

# *How to Characterize Rust Isolates: Genome Sequencing, Molecular Markers and Differentials*

**Dr. Reid D. Frederick  
USDA-Agricultural Research Service  
Foreign Disease-Weed Science Research Unit  
Fort Detrick, MD**

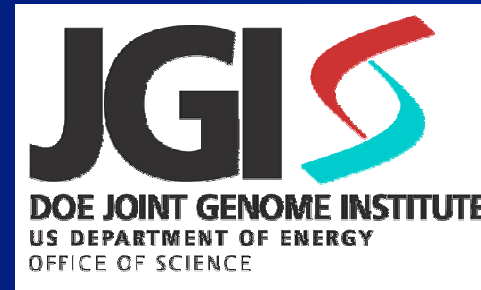
**Phone: 301-619-7386**

**Email: [rfrederick@fdwsr.ars.usda.gov](mailto:rfrederick@fdwsr.ars.usda.gov)**



# **Pathogen Genome Sequencing Project**

# Cooperative Agreement between USDA-ARS & DOE/Joint Genome Institute



- Identify target sequences for molecular diagnostics and isolate identification.
- Identify biochemical pathways as targets for new fungicides.
- Identify candidates for genes mediating host-pathogen responses
- Determine evolutionary relationships with other fungi.

# Genomic Approaches

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- Comparative genomic sequencing project
  - *P. pachyrhizi* & *P. meibomia*e
- Gene expression (Expressed Sequence Tags)
  - Germinating urediniospores
  - cDNA libraries from infected soybean plants

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# Genome sequencing project

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|                       | Library<br>(Insert size) | Bases<br>sequenced |
|-----------------------|--------------------------|--------------------|
| <i>P. pachyrrhizi</i> | 3 Kb                     | 153 MB             |
| <i>P. pachyrrhizi</i> | 8 Kb                     | 546 MB             |
| <i>P. pachyrrhizi</i> | 40 Kb                    | <u>6 MB</u>        |
|                       |                          | 705 MB             |
| <i>P. meibomiaae</i>  | 3 Kb                     | 125 MB             |
| <i>P. meibomiaae</i>  | 8 Kb                     | <u>6 MB</u>        |
|                       |                          | 131 MB             |

Trace records (raw single-pass reads of DNA sequence) released by the DOE-JGI, available at the GenBank: **840.789 Mbp**

<http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=170000>

# Fosmid Sequencing Strategy

**Random fosmids:** Finishing at Stanford Genome Technology Center (SGTC): 109 complete; 6 incomplete

**Selected fosmids:** Hybridizations at Lawrence Livermore National Laboratory (LLNL). Probes designed to 100 “genes” with high homology to pathogenicity related genes, important metabolic pathway genes or genes highly expressed during spore germination.

65 fosmids selected; 17 finished at SGTC.



*P. pachyrhizi* (31.8kb)

*P. meibomiaae* (32.5kb)

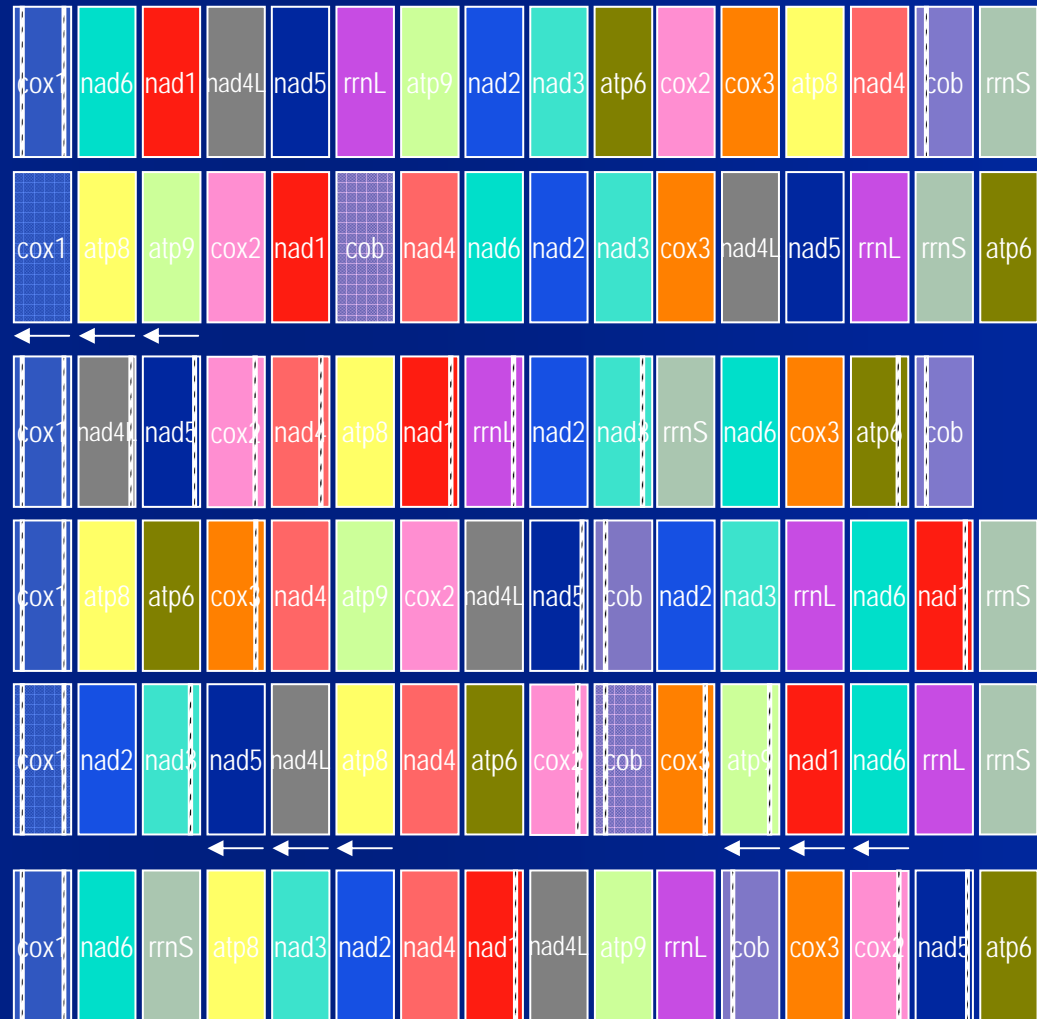
*S. commune* (49.7kb)

