Proteome profile analysis of *Medicago truncatula* leaves in response to *Uromyces striatus*

Mª Ángeles Castillejo[1], Jesús V. Jorrín[2], Diego Rubiales[1]

[1] Departamento de Agronomía y Mejora, IAS (CSIC), Córdoba. [2] Departamento de Bioquímica y Biología Molecular, ETSIAM, Universidad de Córdoba

Summary

The two-dimensional electrophoresis (2-DE) leaf protein profile of four Medicago truncatula genotypes displaying different phenotypes in response to rust (Uromyces striatus) inoculation has been studied. Quantitative as well as qualitative differences were observed between non-inoculated and inoculated plants. Differential spots were analyzed by MALDI-TOF/TOF mass spectrometry and a total of 17 proteins were identified belonging to the functional category of metabolism with a high proportion being photosynthetic and stress-related proteins.

Introduction

Alfalfa rust (*U. striatus*) is an important disease of worldwide distribution, being particularly damaging in alfalfa (*Medicago sativa*) grown for seed production [1]. We have chosen *M. truncatula* as a more tractable biological system to study the proteomic response to rust. Genotypes were selected based on its differential responses to *U. striatus* infection [1, 2]: A17, susceptible; Grc.098 showing post-haustorial resistance; F11.008 and Paraggio showing pre-haustorial resistance. Leaf proteins from non-inoculated and rust inoculated *M. truncatula* plants were extracted and resolved by 2-DE, and the differential spots were analyzed by mass spectrometry.

Methodology

M. truncatula seedlings were inoculated when the third trifoliate leaf was completely expanded. Leaves were collected 48 hours post inoculation (hpi) for microscopic observations [1]. For proteomics analyses, inoculated and non-inoculated plants were sampled 24 and 48 hpi. Proteins from leaf tissue were TCA/acetone extracted [3] and resolved by 2-DE. Gels were silver and SyproRuby stained and the images were analysed with the PD-Quest software (BioRad). Those spots that showed statistically significant differences ($p \leq 0.05$) in intensity were considered for further analysis. Differential spots were excised from gels and digested with trypsin. Peptide fragments from digested proteins were then subjected to mass spectrometry. A PMF (peptide mass fingerprinting) search was performed over non-redundant MSDB database using the MASCOTsearch engine.

Results and Discussion

Differences in rust responses among genotypes were evident 48 hpi (table 1). Of the appressoria successfully formed on a stoma, less were able to penetrate the stoma and form a substomatal vesicle (SV) on F11.008 and Paraggio than on the other genotypes. Differences were also found in the rate of haustoria formation, which was significantly lower in F11.008 and Paraggio genotypes. However units associated with host cell necrosis was markedly higher in Grc.098. Of a total of 37 differential spot proteins between treatments, 17 were identified after MALDI-TOF/TOF and database search. We found an increase of stress-related proteins in pre-haustorial genotypes (table 2, figure 1) and two proteins belonging to other functional categories (glycine-rich RNA binding protein and malate dehydrogenase) which have been involved in transcriptional regulation by different effectors related to plant defense responses [4, 5]. We conclude that the expression of proteins was different in relation to susceptibility/resistance of the genotypes studied at early stage of the infection and the clear increase of stress-related proteins only in the genotypes with pre-haustorial resistance makes us suspect that these proteins may be involved in the stop of parasite development before forming haustoria.

Table 2. *Identified proteins that changed in response to U. striatus inoculation* [a]

Molecular function	*Medicago truncatula* genotypes			
	AI	**Grc.098**	**F11.008**	**Paraggio**
Metabolism	RuBisCO small chain [↑]	RuBisCO small chain (2) [↓]	RuBisCO small subunit (4) [↓]	Malate dehydrogenase [↑]
	RuBisCO large subunit [↓]	RuBisCO activase [↑]		
	Ferredoxin-NADP reductase [↑]			
Nucleic acid binding/ Transcription	Glycine-rich RNA binding protein [↑]	Elongation factor Tu, chloroplast precursor [↓]	Glycine-rich RNA binding protein [↑]	
Defense and stress-related			Ascorbate peroxidase [↑]	Glutathione peroxidise [new]
				Glutathione transferase [↑]

[a] *In parentheses indicate number of times this protein has been identified; [↑] indicate increase and [↓] decrease in intensity of protein.*

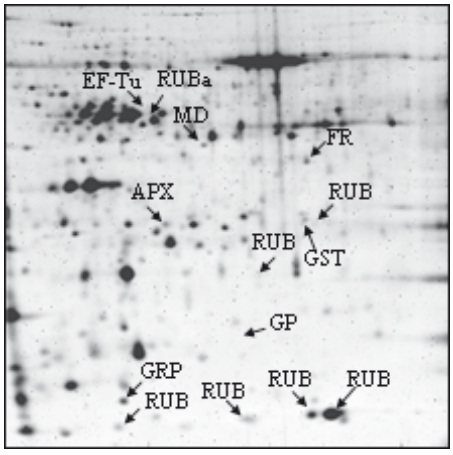

Figure 1. *2DE image gel of M.* **truncatula** *leaves proteome. Arrows indicates identified proteins: APX (ascorbate peroxidase), EF-Tu (elongation factor Tu), FR (ferredoxin-NADP reductase), GP (glutathione peroxidise), GRP (glycine-rich RNA binding protein), GST (glutathione transferase), MA (malate dehydrogenase), RUB (RubisCo), RUBa (RubisCo activasa).*

Table 1. *Microscopical analysis of the early development of U. striatus on M. truncatula* [x]

Genotypes	Appr. penetrated forming SV (%)	Number of haustoria/ colony	Colonies whit necrosis
A17	75.0 a	1.8 a	0 b
Grc.098	76.1 a	1.5 a	51 a
F11.008	42.3 b	1.0 b	0 b
Paraggio	31.8 b	0.03 c	0 b

[x] *Values with letters in common in each column are not significantly different ($p<0.05$, LSD test).*

Acknowledgments

The authors thanks to European Union Grain Legumes Integrated Project, Spanish project AGL2008-01239/AGR and Andalusian project P07-AGR-2883 for financial support.

References

[1] Rubiales D and Moral A. Mechanisms of resistance against alfalfa rust (Uromyces striatus) in Medicago truncatula. Eur J Plant Pathol 2004; 110:239-243.

[2] Kemen E, Hahn M, Mendgen K and Struck C. Different resistance mechanisms of Medicago truncatula ecotypes against the rust fungus Uromyces striatus. Phytopathology 2005; 95:153-157.

[3] Damerval C, de Vienne D, Zivy M and Thiellement H. Technical improvements in two-dimensional electrophoresis increase the level of genetic variation detected in wheat-seedling proteins. Electrophoresis 1986;7:52-54.

[4] Maurino VG, Saigo M, Andreo CS and Drincovich MF. Non-photosynthetic 'malic enzyme' from maize: a constituvely expressed enzyme that responds to plant defence inducers. Plant Mol Biol 2001; 45:409–420.

[5] Amey RC, Schleicher T, Slinn J, Lewis M, Macdonald H, et al. Proteomic analysis of a compatible interaction between Pisum sativum (pea) and the downy mildew pathogen Peronospora viciae. Eur J Plant Pathol 2008; 122:41-55.