5-1-2015

ASSESSING THE ROLE OF GENE AT4G29000 IN STOMATAL IMMUNITY IN ARABIDOPSIS THALIANA

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ASSESSING THE ROLE OF GENE AT4G29000 IN STOMATAL IMMUNITY IN *ARABIDOPSIS THALIANA*

by

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Presented to the Faculty of the Honors College of

The University of Texas at Arlington in Partial Fulfillment

of the Requirements

for the Degree of

HONORS BACHELOR OF SCIENCE IN BIOLOGY

THE UNIVERSITY OF TEXAS AT ARLINGTON

May 2015

ACKNOWLEDGMENTS

I owe a great deal of gratitude to my mentor Dr. Maeli Melotto for encouraging me to be independent and efficient in my research. From this opportunity to conduct research under her guidance, I take away a wealth of knowledge, team spirit and a constant drive of self-improvement. Over the years, I've learned to keep working hard steadily and learn as much as I can moving along the way.

I would also like to thank all the members of the Melotto lab team (Nisita Obulareddy, Shweta Panchal, Debanjana Roy, Anh Le, Samantha Trinh, Davina Sassoon, Jennifer La, Karen Truong, Mark Torres, Paula Oblessuc and Cleverson Matiolli) for their constant support and for helping me transition into the research atmosphere in the lab. It's from them that I learned hands on research. I've been really lucky to have been part of this hardworking and committed team of researchers.

Finally, I would like to thank and commend the Honors College at UTA for supporting and encouraging students to actively pursue research. My research in the Melotto lab has been a great learning experience that has laid the foundation for my future career goals.

May 15, 2015

ABSTRACT

ASSESSING THE ROLE OF GENE AT4G29000 IN STOMATAL IMMUNITY IN *ARABIDOPSIS THALIANA*

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The University of Texas at Arlington, 2015

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Stomata are pores present on the surface of plants and are primarily involved in transpiration. However, due to their abundance and their location on the surface of leaves, they become potential sites of entry for pathogens introduced on the plant surface. Stomata however, possess the innate ability to open and close in response to a pathogen. They can do this by recognizing molecules called pathogen associated molecular proteins (PAMPs) present on the surface of the pathogens. This constitutes the first line of defense for the plant. My research is focused on examining the role of the Tesmin/TSO1-like protein in stomatal immunity. The gene responsible for the production of this protein in *Arabidopsis thaliana* (Arabidopsis) is At4g29000. Homozygous T-DNA insertion mutant was used for experimentation. Thermal imaging, which is used to monitor stomatal opening and closing, has shown that stomatal pores in this mutant have small aperture width as indicated by a hotter leaf surface temperature two and four hours post

inoculation with *Pseudomonas syringae pv. tomato* DC3118 and DC3000 (*Pst* DC3118 and *Pst* DC3000). The mutant has also shown significantly lower levels of infection when inoculated with *Pst* DC3118 and *Pst* DC3000. Further research and confirmation of the role of the Tesmin/TSO1-like protein in stomatal immunity can have potential applications in developing plant lines that have enhanced stomatal immunity, thus, providing opportunities for employing additional control measures to decrease the impact of plant diseases on food production.

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CHAPTER 1

INTRODUCTION

Stomata are pores present on the surface of plants. The stomata are surrounded by guard cells that regulate the opening and closing of the stomata through osmosis. Stomata are primarily involved in transpiration and the exchange of gases. However, due to their abundance and their location on the surface of leaves, they become potential sites of entry for pathogens that are introduced on the surface of the plant. Arabidopsis is a model plant that we use for our research. Research has shown that stomata possess the innate ability to open and close in response to a pathogen. They can do this by recognizing molecules called pathogen associated molecular proteins (PAMPs) that are present on the surface of the pathogens. This constitutes the first line of defense for the plant (Melotto *et al.* 2006).

