Phaseolus vulgaris GENES OBTAINED BY SUBTRACTIVE HYBRIDIZATION UNDER Macrophomina phaseolina INFECTION

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Phaseolus vulgaris GENES OBTAINED BY SUBTRACTIVE HYBRIDIZATION UNDER Macrophomina phaseolina INFECTION

Cuevas-Moreno J. Angel¹, Méndez-Moran Lucila², González-Prieto Juan Manuel¹.


INTRODUCTION

The fungus Macrophomina phaseolina (Tassi) Goid. causes charcoal rot in bean (Phaseolus vulgaris L.). The fungus causes dark lesions on epicotyls and hypocotyls of seedlings and they die due to obstruction of the xylem vessels and vascular wilting. In adult plants, the pathogen causes yellowing of roots, the stems show dark longitudinal lesions and the plant was defoliated and withered. The charcoal rot is promoted by high temperatures and drought conditions, which are frequent in beans growing areas in Mexico. M. phaseolina is responsible for the 65% on loss of bean yield (Abawi and Pastor-Corrales, 1990; Mayek-Perez et al., 1995,, Mayek-Perez et al., 1997). The study of genes differentially expressed in plants under M. phaseolina infection, will allow to gain a better understanding about plant defense mechanism. The objective of this study is the identification of P. vulgaris genes related to plant defense after fungal infection by subtractive hybridization (SSH) technique.

MATERIALS AND METHODS

The HMP05 strain of M. phaseolina and Pinto UI-114 P. vulgaris plants (susceptible variety to M. phaseolina) were grown in MS medium and incubated in a climate chamber at 25º C with 16-h light and 8-h darkness period, during 5 and 15 days for the fungus and plantlets, respectively. For interaction assays, the plants were place on the mature mycelium and incubated in a climate chamber at same conditions of growth. After 48 h three replicates of the interaction samples, and the controls (only fungus and only plants without interaction) were collected. For expressed assays, RNA from interactions and control samples was extracted by TRIzol® (Invitrogen®) method. The SSH was performed with the PCR-Select™ cDNA Subtraction Kit as describe the manufacturer (637401, Clontech®). SSH library products were sent to Eurofins MWG Operon for sequencing. The expressed sequences tag (EST) obtained from the SSH were analyzed using Blastn and Blastx algorithms against the complete transcriptome of P. vulgaris (NCBI repository). Thereafter the ESTs corresponding with P. vulgaris genes that coding to hypothetic or unknown protein were analyzed using Blastp algorithm.

RESULTS AND DISCUSSION

A total of 68 ESTs from the P. vulgaris were obtained during the interaction with M. phaseolina and displayed a significant match against deposited sequences in the no redundant protein database. The sequences identified were related to senescence-related proteins like glyoxysomal malate dehydrogenase, several proteases like aleurain, asparagine synthetase, auxin response factor, chloroplastic pyruvate phosphate dikinase 1, glyoxysomal malate synthase, beta-galactosidase, catalase, ATP sulphurylase, isocitrate lyase and osmotin. A fraction of the ESTs obtained (26.4 %) matching with several metabolic process proteins (e.g. ubiquitination, glycolysis or fatty acid oxidation) like ubiquitin activating enzyme 1, ATP synthase, P450 cytochrome protein and carnitine acyl carrier protein. Additionally, 3 ESTs corresponding to proteins involved in the cell wall biosynthesis like a hydroxyproline-rich glycoprotein, synthase
1 and proline-rich glycoprotein. Finally, we identified 5 ESTs with similarity to plant defense genes previously reported in other plants (such as Arabidopsis thaliana) during pathogen interaction. The feature of the identified genes are shown in table 1.

Table 1. Some Phaseolus vulgaris genes obtained during the interaction with Macrophomina phaseolina by SSH.

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Protein coding</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pvped1</td>
<td>3-ketoacyl-CoA 2-peroxisomal thiolase</td>
<td>Mitochondrial protein involved in fatty acids beta-oxidation and is required for induce the jasmonic acid local and systemic biosynthesis on pathogen (e.g. Botrytis cinerea) and mechanic stresses.</td>
</tr>
<tr>
<td>Pvext3</td>
<td>Extensin 3</td>
<td>A glycoproteins constituent of the plant cell walls that differentially express during mechanic and pathogen induced stresses.</td>
</tr>
<tr>
<td>Pvlfs2</td>
<td>2-hydroxy Isoflavone synthase 1</td>
<td>An enzyme involved in the flavonoids, isoflavonoids and phytoalexins biosynthesis (antimicrobial secondary metabolites). It is express on hydric, mechanic, thermic and pathogen induced stresses.</td>
</tr>
<tr>
<td>Pvebgla</td>
<td>Endo 1,3-Beta-D-glucanase</td>
<td>Hydrolyzing essential components of the fungal and bacteria cell walls. And is co-express with chitinases by pathogen elicitors induction.</td>
</tr>
<tr>
<td>Pvuvpr</td>
<td>Protein induced by UV-B</td>
<td>Cytoplasmic protein involved in the possible NADH-depending ROS regulation and also participate in the jasmonic acid and ethylene signaling.</td>
</tr>
</tbody>
</table>

These results are reported by first time in P. vulgaris under the interaction with the phytopathogen fungus M. phaseolina. The information obtained shows a panorama about particular genes that are involved in P. vulgaris defense under fungus infection. This work represent a new insight for new studies about the differential expression of plant defense genes, and could elucidate the mechanisms of P. vulgaris defense against this important pathogen.

REFERENCES