*P. anserina* (100.3kb)

*Y. lipolitica* (47.9kb)

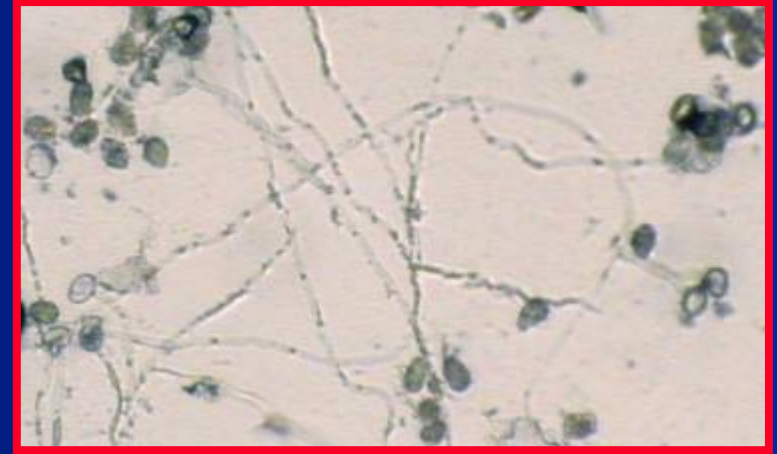
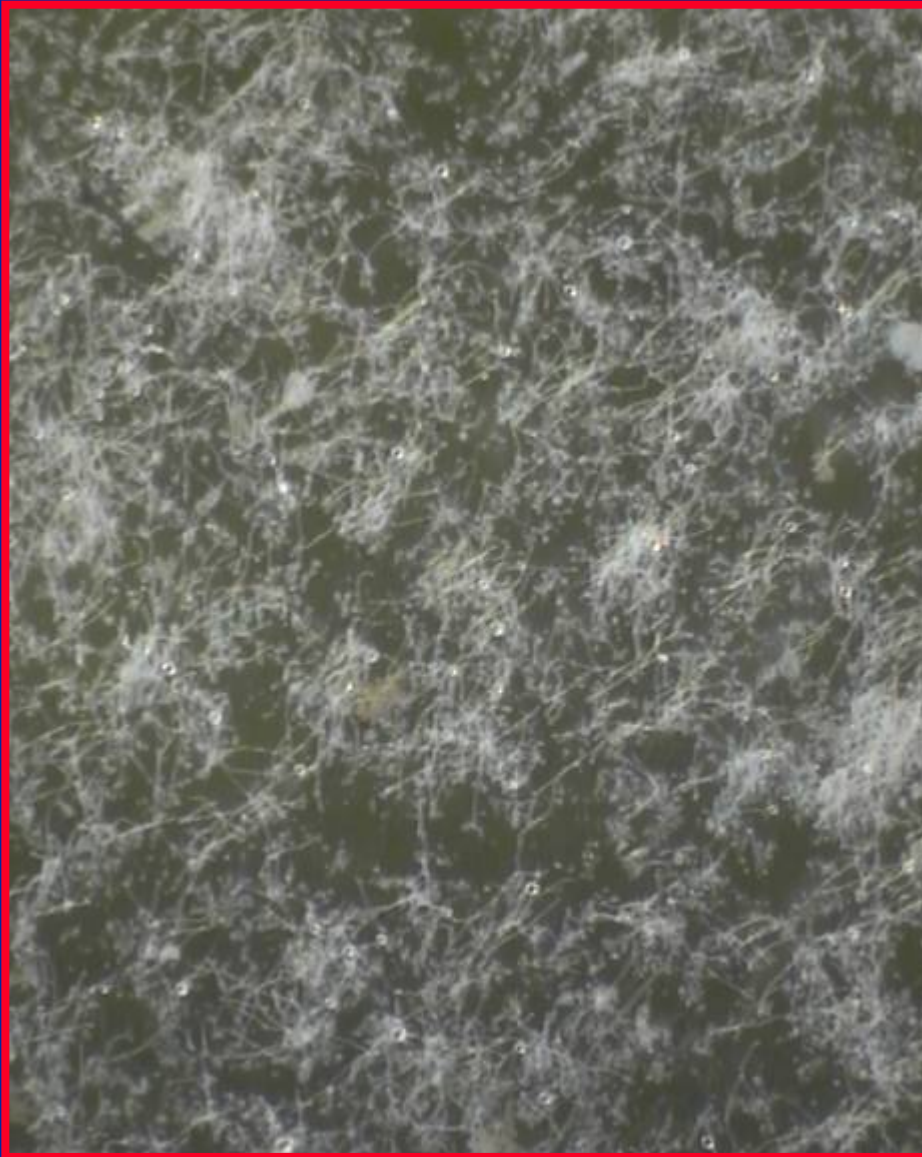
*R. stolonifer* (54.2kb)

