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

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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≤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 MASCOT search 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 1. Microscopical analysis of the early development of U. striatus on M. truncatula

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

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

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References