Arabidopsis thaliana-Pseudomonas syringae interaction is a model system I use to study host-pathogen interaction among plants. *Pst* DC3000 and *Pst* DC3118 are the two strains of *P. syringae* that were used for this research. *Pst* DC3000 has very clear physical symptoms of infection on Arabidopsis. Hence, these two strains are widely used to study the comparative effects of infection on Arabidopsis. *Pst* DC3000 produces coronatine. Coronatine is a toxin which induces chlorosis in leaves (Benedetti *et al*. 1998). It also has the ability to reopen stomata after the initial encounter of the plant with the pathogen (Melotto *et al.* 2006). *Pst* DC3118 which was created from *Pst* DC3000 (Ma *et al.* 1991), lacks the ability to produce coronatine and as a result, cannot reopen the

stomata. Hence, *Pst* DC3000 is more virulent compared to *Pst* DC3118 (Katagiri *et al.* 2002). It is thus necessary to consider both these factors while examining the resistance or susceptibility of a mutant, and hence, both these bacterial strains were for this research.

This research is focused on examining the role of the gene At4g29000 in Arabidopsis by using a mutant plant (SALK_020712C) line that contains T-DNA insertion which prevents the expression of this gene. This mutant was selected for intense experimentation based on the previous screening done with over two thousand confirmed homozygous T-DNA insertion mutant lines of Arabidopsis available in our lab. Previous screenings have indicated that this mutant has relatively more closed stomata compared to the control after infection (M. Melotto, Unpublished).

The gene At4g29000 is involved in the regulation of transcription. It is expressed in guard cells among other places in the plant. It codes for a Tesmin/TSO1-like CXC domain containing protein. Homozygous mutants for this gene have a mutation in a conserved cysteine-rich domain (Andersen *et al*. 2007). This gene has been shown to play a role in male and female fertility in Arabidopsis. This cysteine-rich domain binds to zinc which correlates to its affecting the cysteine residues. The preliminary mutant screening process done in our lab has shown that the mutant also shows sensitivity to bacterial inoculation which triggers stomatal closure (M. Melotto, Unpublished).

Understanding and identifying the level of stomatal immunity in Arabidopsis mutants can be very instrumental in improving our knowledge about plant-microbe interaction. Thus, this research will probably help to reduce the amount of chemical

control measures used in food production currently by harnessing the plant's innate immunity to defend itself from pathogens.

CHAPTER 2

METHODOLOGY

2.1 Overview

The goal of this project is to assess, measure and replicate the stomatal response to bacteria in Arabidopsis mutant plants having a T-DNA insertion in the At4g29000 gene (SALK_020712C). Only homozygous plant lines were used for experimentation (TAIR). *Pst* DC3118 and *Pst* DC3000 were the two strains of bacteria used. Three different methods were used to assess the stomatal response to the bacteria. Thermal imaging was the preliminary method to check for comparative levels of stomatal closure/opening in response to bacteria being introduced on the surface, compared to the control (Col-0). Col-0 is the wildtype line of Arabidopsis. The surface temperature and level of stomatal closure/opening were correlated in this method.

Another method used to check for stomatal response was the bacterial enumeration in the leaf tissue. The level of bacteria growing in the leaf apoplast was correlated with the level of stomatal closure/ opening in this method.

Finally, the most direct method used to check for stomatal response was a stomatal assay, in which, a microscope was used to take pictures of the stomata. The size of the stomatal aperture was measured to give a direct quantification of the level of stomatal response to the bacteria.

2.2 Growing Plants

Plants were grown in 5cm X 5cm plastic pots. All seeds were suspended in a 0.1% agarose solution and pipetted onto five spots on the pots filled to the brim with a soil mixture (1:1:1 mix of vermiculite, perlite and potting soil), topped with vermiculite and covered with a mesh. Two pots containing Col-0 plants and two pots containing SALK_020712C plants were sown for each batch. Each replicate consisted of two batches of plants: one inoculated using *Pst* DC3000 and one inoculated using *Pst* DC3118. The overall experiment consisted of three such biological replicates.

