AFLP FINGERPRINTING FOR IDENTIFICATION OF ANASTOMOSIS GROUPS OF 
*Rhizoctonia solani* ISOLATES FROM COMMON BEAN IN MEXICO^1

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*Rhizoctonia solani* (Rs) is a basidiomycete anamorph that does not produce any asexual spores (conidia) and occasionally produces sexual spores (basidiospores). Parmeter (4) indicated that hyphal anastomosis is a useful characteristic to identify Rs, and implies that genetically related isolates can recognize and fuse ("anastomose") with each other. Determination of anastomosis is done by microscopic monitoring of hyphal fusion which is a laborious and subjective method. Our study was carried out in order to obtain the AFLP genotype of nine *R. solani* isolates from common beans and 17 AG testers and determine the genetic relationship between anastomosis group (AG) and isolates of the pathogen; and determine the pathogenicity of RS isolates and then establish the association between anastomosis group, pathogenicity and AFLP genotype.

Nine Rs isolates were obtained from bean plants collected in Estado de Mexico and Veracruz, Mexico. DNA from each Rs isolate and 17 AG testers (AG 1A, AG B1, AG C1, AG 2.1, AG 2.2, AG 2.3, AG 3, AG 4, AG 5, AG 6, AG 7, AG 8, AG 9, AG 10, AG 11, AG 12, AG 13) was extracted (5). AFLP analysis (6) was performed and amplified products were separated in acrylamide gels and visualized using silver staining. The AG of each Rs isolate was performed on *in vitro* conditions (1). Each isolate was cultured on PDA and 10 seeds of each of the bean cultivars Pinto Villa, Río Tibagi, Pinto UI-114, Bayo Durango, Azufrado Tapatío, Bayo Mecentral, Negro 8025, SEQ 12, TLP 19, and BAT 477 were placed over the mycelia. Pathogenicity was scored three days after placing the seeds using a six level scale (from 0 to 5, where 0 = No infection and 5 = 81-100 % of infected seed). Values from 0 to 2.0 were classified as a resistant reaction and values from 2.1 to 5 as a susceptible reaction.

Five isolates of Rs were from Veracruz (RS010, RS011, RS012, RS013, RS014) and four from México (RS001, RS002, RS003, RS009). AFLP analysis produced 415 bands including 3 monomorphic bands (0.72 %) among the nine *Rhizoctonia* isolates, while among the AG testers no monomorphic bands were found (data not shown). One isolate from Veracruz (RS010) and four isolates from México (RS001, RS002, RS003, RS009) were similar to AG-2 type 3; three isolates from Veracruz were similar to AG-BI, and one isolate from Veracruz (RS013) grouped with AG-5 (Fig. 1). Test for identification of anastomosis groups *in vitro* showed the complete coincidence between the AGs determined by AFLPs and by *in vitro* tests. BAT 477 showed the highest mean of severity by Rs while the other nine bean cultivars exhibited severity ratings lower than 2.0. Common bean germplasm from races Durango and Jalisco showed a higher frequency of resistance (7 to 9 isolates) than whose from race Mesoamerica (5 to 8 isolates). Isolates 011 (AG-B1), 002 and 003 (AG 2.3) were the more aggressive in common bean seeds (Table 1). No association was found between the anastomosis group of each Rs isolate and pathogenicity on common bean seeds. An inverse resistance/susceptibility relationship as the one
showed here have been found between *P. vulgaris* gene pools and *Macrophomina phaseolina* (3) and *Fusarium* (2). Resistance to Rs in Durango germplasm may have been produced through selective pressure since the fungus is the major causal agent of root rots in cultivated *P. vulgaris* in the states of Durango and Zacatecas, México from where the germplasm was originated. The grouping of each isolate to its corresponding AG indicated that AFLP could be a reliable method for molecular identification of AGs of *Rhizoctonia* when the technique is available. The AFLP technique offers several advantages as a molecular test for identification of anastomosis groups of Rs isolates such as the short time needed to obtain results; the possibility to rapidly classify large numbers of isolates; the establishment of a data base that includes banding patterns of AGs for further identification of isolates (using automatic sequencing systems); and the elimination of the laborious identification of AGs under the microscope.

**LITERATURE CITED**


**Table 1. Resistance/susceptibility of 10 common bean cultivars to nine isolates of *R. solani***

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Race²</th>
<th>R. solani isolates</th>
<th>Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>001</td>
<td>002</td>
<td>003</td>
</tr>
<tr>
<td>B. Durango</td>
<td>D</td>
<td>0.6</td>
<td>0.3</td>
</tr>
<tr>
<td>P. Villa</td>
<td>D</td>
<td>1.0</td>
<td>3.7</td>
</tr>
<tr>
<td>P. U1-114</td>
<td>D</td>
<td>1.0</td>
<td>0.9</td>
</tr>
<tr>
<td>A. Tapatio</td>
<td>J</td>
<td>0.4</td>
<td>0.3</td>
</tr>
<tr>
<td>SBQ 12</td>
<td>M</td>
<td>0</td>
<td>1.5</td>
</tr>
<tr>
<td>BAT 477</td>
<td>M</td>
<td>3.4</td>
<td>3.4</td>
</tr>
<tr>
<td>TLP 19</td>
<td>M</td>
<td>1.1</td>
<td>2.3</td>
</tr>
<tr>
<td>N. 8025</td>
<td>M</td>
<td>0.5</td>
<td>2.3</td>
</tr>
<tr>
<td>Rio Tibagi</td>
<td>M</td>
<td>1.6</td>
<td>2.4</td>
</tr>
<tr>
<td>B. Mecentral</td>
<td>J</td>
<td>0.7</td>
<td>3.1</td>
</tr>
</tbody>
</table>

Mean ± SE 1.0±0.9 2.0±1.2 1.9±0.7 0.6±0.5 0.6±0.5 2.1±0.9 1.2±0.7 0.8±1.0 0.5±0.4 1.2±0.7

²D = Durango, J = Jalisco, M = Mesoamerica.

*Where reaction of resistance were from 0 to 2.0 and susceptibility were from 2.1 to 5.*

![Fig. 1. Dendrogram produced from AFLP data obtained from nine Mexican *R. solani* isolates and 17 AG testers.](image-url)