Apple Cultivars Use Varying Mechanisms in Response to Fire Blight Infection

Awais Khan and Jugpreet Singh
Plant Pathology and Plant-Microbe Biology Section, Cornell University, Geneva, NY, 14456, USA Khan email: mak427@cornell.edu

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Fire blight, caused by the bacterium *Erwinia amylovora*, poses a major threat to apple production in the USA and globally. Pruning infected organs, application of chemicals, and bio-control are fairly effective preventive measures, but sometimes fire blight infection can take place even with a good disease management program. Bacteria can overcome control efforts by developing antibiotic resistance or through contaminated orchard management tools and can then invade a susceptible host under favorable environmental conditions during spring and early summer, causing severe outbreaks (McManus et al., 2002; Norelli et al., 2003). The most sustainable option for fire blight control is the use of cultivars with durable resistance (Desnoues et al., 2018; Khan and Chao, 2018). Genetic resistance to fire blight has been identified in both wild and cultivated apples, which have varying responses under specific environments through complex host-pathogen interactions (Baldo et al., 2010; Emeriewen et al., 2014). These studies indicate the need for integrated genetic and molecular research in specific environmental conditions to understand the complex mechanisms associated with differential fire blight response in host plants. Fire blight bacteria release molecules required for infection directly into the host plant cells where they need to interact with disease-specific proteins for successful infection (Ancona et al., 2015; Khan et al., 2012). Apple plants activate several defenses against fire blight infection. First, bacteria must overcome different physical barriers on the plant surface including wax layers, rigid cell walls surrounding the plant cell, cuticular lipid layer, trichomes present on the leaf surface, and antimicrobial enzymes or secondary metabolites (Malnoy et al., 2012). In addition, resistant plants can effectively recognize the pathogens and actively respond using innate immune system or by triggering other defense responses to control their spread. Different cultivars grown in commercial apple orchards use diverse mechanisms to respond to fire blight infection. Therefore, understanding cultivar-specific responses can improve assessment of infection risk and severity, improving precision of forecasting models and allowing more precise preventive spray and pruning programs for better fire blight management.

"Our research has shown that Empire (fire blight tolerant) and Gala (fire blight susceptible) have different genetic responses to fire blight infection indicating they have different methods of combating the disease. The different responses to disease, from whole-plant to molecular level, have implications for disease management. A specific response mechanism may determine which methods are best for deterring or controlling disease for a particular cultivar."

Materials and Methods

The main objective of this experiment was to compare gene expression profiles of both cultivars to identify mechanisms that respond to fire blight infection. We have sequenced the RNA (Ribonucleic Acid) of two apple cultivars 'Empire' and 'Gala' apple at three timepoints after artificial infection to fire blight. RNA is an intermediate molecule derived from DNA that translates important genetic information from DNA into proteins for specific plant functions (Figure 1A). In the nucleus of animal and plant cells, a process called 'transcription' makes a single helix--the RNA from the double helix DNA. Afterwards, in a process called 'Translation', this RNA molecule makes