Immediately after sowing seeds, the pots were transferred to a growth chamber at 4°C for 48 hours for cold treatment in order to help facilitate the germination process. The plants were grown at 21°C under a 12 hour light/ dark cycle with 59% relative humidity and a photosynthetic photon flux of 79 μmol. m^{-2} .s^{-1}. The plants were used for inoculation when they were 4-5 weeks old. Twenty four hours prior to inoculation and stomatal assay, the plants are moved to 25°C and under a 12 hour light/ dark cycle and 70% relative humidity, along with the same light intensity.

2.3 Bacterial Inoculation

For bacterial inoculation, two different bacteria: *Pst* DC3000 and *Pst* DC3118 were used and two different methods of inoculation were performed for each of the bacteria: vacuum infiltration and dipping. Within each batch of plants, one set (Col-0 and Mutant) was inoculated by vacuum infiltration and another by dipping. Only one of the bacteria were used per batch. Each of the inoculation with one bacteria was repeated three times.

For dipping, the inoculum consists of a single bacterial colony of bacteria transferred into Luria-Bertrani (LB) medium mixed with the appropriate antibiotic. For *Pst* DC3118, kanamycin (concentration of 50 µg/mL) and rifampicin (concentration of 100 µg/mL) were the antibiotics that were used. For *Pst* DC3000, only rifampicin (concentration of 100 µg/mL) was used. The optical density (OD_{600}) for the inoculum was normalized using spectrophotometry.

For dip inoculation, plant rosettes (one Col-0 plant and one SALK_020712C plant) were dipped in a bacterial culture of OD_{600} 0.2 along with 0.03% Silwet L-77 used as a surfactant.

For vacuum infiltration, plant rosettes (one Col-0 plant and one SALK 020712C plant) were infiltrated with a bacterial culture of OD_{600} 0.002 and 0.008% Silwet L-77.

2.4 Thermal Imaging and Symptoms Recording

Thermal imaging was conducted using Flir system T300 camera. Images were taken for all of the inoculated plants two and four hours post inoculation. The pictures were compared and analyzed using the Flir Quickreport ™ software. SALK_020712C plants were compared with Col-0 plants after stabilizing and equalizing the temperature range. The relatively higher or lower leaf surface temperature of the mutant plants was noted.

Normal images were taken for every plant seven days post inoculation to check for visual symptoms of infection in the form of chlorosis and/or necrosis typically produced by *P. syringae* in Arabidopsis plants.

2.5 Bacterial Plating

Three inoculated leaves were picked from each plant and the leaves were sterilized in 70% ethanol for three minutes followed by cleaning them in sterile water for one minute. Four circular sections were cut out of the leaf using a leaf borer (#2). The sections were transferred to a microfuge tube containing sterile water and crushed using a pestle. The suspension was serially diluted up to 10^{-6} and each of the dilution was plated on square LB agar plates containing appropriate antibiotics. For *Pst* DC3118, kanamycin (concentration of 50 µg/mL) and rifampicin (concentration of 100 µg/mL) were the antibiotics used. For *Pst* DC3000, only rifampicin (concentration of 100 µg/mL) was used (Katagiri *et al.* 2002).

The plates were then inoculated at 30°C for 24 hours and then stored at 4°C for 24-48 hours until the bacterial colonies are big enough to be counted.

The colonies were then counted under a dissection microscope and the data was recorded in order to conduct a two tailed student's t-test to measure the level of significance correlating to either immunity or susceptibility of the mutant plant compared to the control plant.