*A. macrogynus* (57.5kb)

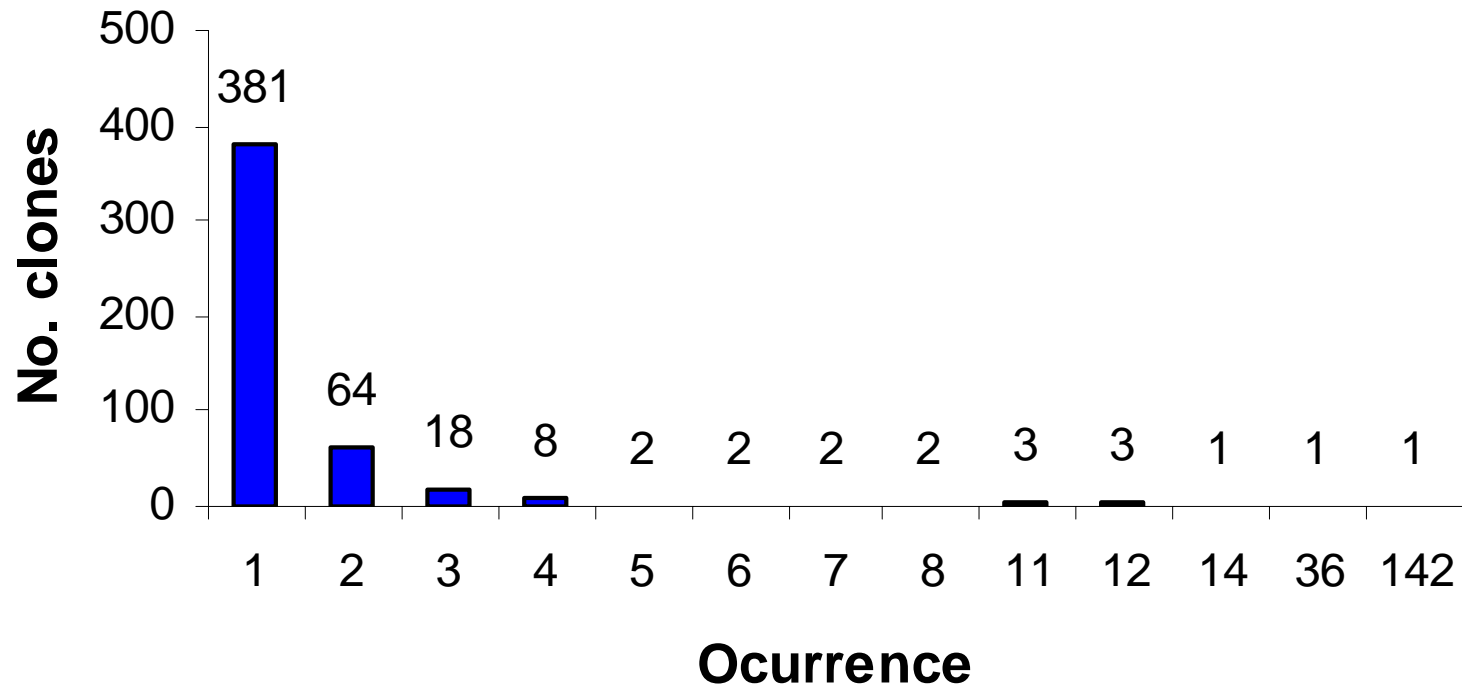


Comparison of mitochondrial genomes from the four phyla of fungi. Protein-coding and rRNA genes are represented by boxes; arrows indicate the direction of transcription. Lines within genes represent presence of intron(s).

