Plants possess innate immune system that efficiently detects microbial invasions. The primary innate immune responses are triggered by microbe associated molecular patterns (MAMPs) and play important roles in broad-spectrum defenses. The MAMPs are perceived by cell surface receptors that activate a complex cascade of reactions and expression of defense genes.

The undergraduate research project of Summer 2012 is designed to develop a high-throughout screening with model plant Arabidopsis to identify the regulators in plant immune signaling. We have obtained an EMS-mutagenized FRK1-LUC transgenic mutant population and aim to identify immune response regulatory genes named as Arabidopsis genes governing immune gene expression (Aggie). By using Pseudomonas syringae pv. tomato DC3000 hrcC, a bacteria mutant incapable of type III effectors secretion, to activate defense responses in plants, we got three mutants (aggie 5, 6 and 7) that display significantly enhanced or reduced FRK1-LUC induction upon hrcC inoculation. The three mutants were more resistant or susceptible to DC3000 infection, suggesting that the mutated genes may have important functions in plant immunity.

The aggie5 mutant exhibited an enhanced MAPK activation and FRK1 induction upon MAMP perception, indicating that the mutated genes in aggie5 may play negative roles in plant immune response. Consistently, it possessed an enhanced ROS production upon treatment with flg22. More importantly, it showed resistance to pathogenic bacterial infection.

The aggie7 mutant displayed reduced MAPK activation and FRK1 induction upon MAMP perception, suggesting that the mutated genes in aggie7 may play positive roles in plant immune response. In addition, aggie7 is strongly impaired in flg22-induced ROS burst, indicating the causal mutated gene is required for controlling plant immune responses.

The aggie6 mutant displayed a reduced MAPK activation; yet it showed a high FRK1 induction upon MAMP perception. Also, aggie6 mutant are insensitive to prolonged MAMP treatment as indicated that the seedling growth was not inhibited upon flg22 treatment. Thus it is likely that the mutated gene in aggie6 plays complicated roles in plant immune response.

In conclusion, isolation and identification of various distinct aggie mutants provide us invaluable genetic resource to further elucidate the immune signaling networks at the molecular and biochemical level and improve our ability to engineer crops with broad spectrum and durable resistance. From the genetic information retrieved from the three aggie mutants, we could genetically modify commercial crops to possibly improve their resistance and further guarantee their crop yields.

**Reference**


**Abstract**

Plants possess innate immune system that efficiently detects microbial invasions. The primary innate immune responses are triggered by microbe associated molecular patterns (MAMPs) and play important roles in broad-spectrum defenses. The MAMPs are perceived by cell surface receptors that activate a complex cascade of reactions and expression of defense genes.

The undergraduate research project of Summer 2012 is designed to develop a high-throughout screening with model plant Arabidopsis to identify the regulators in plant immune signaling. We have obtained an EMS-mutagenized FRK1-LUC transgenic mutant population and aim to identify immune response regulatory genes named as Arabidopsis genes governing immune gene expression (Aggie). By using Pseudomonas syringae pv. tomato DC3000 hrcC, a bacteria mutant incapable of type III effectors secretion, to activate defense responses in plants, we got three mutants (aggie 5, 6 and 7) that display significantly enhanced or reduced FRK1-LUC induction upon hrcC inoculation. The three mutants were more resistant or susceptible to DC3000 infection, suggesting that the mutated genes may have important functions in plant immunity.

**Results**

<table>
<thead>
<tr>
<th>FRK1-LUCRescreen (fold)</th>
<th>CoiFRK1-LUC</th>
<th>aggie5</th>
<th>aggie6</th>
<th>aggie7</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0000</td>
<td>14.3739</td>
<td>17.2678</td>
<td>0.2244</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1. aggie5 and aggie6 showed a stronger pFRK1-LUC activity after the treatment with hrcC; aggie7 showed a reduced pFRK1-LUC activity. 4-5 weeks old Arabidopsis leaves were inoculated with hrcC (OD600=0.5) and luciferase activity was measured 12 hours later. Individual leaves of each plant were put into a 96-well plate and sprayed with Luciferin substrate plus 0.02% Silwet L-77.

Figure 2. aggie6 is insensitive to flg22. After three days, seedlings grew on 1/2MS agar plates were transferred into 24-well plates with 400µl liquid 1/2MS and grew at 12h/12h light room for 10 days.

Figure 3. The MAPK activation is enhanced in aggie5, while it is reduced in aggie6 and aggie7. The MAPs were activated by 100nM flg22 for 15 min in 12-day old seedlings, and were detected by α-pFRK antibody.

Figure 4. aggie7 is strongly impaired in flg22-induced ROS burst, while aggie5 shows enhanced ROS production. Reactive oxygen species (ROS) burst in Arabidopsis leaves was triggered by flg22. Leaf discs were treated with H2O (Ctrl) or 100 nM flg22.

Figure 5. aggie5 is more resistant to Pseudomonas syringae pv. maculicola ES 4326, while aggie7 shows susceptibility. Leaves inoculated with hrcC (OD600=0.005) were hand-drilled and mixed with H2O and after serial dilutions, plate streaking was conducted and incubated at 28°C chamber. Bacterial counting was performed three days after the inoculation.