2.6 Stomatal Assay

Stomatal assay is a direct method of measuring the size of the stomatal aperture (Chitrakar and Melotto 2010). For the overall experiment, three replicates were conducted for using *Pst* DC3118 and *Pst* DC3000 respectively, adding to a total of six batches of inoculation. For each inoculation, a bacterial inoculum with an OD_{600} of 0.2 was used without a surfactant. Leaves floated in water were used as a control to the

leaves floated in the inoculum. Two sets comprising of three leaves each were collected from both plants: Col-0 and SALK_020712C. One batch from each plant was floated in autoclaved DI water and the other batch was floated in the inoculum. Using fluorescent microscopy, pictures were taken of various sections on all the leaves two and four hours post inoculation, and a minimum of sixty stomatal apertures were measured for each set.

The measurements from the stomatal apertures were then used to conduct statistical analysis.

2.7 Statistical Analysis

The data collected from the different methodologies were subjected to different statistical analysis. The bacterial colony counts obtained from bacterial plating were used to conduct a two tailed student's t-test using the averages and by calculating the standard error (SE) and standard deviation (SD). In this case, the bacterial growth in Col-0 was compared to that in SALK_020712C. If the P-value obtained from the student's t-test was less than 0.05, then the difference was considered significant.

A similar student's t-test was conducted on the data obtained from stomatal assay. The stomatal aperture size for mock inoculation of Col-0 and SALK_020712C was compared with the stomatal aperture size for the respective plants inoculated with bacteria. The averages, standard deviation (SD) and standard error (SE) were calculated for the data as well. If the P-value obtained from the student's t-test was less than 0.05, then the difference was considered significant.

Finally, all the statistical analysis along with the both thermal and regular pictures were compared to correlate the level of stomatal opening/ closure to the level of infection.

CHAPTER 3

RESULTS

3.1 Thermal Imaging

In order to accurately understand the effect of different conditions on the leaf surface temperature, different comparisons have to be made for categories based on the type of bacteria used, the type of inoculation method used and the duration after inoculation at which the images were taken. In the following figures, different factors mentioned above have been compared. While analyzing the thermal pictures, a normal picture of an Arabidopsis rosette was used as a reference (Figure 1).

SALK_020712C plants dip inoculated with *Pst* DC3000 show higher leaf surface temperature two hours post inoculation (Figure 2), but, no difference was observed four hours post inoculation (Figure 3). SALK_020712C plants inoculated with *Pst* DC3000 using vacuum infiltration, showed no differences observed two hours post inoculation (Figure 4), however, the mutant displayed a higher leaf surface temperature compared to Col-0 four hours post inoculation (Figure 5). SALK_020712C plants showed higher temperature two and four hours post dip inoculation with *Pst* DC3118 (Figures 6 & 7). The mutant showed higher leaf surface temperature two hours post vacuum infiltration with *Pst* DC3118 (Figure 8), however, no differences were observed four hours post inoculation (Figure 9).

Figure 3.1: A normal image showing the structure of an Arabidopsis rosette. This image can be used as a reference while observing the thermal images that follow. All thermal images were taken with a focus on the central rosette in the pot.

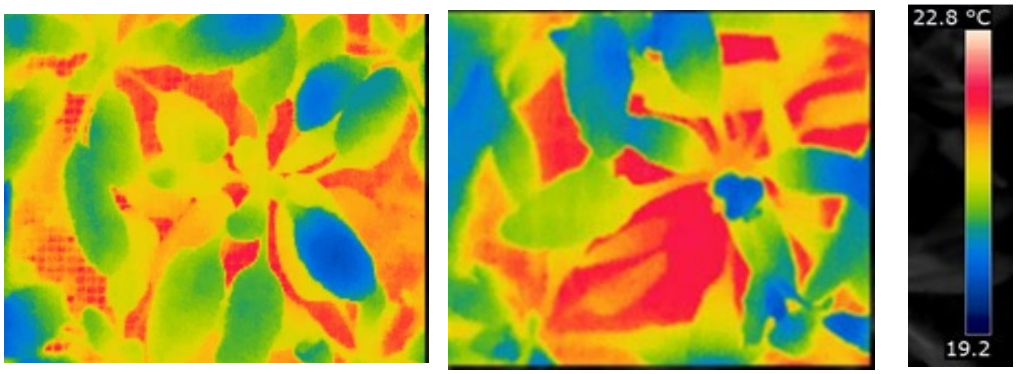

Figure 3.2: Thermal images showing Col-0 (Left) and SALK_020712C (Right) two hours post inoculation with *Pst* DC3000 using dip inoculation.