# *Phakopsora pachyrhizi* EST Project

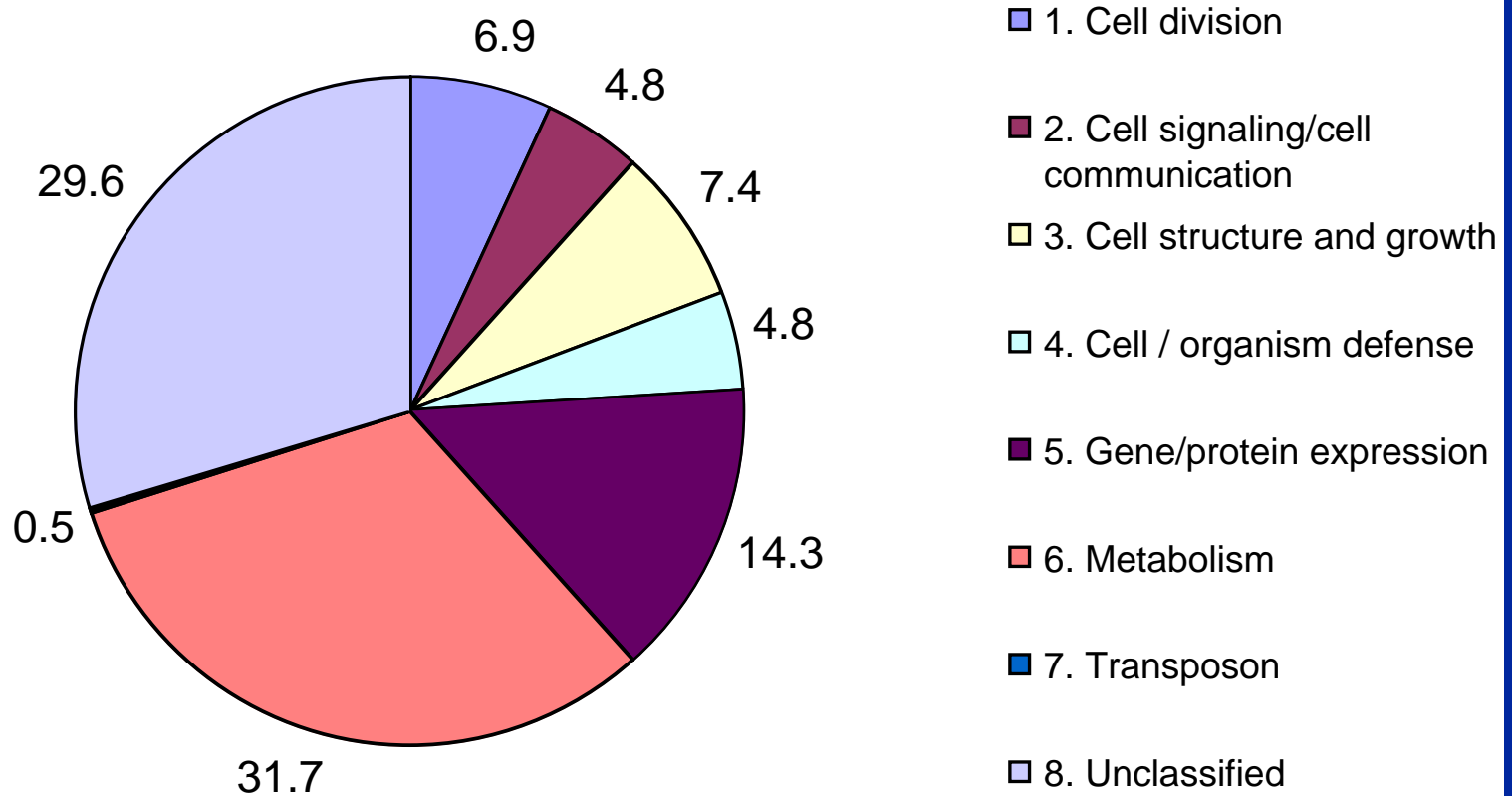


# REDUNDANCY



Frequency of occurrence of multiple clones in the *Phakopsora pachyrhizi* cDNA library from germinating urediniospores.

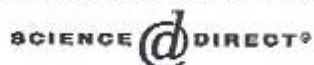
# PUTATIVE FUNCTION



Classification of 189 unique *Phakopsora pachyrhizi* ESTs from germinating urediniospores. ESTs with significant similarities (BLASTX *Evalue* <  $10^{-5}$ ) to the “nr” database grouped into functional categories according to EGAD.



