Title
Soybean Rust Genome Sequencing Project

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Soybean Rust is caused by two closely related fungal pathogens, Phakopsora pachyrhizi and P. mediei. The Asian soybean rust pathogen P. pachyrhizi (ASR) is highly aggressive and is responsible for significant losses of soybean crop in Africa, Asia, Australia and South America (Figure 1). It was discovered for the first time in the continental United States in Louisiana in November 2004. During 2005, the presence of ASR was confirmed in 138 counties across nine southern states. ASR poses a significant threat to the U.S. soybean industry (17 billion dollar annually), depending on the severity and extent of subsequent outbreaks.

Currently, no commercial soybeans are resistant to ASR, and fungicides are generally recognized as the most effective means for controlling the disease. Very little is known about the molecular mechanisms involved in the soybean-rust interaction. In order to develop new strategies to control the disease, it is crucial to increase our understanding of the biology of the pathogen and the infection process.

Here, we present strategies and preliminary results from the P. pachyrhizi Genome Sequencing Project, including the complete mitochondrial genome sequence and the comparative analysis of expressed sequence tags (ESTs) generated from four specific stages of P. pachyrhizi.

Mitochondrial Genome

Known mitochondrial genome sequences were compared to the entire set of genomic reads using the Blast algorithm. Potential mitochondrial sequences were assembled with the Phred Phrap Package. This resulted in single contig assembly for the P. pachyrhizi mitochondrial genome.

The complete nucleotide sequence of the mitochondrial (mt) genome was determined for Phakopsora pachyrhizi. This 32 kb genome contains the genes encoding ATP synthase subunits 6, 8, and 9 (a6p, a8p, and a9p), cytochrome oxidase subunits I, II, and III (cox1, cox2, and cox3), apocytochrome b (cob), reduced nicotinamide adenine dinucleotide ubiquinone oxireductase subunits (nad1, nad2, nad3, nad4, nad4L, nad5, and nad6), the large and small mitochondrial ribosomal RNAs (rns and rrm) and tRNAs for all amino acids.

Acknowledgments

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References


Figure 1. 1. (A) Field of P. pachyrhizi in Zimbabue. C. Inoculated soybean leaf infected with Phakopsora pachyrhizi after three times with fungicide, yellow line showing where the fungus didn’t reach the plant (Zimbabwe).

Figure 2. Phakopsora pachyrhizi mtDNA 31.2 kb

Figure 3. Four P. pachyrhizi unidirectional cdNA libraries from different stages were constructed in pRSETA (Invitrogen). Germinating urediniospores, resting urediniospores, hyphal growth (6-8 days post inoculation) and high sporulation (13-15 days post inoculation).

Table 1. Estimation of P. pachyrhizi genome size

<table>
<thead>
<tr>
<th>Estimation Method</th>
<th>Genomic Size</th>
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</thead>
<tbody>
<tr>
<td>cDNA Coverage</td>
<td>720 Mb</td>
</tr>
<tr>
<td>All-Pairs Read Alignment</td>
<td>500-800 Mb</td>
</tr>
<tr>
<td>Gene Density</td>
<td>300-700 Mb</td>
</tr>
<tr>
<td>Shotgun Fosmid Coverage</td>
<td>600-950 Mb</td>
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</tbody>
</table>

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The available genomic sequences and the ESTs represent an important collection that provides new molecular markers as microsatellites and SSR for diversity and phylogenetic studies. Further analysis of these data will also provide a new genome size estimation, information on gene and non-coding regions distribution and repeat families.