Research article

Proteomic investigation of response to forl infection in tomato roots

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A R T I C L E   I N F O

A B S T R A C T

Fusarium oxysporum f. sp. radicis-lycopersici (FORL) leading to fusarium crown and root rot is considered one of the most destructive tomato soilborne diseases occurring in greenhouse and field crops. In this study, response to FORL infection in tomato roots was investigated by differential proteomics in susceptible (Monalbo) and resistant (Momor) isogenic tomato lines, thus leading to identify 33 proteins whose amount changed depending on the pathogen infection, and/or on the two genotypes. FORL infection induced accumulation of pathogen-related proteins (PR proteins) displaying glucanase and endochitinases activity or involved in redox processes in the Monalbo genotype. Interestingly, the level of the above mentioned PR proteins was not influenced by FORL infection in the resistant tomato line, while other proteins involved in general response mechanisms to biotic and/or abiotic stresses showed significant quantitative differences. In particular, the increased level of proteins participating to arginine metabolism and glutathione S-transferase (GST; EC 2.5.1.18) as well as that of protein LOC544002 and phosphoprotein ECPP44-like, suggested their key role in pathogen defence.

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1. Introduction

Tomato (Solanum lycopersicum) is one of most important vegetable crops worldwide and it can be considered a suitable model to study genetics and pathogen resistance processes in plants (Ercolano et al., 2012). This species is susceptible to over 200 diseases caused by all kinds of pathogens, including viruses, bacteria, fungi and nematodes (Lukyanenko, 1991). Among them, Fusarium oxysporum is responsible for two devastating diseases: tomato vascular wilts and crown and root rot. Three resistance genes against Fusarium oxysporum f. sp. lycopersici FOL (I, I2 and I3), have been identified in tomato wild species and introgressed into tomato commercial cultivars (Hemming et al., 2004). Moreover, the interaction between I2 gene and corresponding FOL Avr2 was used as a model system to study NLR-mediated recognition of secreted effectors (Takken and Rep, 2010).

On the contrary, information on Fusarium oxysporum f. sp. radicis-lycopersici (FORL) resistance sources is still fragmented (Fazio et al., 1999). FORL disease management has proved to be difficult. Conventional practices, including crop rotation, soil solarization and fungicides, have been applied with limited efficacy (Rekah et al., 1999). Few studies on FORL resistance mechanisms have so far been carried out. In particular, a resistance gene (Frl) conferring resistance to Fusarium crown and root rot and located on tomato chromosome 9, was identified and used for tomato breeding programs (Fazio et al., 1999).

Molecular investigation of resistant and/or susceptible genotypes could contribute to develop a modern integrated disease management system. In the last decades, several studies have focused on plant–pathogen interaction and on molecular mechanisms at the basis of plant-microbe interplay. The first active line of defence occurs on the plant cell surface, when pattern-recognition receptors (PRRs) recognize conserved pathogen-associated molecular patterns (PAMPs), leading to PAMP-triggered immunity (PTI). In order to circumvent PTI, pathogens can deliver effector molecules directly into the plant cell. Plants have developed intracellular immune receptors, known as resistance (R) proteins, that can recognize the presence of pathogen effector molecules activating

effector-triggered immunity (ETI) (Jones and Dangl, 2006; Spoel and Dong, 2012).

Resistance genes in tomato have been extensively explored. High-throughput DNA sequencing techniques, large-scale micro-array analyses and computational technologies are providing a growing understanding of the molecular basis of tomato–pathogen interaction and resistance mechanisms to different pathogens [1 and references herein]. On the contrary, a limited number of pro-teomic studies that investigated the molecular basis of pathogenic fungal attack in roots have been so far performed (Mathesius, 2009; Mehta et al., 2008). Furthermore, proteomic studies were carried out to elucidate tomato response and resistance mechanisms to bacterial infection (Afroz et al., 2009; Dahal et al., 2009; Dahal et al., 2010; Savidor et al., 2012), virus invasion (Casado-Vela et al., 2006), and the effect of FOL infection on the xylem sap protein composi-tion (Houterman et al., 2007). Proteins induced in roots after the infection with the oomycete *Aphanomyces euteiches* were identified in *Medicago truncatula*, thus providing proteomic evidence of the relation between abundance levels of specific pathogenesis-related (PR) proteins and infection level or severity (Colditz et al., 2005; Colditz et al., 2007; Trapphoff et al., 2009). More recently, the response of avocado plants to *Phytophthora cinnamoni* infection has been also studied through proteomics (Acosta-Muñiz et al., 2012).