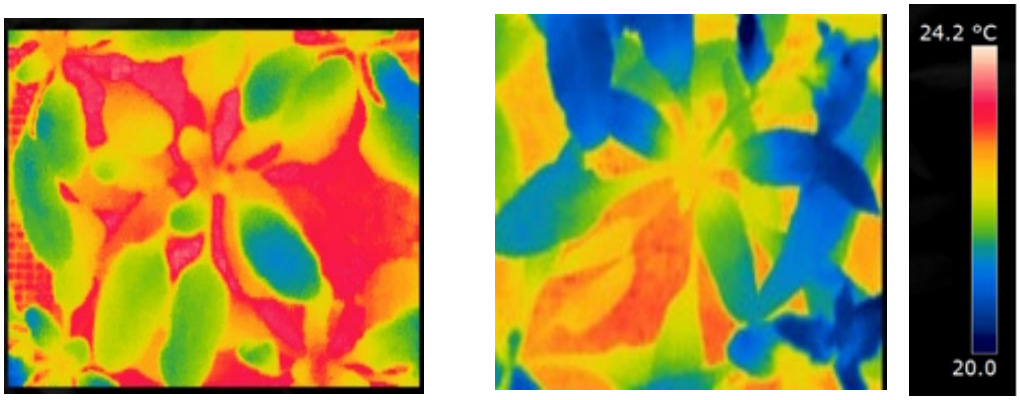

Figure 3.3: Thermal images showing Col-0 (Left) and SALK_020712C (Right) four hours post inoculation with *Pst* DC3000 using dip inoculation.

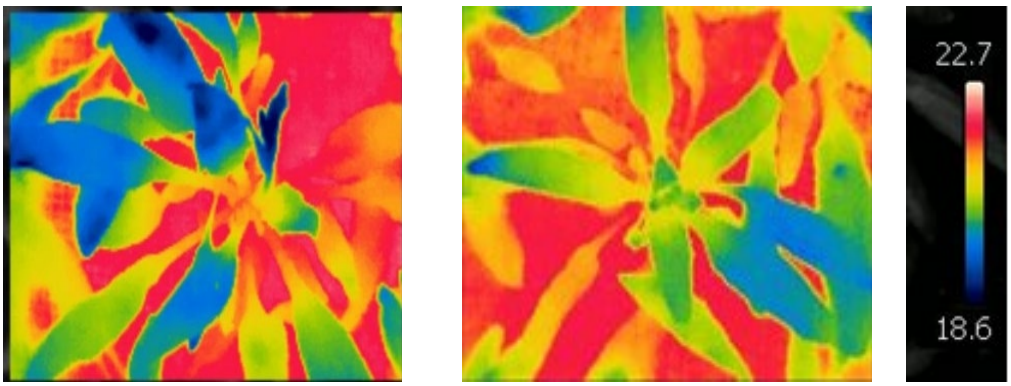

Figure 3.4: Thermal images showing Col-0 (Left) and SALK_020712C (Right) two hours post inoculation with *Pst* DC3000 using vacuum infiltration.