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FUNGAL GENETICS  
AND BIOLOGY[www.elsevier.com/locate/yfghi](http://www.elsevier.com/locate/yfghi)

## Expressed sequence tag analysis of the soybean rust pathogen *Phakopsora pachyrhizi*

Martha Lucia Posada-Buitrago<sup>1</sup>, Reid D. Frederick<sup>\*</sup>*USDA-Agricultural Research Service, Foreign Disease-Weed Science Research Unit, 1301 Ditto Avenue, Fort Detrick, MD 21702, USA*

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### Abstract

Soybean rust is caused by the obligate fungal pathogen *Phakopsora pachyrhizi* Sydow. A unidirectional cDNA library was constructed using mRNA isolated from germinating *P. pachyrhizi* urediniospores to identify genes expressed at this physiological stage. Single pass sequence analysis of 908 clones revealed 488 unique expressed sequence tags (ESTs, unigenes) of which 107 appeared as multiple copies. BLASTX analysis identified 189 unigenes with significant similarities ( $E_{\text{value}} < 10^{-5}$ ) to sequences deposited in the NCBI non-redundant protein database. A search against the NCBI dbEST using the BLASTN algorithm revealed 32 ESTs with high or moderate similarities to plant and fungal sequences. Using the Expressed Gene Anatomy Classification, 31.7% of these ESTs were involved in primary metabolism, 14.3% in gene/protein expression, 7.4% in cell structure and growth, 6.9% in cell division, 4.8% in cell signaling/cell communication, and 4.8% in cell/organism defense. Approximately 29.6% of the identities were to hypothetical proteins and proteins with unknown function.

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**Keywords:** *Phakopsora pachyrhizi*; Genome analysis; cDNA sequencing; Expressed sequence tags; Gene expression

# cDNA Libraries

Germinating  
Spores

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16 Hours on  
water surface

**18638**

Resting spores

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Kept at  
- 80°C

**1762**

Hyphal growth

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6  
7  
8 Days after  
inoculation

**5374**

High sporulation

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13  
14  
15 Days after  
inoculation

**4610**

mRNA was extracted from infected leaf at each time point and pooled together for the construction of the cDNA libraries.

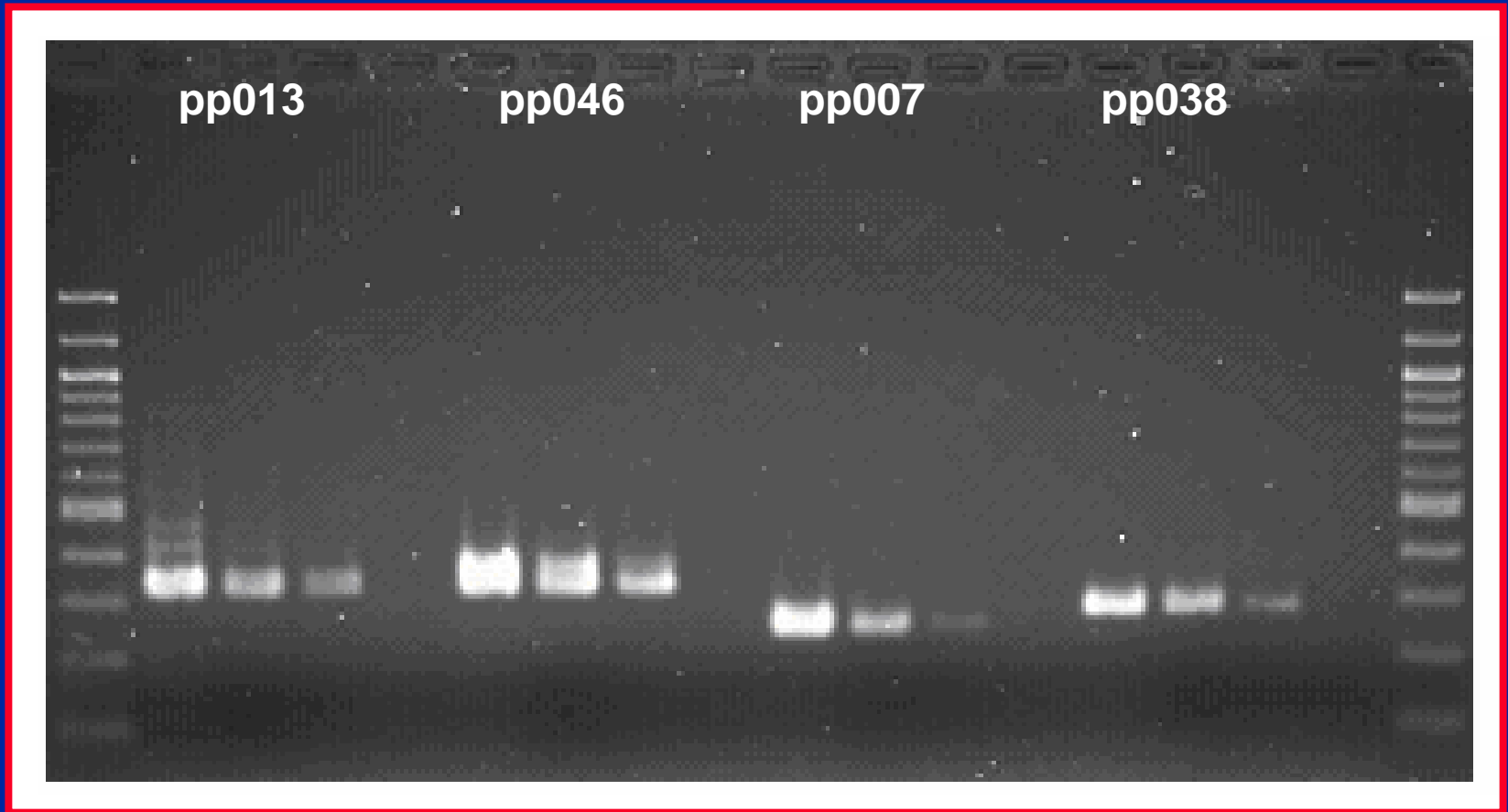
Unidirectional cDNA libraries constructed in plasmid pSPORT1.