A differential proteomic approach was applied in the present study to investigate changes occurring in protein profiles of roots from resistant (*Momor*) and susceptible (*Monalbo*) isogenic tomato lines after the infection by FORL. Results clearly indicated that in *Monalbo* line the infection triggered accumulation of several PR proteins displaying chitinase, glucanase and protease activity, or involved in redox reactions. As to the resistant tomato line, our results pointed out that the infection caused higher levels of pro-teins involved in general response mechanisms to biotic and/or abiotic stresses, and might also suggest a key role of proteins belonging to arginine metabolism and glutathione S-transferase in conferring resistance to fungal pathogen attack.

To our knowledge, this study has provided the first proteomic investigation of resistance mechanisms at the basis of FORL-tomato roots interaction.

2. Results

Proteomic analyses performed by integrating two-dimensional electrophoresis (2-DE) and mass spectrometry, led to the identifi-cation of 33 proteins contained in 44 spots (Fig. 1, Supplementary Table A and B) showing different accumulation patterns in rela-tion to FORL infection and/or to genotype. Reliable identification of
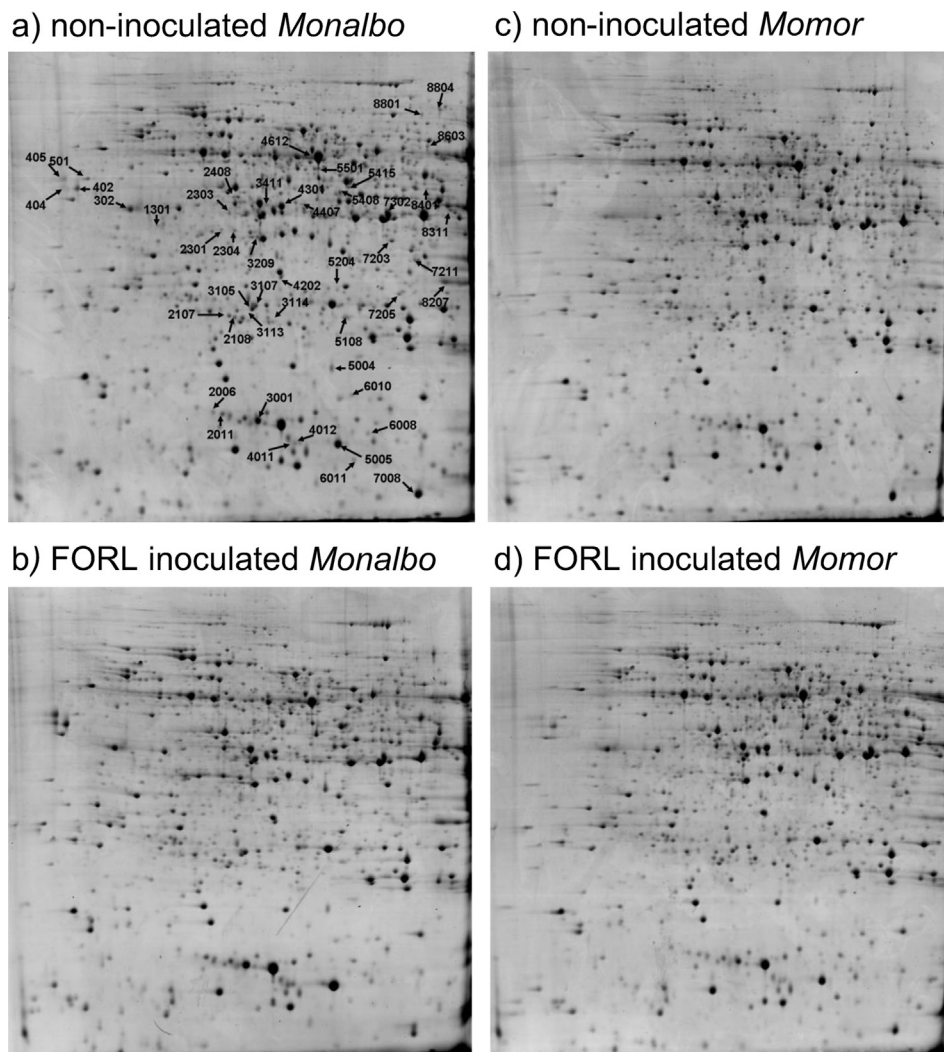
Fig. 1. Representative 2-DE gels of tomato roots proteome: a) non-inoculated *Monalbo* (susceptible genotype) (sample A); b) FORL inoculated *Monalbo* (sample B); c) non-inoculated *Momor* (resistant genotype) (sample C); d) FORL inoculated *Momor* (sample D). Spots displaying significant differences in mean intensity are indicated.