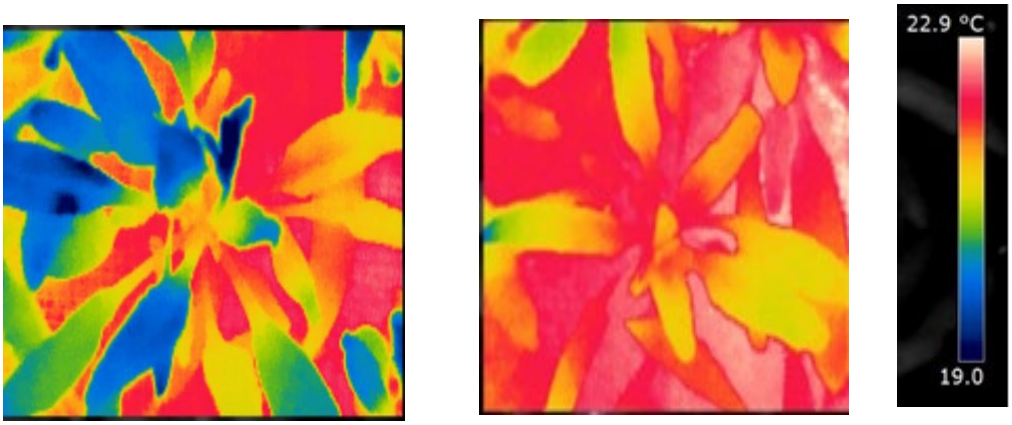

Figure 3.5: Thermal images showing Col-0 (Left) and SALK_020712C (Right) four hours post inoculation with *Pst* DC3000 using vacuum infiltration.

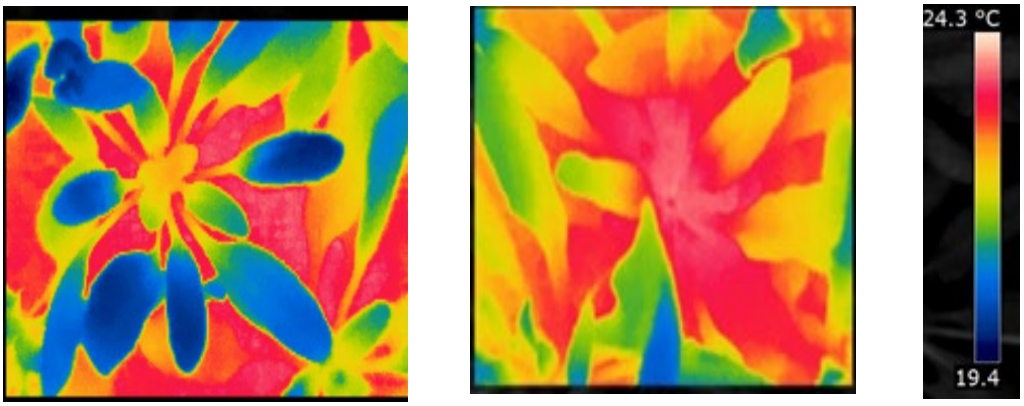

Figure 3.6: Thermal images showing Col-0 (Left) and SALK_020712C (Right) two hours post inoculation with *Pst* DC3118 using dip inoculation.

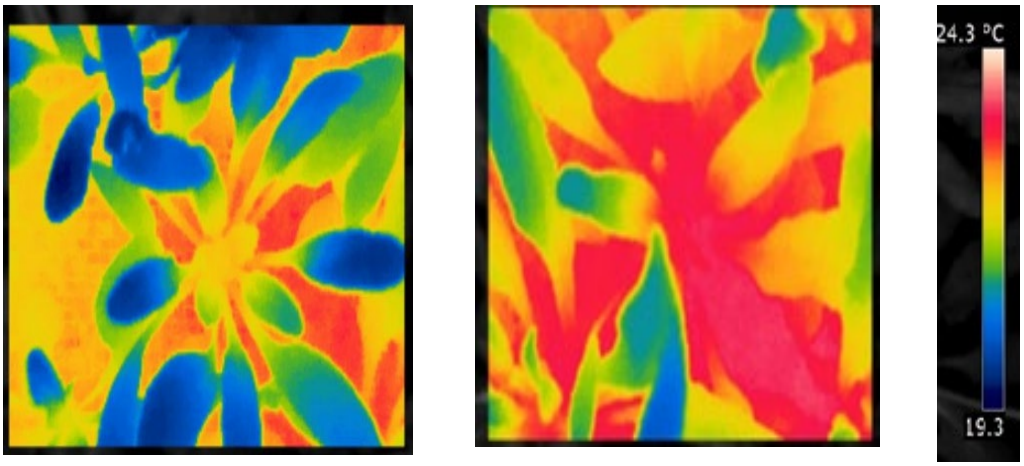

Figure 3.7: Thermal images showing Col-0 (Left) and SALK_020712C (Right) four hours post inoculation with *Pst* DC3118 using dip inoculation.