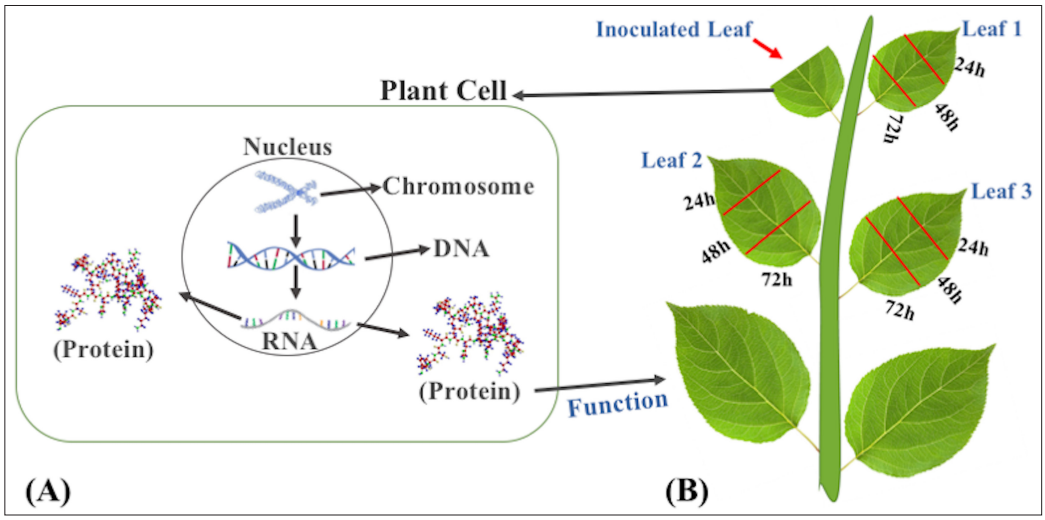

Figure 1. (A) Process of transcription of DNA into RNA and translation into proteins in the nucleus of a plant cell for specific plant functions. (B) Procedure for fire blight inoculation of 'Empire' and 'Gala' leaves with bacterial strain Ea2002A and sampling for RNA sequencing at 24, 48, and 72 hours after infection.

proteins to perform specific functions in the living organisms. Therefore, RNA sequencing is used to quantify the expression level of transcripts that will ultimately translate into proteins and will be responsible for functions. Different plants with different amounts of a specific RNA are demonstrating a difference in the way each plant responds to a disease. We have extracted RNA from leaves of both 'Empire' and 'Gala' cultivars 24, 48, and 72 hours after artificial infection with one highly aggressive fire blight bacterial strain (Figure 1B). Below are the details of the experiment.

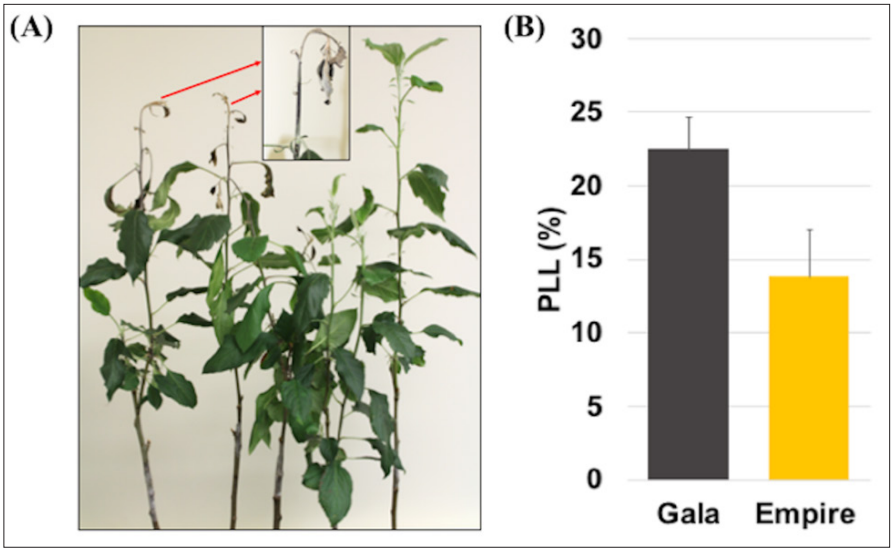

Figure 2. (A) Visual symptoms (light brown to reddish discoloration) from fire blight infection in both cultivars. The three plants on left are 'Gala' and two plants on right are 'Empire'. (B) The percentage (%) of fire blight infection for 'Gala' and 'Empire' at 3 days (72 hours) after infection.