**Simple Sequence  
Repeats  
(Microsatellites)**

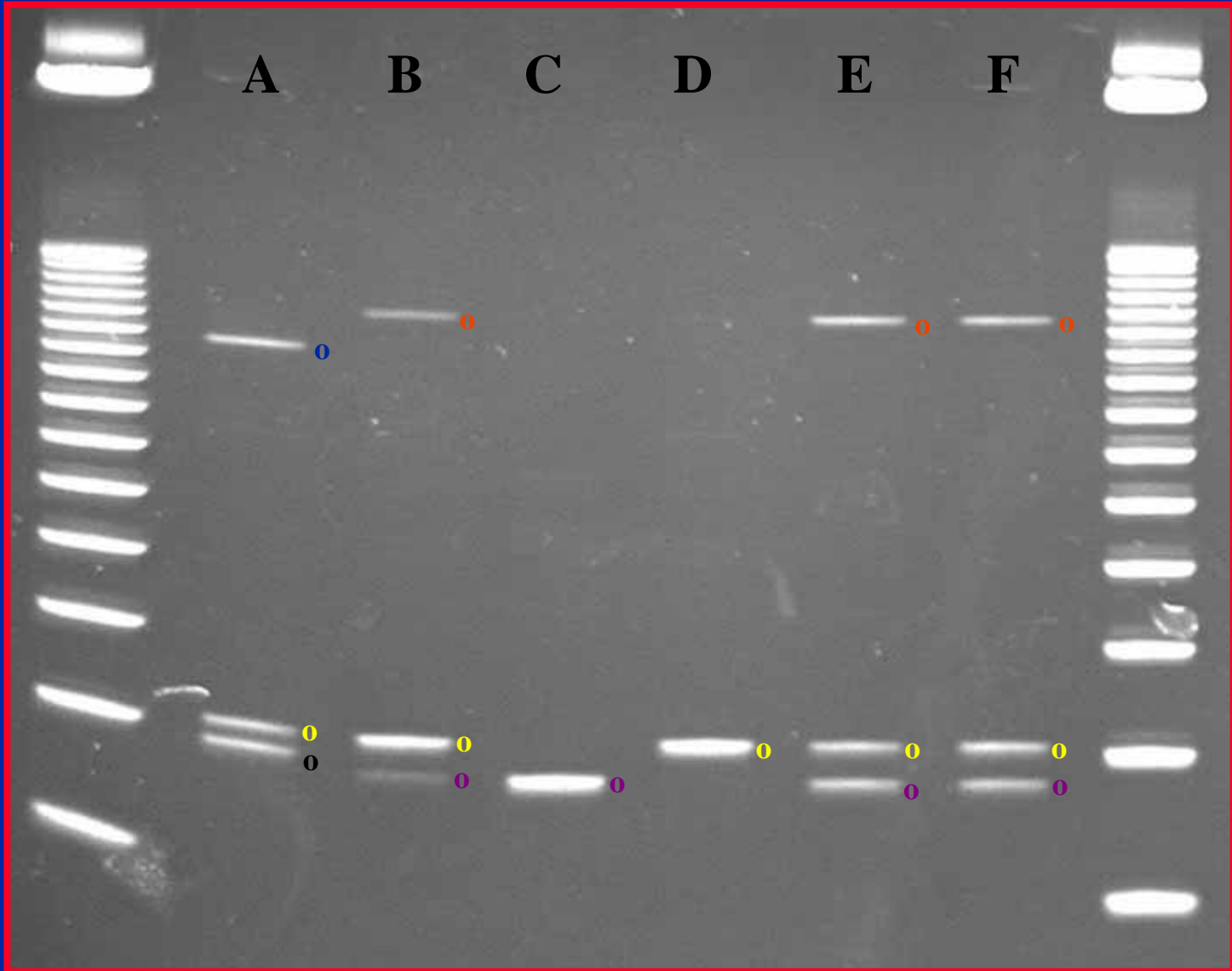
# Microsatellites/SSRs

| <b>Class</b> | <b>Example Motif</b> | <b>Repeat Threshold</b> |
|--------------|----------------------|-------------------------|
| <b>Di</b>    | <b>CA</b>            | <b>12</b>               |
| <b>Tri</b>   | <b>ATG</b>           | <b>6</b>                |
| <b>Tetra</b> | <b>ATCG</b>          | <b>5</b>                |
| <b>Penta</b> | <b>AGTTG</b>         | <b>4</b>                |
| <b>Hexa</b>  | <b>CCCCAA</b>        | <b>3</b>                |

