQ plants. Differences found in the 2-DE from the two cultivars and from the YQC-L1- inoculated plants or non-inoculated plants, respectively, were compared. The identified differentially expressed proteins were further characterized using MALDI-TOF/TOF mass spectrometry. As a result, a total of 93 differentially expressed proteins were identified in roots and/or in leaves. Analysis of these identified proteins allowed us to place them into different functional categories including metabolism, photosynthesis, energy biosynthesis, protein synthesis, protein modification and degradation, defense and stress responses. We consider that the information presented in this paper to benefit researchers who are interested in molecular breeding for resistance to *Fusarium* spp. infection in alfalfa.

2. Materials and methods

2.1. Source of *F. proliferatum* strain YQC-L1 and inoculum preparation

The source of *F. proliferatum* strain YQC-L1 was reported previously (Cong *et al.* 2016). This YQC-L1 strain was confirmed to be the main causal agent of alfalfa Fusarium root rot disease. Liquid inoculum of YQC-L1 was initiated by growing its mycelium in potato dextrose broth (PDB) medium as previously described (Gloria 2012). The culture was then grown at 25°C for 10 days in the dark on a rotary shaker (~180 r min^{-1}). Conidia were separated from the mycelia mass by filtering the culture through four layers of sterilized gauze. The resulting spores were pelleted through 10-min centrifugation at 8 000 r min^{-1} (3K30, SIGMA, Germany) and re-suspended in sterile water for inoculation.

2.2. Plant materials, growth conditions and inoculation

Alfalfa cultivars AmeriGraze 401+Z and WL656HQ were selected for the study based on our previous cultivar screen study. In that screen, cultivar AmeriGraze 401+Z was determined to be tolerant to YQC-L1 strain infection while cultivar WL656HQ was highly susceptible to the pathogen. The inoculation method used was a modified version of that described by Ramírez-Suero (2009). Seeds of these two alfalfa cultivars were sterilized in a concentrated sulphuric acid solution for 10 min. After five rinses in sterile water, the seeds were placed on two layers of wet filter papers inside petri dishes and allowed to germinate under a controlled condition (25/20°C (day/night), 80% relative humidity, 16 h/8 h (light/dark)). Alfalfa seedlings of the same size were transplanted into pots. The outside surfaces of the pots were taped with black adhesive tape to keep the roots in the dark. The pots were then covered with a high-density foam board with 70 evenly spaced holes punched with a cork borer before use. The pot, measuring 26.5 cm×18.5 cm×7 cm, was filled with 2.1 L of half-strength Hoagland's solution (Hoagland and Arnon 1950). The seedlings were transplanted into pots and held in position using a 3 cm×1 cm×0.5 cm sponge strip. All seedlings were grown in plant growth chamber under the same condition as during germination. At 7 days post transplantation, the plants were inoculated with a spore suspension containing 5×10^6 conidia mL^{-1}. Plants inoculated with sterile water were used as the