Plant material, fire blight inoculations, and disease assessment

We grafted scion wood of 'Empire' and 'Gala' on 1/4" M.7 rootstocks. Grafted plants were grown in pots (Stuewe and Sons, Tangent, OR) using Cornell potting mix containing 50:50 ratio of vermiculite and peat moss with 6.2 kg-m-3 lime, 0.62 kg-m-3 calcium nitrate, and 1.25 kg-m-3 superphosphate at Cornell AgriTech (Geneva, New York) greenhouse. Plants were grown in pots for 6 weeks under natural light, and 24°C temperature along with approximately 50% relative humidity. Eight replicates and two controls of four-month old plants were inoculated by cutting the youngest leaf with a scissor that have been dipped either in water or the bacterial inoculum (Figure 1B). Inoculum of E. amylovora strain "Ea2002A" was prepared at a concentration of 109 CFU/ml to inoculate the plants. Ea2002A is a highly aggressive bacterial strain causing fire blight infection in many apple cultivars (Norelli et al., 1984; Momol et al., 1997; Jensen et al., 2012). It was initially isolated in 1980-81 from "Jonathan" Apples in Ontario, Canada. The fire blight infection severity was evaluated by measuring the length of necrotic lesions (cm) at 1, 2, 3, 4, 6, 9, 12, and 15 days after inoculation (dai). Percentage of fire blight infection was calculated as percent lesion length (PLL) by dividing the necrotic shoot length with the total shoot length and multiplying the output by 100.

Sample harvesting, RNA isolation, and sequencing

Three leaves below each inoculated leaf were used to sample a ~1cm leaf strip (Figure 1B). The samples were collected at 1, 2, and 3 days after inoculation from the same plants to keep uniformity across the time points. The harvested leaf samples were placed in liquid nitrogen during sampling and transferred later to -80°C until RNA extraction. Total RNA was isolated from infected and control samples using a commercial RNA extraction kit and sequenced at Genomics Facility in the Biotechnology Institute at Cornell University, Ithaca, NY, USA. Statistical analysis was conducted to evaluate the differential response of 'Empire' and 'Gala' to fire blight infection in terms of RNA expression differences between cultivars and individual timepoints, in comparison with control.

Results and Discussion

There was a clear difference in fire blight response of 'Empire' and 'Gala' at different time points post inoculation (Figure 2A). The necrotic length and percentage of fire blight infection were almost similar in 'Gala' and 'Empire' until three days post inoculation. At four days and afterwards, 'Empire' was able to suppress the bacterial infection better and showed comparatively less symptoms than 'Gala'. Shoot blight was first prominent 3 days (72 hours) after infection in both cultivars and presented similar percentage of lesion length (PLL), but at 4 dai, 'Empire' showed significantly ($p<0.05$) less PLL (3.63 ± 0.27%) than 'Gala' (6.58 ± 0.59 %). At 15 dai, the average PLL was significantly lower, 35% and 38%, in 'Empire' (Student t test p-value < 0.001) (Figure 2B), indicating 'Gala' as the more susceptible cultivar. Visual detection of light brown to reddish discoloration and bacterial ooze from the point of inoculation were also observed at 72 hpi after infection in both cultivars.

Cultivar and time-point specific gene expression differences identified potential gene classes associated with disease resistance. Differences in gene expression patterns between 'Empire' and 'Gala' were already evident at 24 hours post inoculation and proceeded until 72 hours post inoculation. These gene expression changes were more severe in 'Gala' at earlier stages of infection, but 'Empire' had more transcriptional activity at 72 hours post inoculation, suggesting that the two cultivars have different mechanisms in response to fire blight infection. These responses can lead to either resistance or susceptibility, but in this experiment, we only looked into two examples. Other apple cultivars may have a broad range of responses to infection, and if we chose two other cultivars, they could have completely different responses; several of which could lead to resistance, and others could make the plant susceptible.

The differences in necrotic lesion length after 72 hpi are likely due to earlier active processes, although later gene expression changes might also contribute to levels of disease susceptibility in two apple cultivars. Overall, the broad level of gene activation of both general and disease-specific pathways and metabolic processes illustrates the constraints that bacterial infection puts on normal functioning of the plant (Figure 3). For example, the gene pathways related to plant growth (cell

cycle), metabolism and plant cellular skeleton were suppressed, whereas genes in defense, stress response and biotic stimulus pathways show induction upon fire blight infection (Figure 3). These patterns indicate the presence of a distinct molecular response of 'Empire' and 'Gala' to fire blight infection. 'Empire' and 'Gala' also have distinct pedigrees. 'Empire', a sweet-tart apple cultivar, was developed at Cornell University in the 1940s by crossing 'McIntosh' and 'Red Delicious', whereas 'Gala', mild flavor bright yellow-red color, was developed in New Zealand by crossing 'Kidd's Orange Red' and 'Golden Delicious' (Evans et al., 2011). These different parentages might have contributed to their differential responses to fire blight. There can be many roads to resistance, and to susceptibility as well, which is a major difficulty in determining disease resistance through genetic testing alone.

