Investigating the Role of the Circadian Clock Genes PRR5, PRR7, and PRR9 in Regulating Plant Immunity

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Abstract
Successful defense against pathogens is critical for plant survival. Recent studies have shown that the circadian clock, the internal time measuring machinery, is involved in disease resistance in addition to its roles in plant development. One such protein, LUX, binds to the promoters of the clock genes PRR5, 7, and 9; we have preliminary results to show that these genes are involved in defense. To confirm if these genes affect SA-mediated defense, we introduced individual single mutants of the genes PRR5, 7, and 9 into acd6-1, a small mutant plant with constitutive defense whose size change predicts the defense levels. We have isolated the double mutants (acd6-1prr5, acd6-1prr7, acd6-1prr9), two triple mutants (acd6-1prr5prr9 and acd6-1prr7prr9), and the quadruple mutant (acd6-1prr5prr7prr9). We are currently assessing the plant phenotypes by measuring their sizes, cell death levels, SA levels, and the expression of defense genes. Analysis of acd6-1 phenotype suppression, if any, exists, will show whether the PRR5, 7, and 9 genes act in a synergistic manner in the SA pathway. Significant phenotypic recovery would be evidence for roles of these genes in defense control.

Approaches and Results
We obtained knockout mutants for each PRR gene, and crossed the mutations into the acd6-1 background. We genotyped seedlings by PCR to find individuals homozygous for each combination of mutations. In addition to the double mutants (acd6-1 and one PRR gene knockout), we also identified two triple mutants and the quadruple mutant.

Table 1: acd6-1 PRR Gene Mutants Identified
<table>
<thead>
<tr>
<th>Genotype</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>acd6-1prr5-1</td>
<td>acd6-1prr5-1prr9-1</td>
</tr>
<tr>
<td>acd6-1prr7-3</td>
<td>acd6-1prr7-3prr9-1</td>
</tr>
<tr>
<td>acd6-1prr9-1</td>
<td>acd6-1prr5-1prr7-3prr9-1</td>
</tr>
</tbody>
</table>

We visually screened changes in the mutants. Most of the phenotypic differences between the strains were suppressed by the growth conditions, but there were small differences in leaf color and shape between the genotypes.

Table 2: Initial Phenotype Results (obtained 02/07)
<table>
<thead>
<tr>
<th>Genotype Name</th>
<th>Phenotype</th>
<th>Genotype Name</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colombia-0</td>
<td>Dark green, rounded shape</td>
<td>acd6-1</td>
<td>Light green, elongated leaves</td>
</tr>
<tr>
<td>acd6-1prr5-1</td>
<td>Light green, elongated leaves</td>
<td>acd6-1prr5-1prr9-1</td>
<td>Leaves slightly less light green than acd6-1</td>
</tr>
<tr>
<td>acd6-1prr7-3</td>
<td>Light green, elongated leaves</td>
<td>acd6-1prr7-3prr9-1</td>
<td>Did not grow</td>
</tr>
<tr>
<td>acd6-1prr9-1</td>
<td>Darker, more rounded leaves</td>
<td>acd6-1prr5-1prr7-3prr9-1</td>
<td>Lighter, more rounded leaves</td>
</tr>
</tbody>
</table>

We have planted another set of mutants and will collect more quantitative phenotype data beginning 04/17. We plan to measure:
- Size
- Cell death in leaves
- Defense levels as seen by callose deposition in leaves
- SA levels
- Expression of the defense gene PR1 (by measuring the amount of its mRNA)

We also plan to measure defense levels by measuring each strain’s resistance to infection by bacterial (Pseudomonas syringae) and oomycete (Hyaloperonospora arabidopsidis) pathogens.

Discussion
If the loss of a gene suppresses the acd6-1 phenotype and exhibits a return toward wild type (Columbia-0) phenotype, then that gene positively regulates the SA-mediated defense.

Future Directions
- Ongoing: quantitatively measure mutant phenotypes
  - Are the PRR genes involved in regulating the SA-mediated immune response?
  - What pathways do they use?
- What are the downstream targets of these genes?
  - Bioinformatics analysis suggests the gene TGA3 may be connected to PRR5.
  - TGA3 is a transcription factor controlling the expression of many SA-mediated immune response genes.
  - It may be regulated by a circadian component.

References
Farre Lab website, http://farrelab.openwetware.org/Research.html

Acknowledgments
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