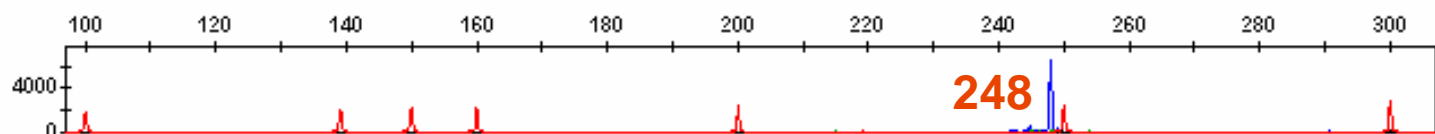
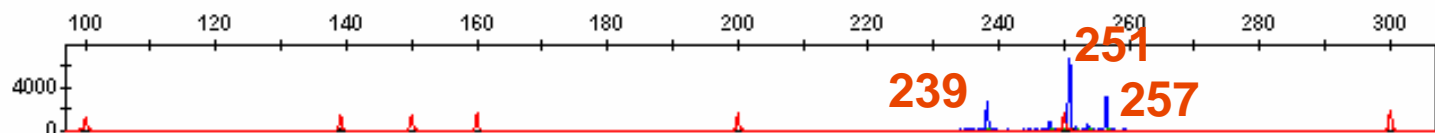
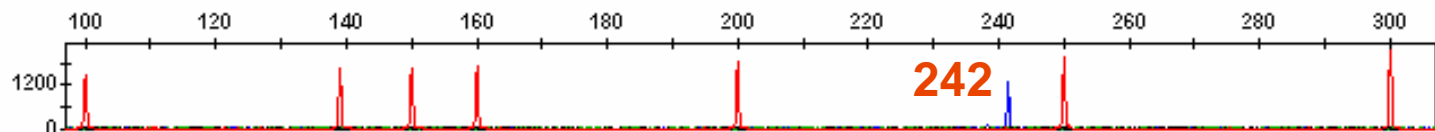
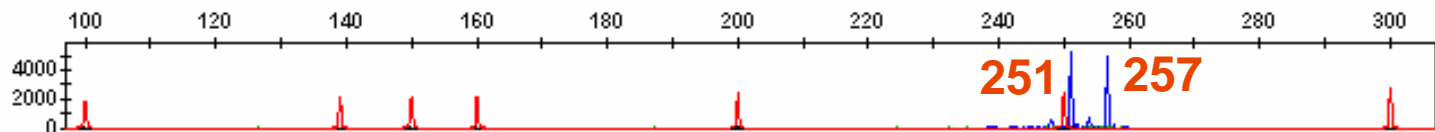
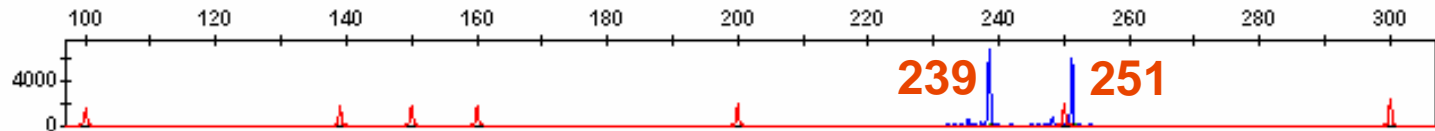
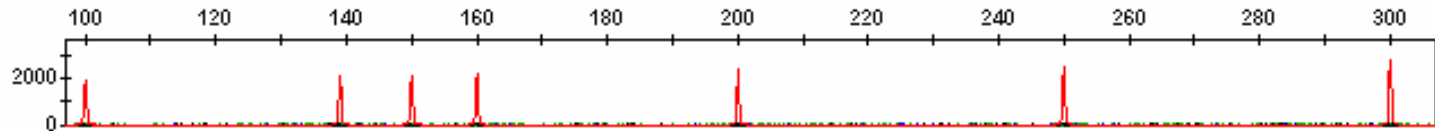
# SSRs: Marker development