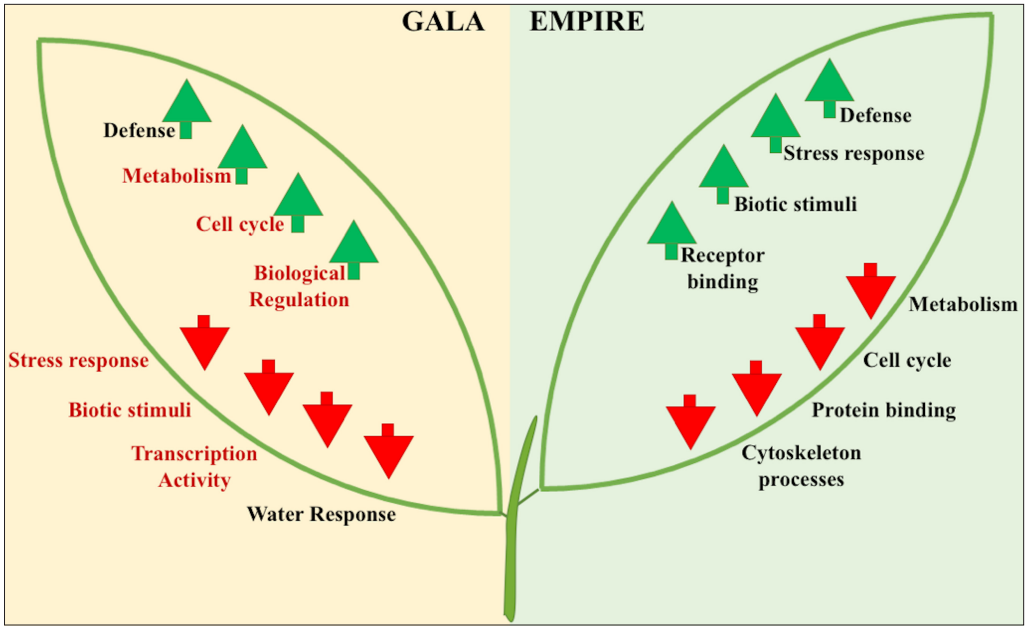

Figure 3. Up-regulated and down-regulated gene pathways in 'Gala' and 'Empire' at 3 days (72 hours) after infection. The up- and down-regulated pathways with red font in 'Gala' showed reversing trends as the disease progresses from 24 to 72 hours after fire blight infection, i.e. the down-regulated pathways at 24 hours in Gala showed up-regulation at 72 hours and vice-versa.

The different responses to disease, from whole-plant to molecular level, have implications for disease management. A specific response mechanism may determine which methods are best for deterring or controlling disease for a particular cultivar. With more research, information on the varying responses could be incorporated into improved risk assessment models, which traditionally use weather data and previous history of the presence of inoculum. For example, a threshold for the amount of bacteria present could trigger different responses for different cultivars, so if we know how much bacteria are present, a lower or higher risk of infection could be calculated for a particular cultivar and an entire orchard. It is also possible that varying nutritional profiles of apple cultivars can restrict or induce bacterial growth at certain physiological stages. Overall, these results and further studies will increase the understanding of interaction between fire blight pathogen and apples, and will allow improvements in durable and sustainable disease control.

Acknowledgements

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Awais Khan is an Associate Professor of Plant Pathology at Cornell AgriTech in Geneva, NY where his research and extension program focuses on mechanisms of disease resistance and disease management through host resistance in fruit crops. **Jugpreet Singh** is a postdoctoral research associate who works with Dr. Khan.

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