Department of Plant Pathology & Microbiology
PLPM Bioenvironmental Sciences BESC

AGRICULTURE & LIFE SCIENCES
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Genetic Dissecting Plant Innate Immune Signaling Networks

Hunting for aggies(***Arabidopsis* genes governing immune gene expressions)**

Liu. Jun[1], Cui. Fuhao[1], He. Ping[2], Shan. Libo[1]

[1]Department of Plant Pathology & Microbiology, [2]**Department of Biochemistry and Biophysics,** Texas A&M University

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

	FRK1-LUC Rescreen (fold)
Col FRK1-LUC	1.0000
aggie5	14.3739
aggie6	17.2678
aggie7	0.2244

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.

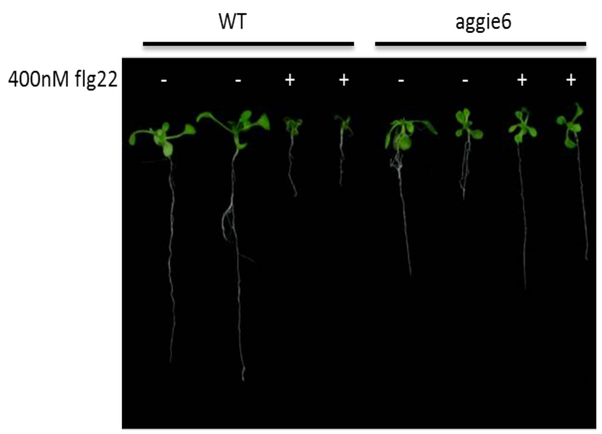

Figure 2. aggie6 is insensitive to flg22. After three days, seedlings grew on 1/2MS agar plates were transferred into 24-well plates with 400µl liquid 1/2MS and grew at 12h/12h light room for 10 days.

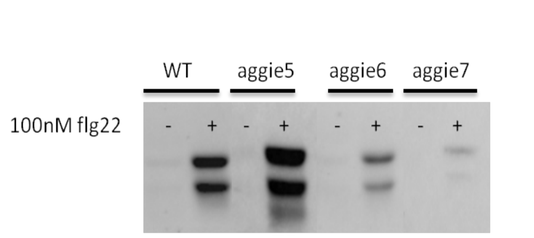

Figure 3. The MAPK activation is enhanced in aggie5, while it is reduced in aggie6 and aggie7. The MAPKs were activated by 100nM flg22 for 15 min in 12-day old seedlings, and were detected by α-pERK antibody.

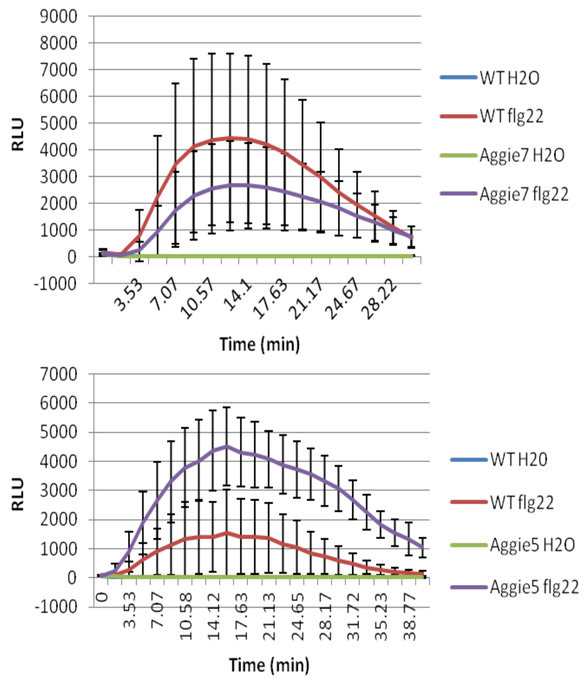

Figure 4. aggie7 is strongly impaired in flg22-induced ROS burst, while aggie5 shows enhanced ROS production . Reactive oxygen species (ROS) burst in *Arabidopsis* leaves was triggered by flg22. Leaf discs were treated with H2O (Ctrl) or 100 nM flg22.

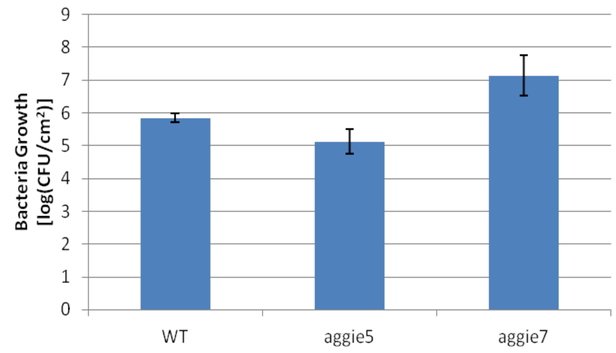

Figure 5. aggie5 is more resistant to *Pseudomonas syringae* pv. maculicola ES 4326, while aggie7 shows susceptibility. Leaves inoculated with *hrcC* (OD600=0.0005) were hand-drilled and mixed with H_2O and after serial dilutions, plate streaking was conducted and incubated at 28°C chamber. Bacterial counting was performed three days after the inoculation.

CONCLUSION AND DISCUSSION

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.

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