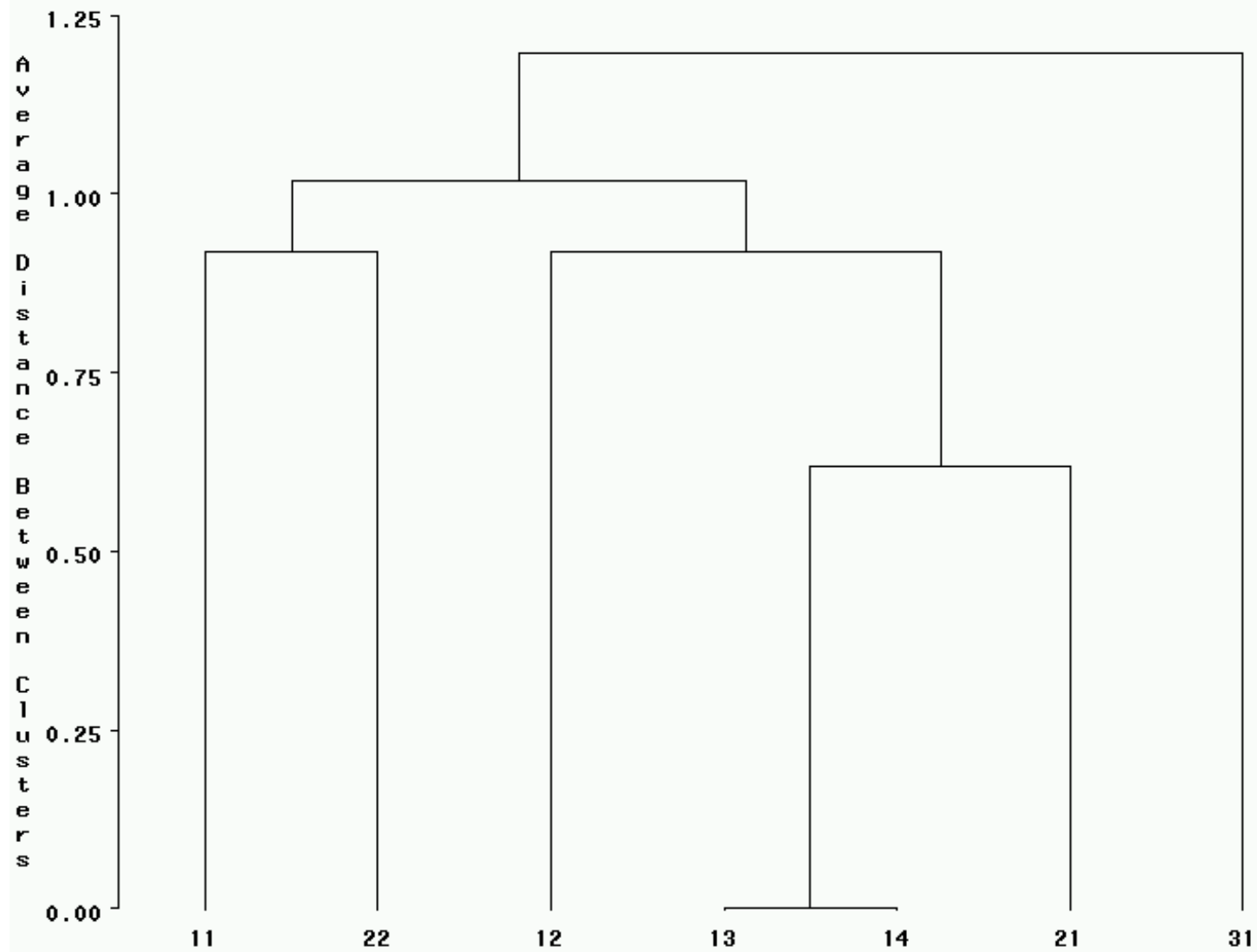
# SSRs: Marker development



# SSR polymorphism: pp002



# upgma cluster and tree



# Reaction Phenotypes



**TAN**



**RB**



**IMMUNE**

# Rust Reactions

| Entry                | <i>P. pachyrhizi</i> |              |              |              |              |              |              |           |              |           | <i>Pm</i> |           |
|----------------------|----------------------|--------------|--------------|--------------|--------------|--------------|--------------|-----------|--------------|-----------|-----------|-----------|
|                      | TW                   | TH           | PH           | ZM           | SA           | BZ           | PG           | AU        | IN           | HW        | BZ        | PR        |
| Williams             | Tan                  | Tan          | Tan          | Tan          | Tan          | Tan          | Tan          | Tan       | Tan          | Tan       | Red-brown | Red-brown |
| PI548628 (Wayne)     | Tan                  | Tan          | Tan          | Tan          | Tan          | Tan          | Tan          | Tan       | Tan          | Tan       | Red-brown | Red-brown |
| PI200492 (Rpp1)      | Tan                  | Tan          | Tan          | Tan          | Tan          | Tan          | Tan          | Immune    | Immune       | Immune    | Red-brown | Red-brown |
| PI230970 (Rpp2)      | Red-brown            | Intermediate | Red-brown    | Intermediate | Intermediate | Intermediate | Intermediate | Red-brown | Intermediate | Red-brown | Immune    | Red-brown |
| PI462312 (Rpp3)      | Tan                  | Tan          | Tan          | Tan          | Red-brown    | Intermediate | Intermediate | Red-brown | Red-brown    | Red-brown | Immune    | Red-brown |
| PI459025B (Rpp4)     | Intermediate         | Red-brown    | Red-brown    | Intermediate | Intermediate | Intermediate | Red-brown    | Tan       | Intermediate | Red-brown | Red-brown | Red-brown |
| PI268039 (Tainung #) | Tan                  | Tan          | Intermediate | Tan          | Red-brown    | Intermediate | Intermediate | Immune    | Immune       | Immune    | Immune    | Red-brown |

 Tan

 Intermediate

 Red-brown

 Immune



# Acknowledgements

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- Tom Slezak & Laurie Gordon



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