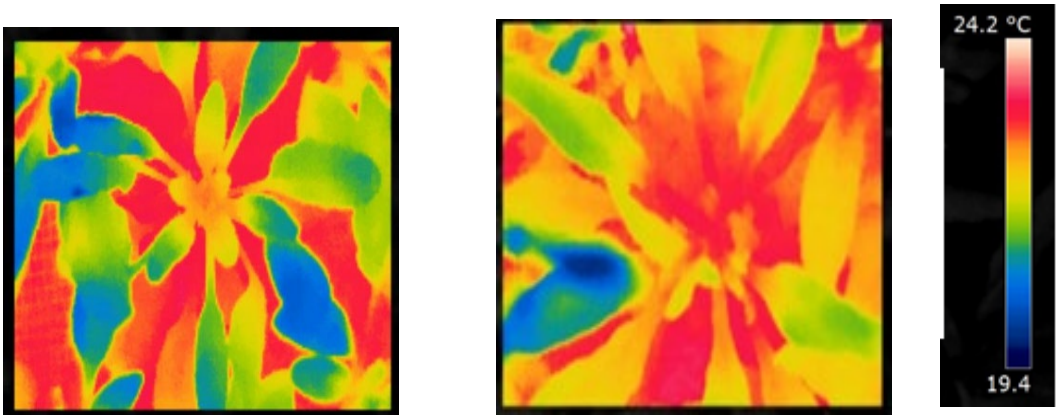

Figure 3.8: Thermal images showing Col-0 (Left) and SALK_020712C (Right) two hours post inoculation with *Pst* DC3118 using vacuum infiltration.

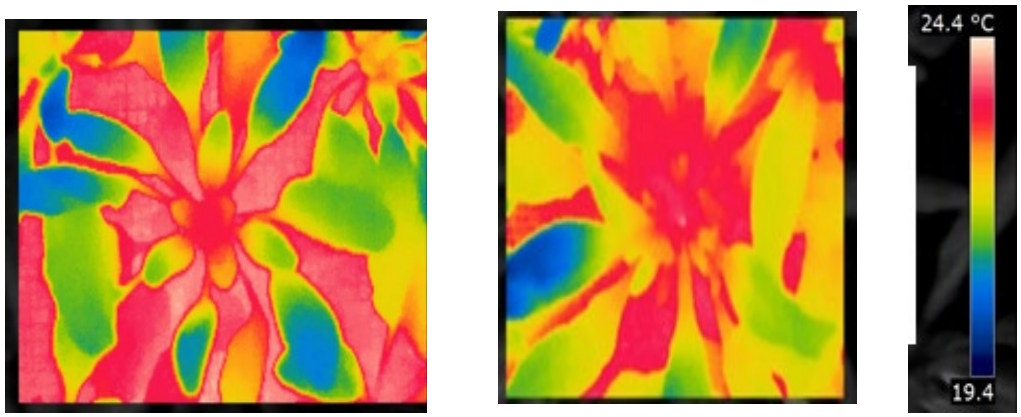

Figure 3.9: Thermal images showing Col-0 (Left) and SALK_020712C (Right) four hours post inoculation with *Pst* DC3118 using vacuum infiltration.

3.2 Bacterial Plating

The results obtained from bacterial plating have been divided into two different categories based on which bacteria was used for inoculation. Each of the graph is then subdivided within by categories based on when the samples were plated.

For inoculation with *Pst* DC3000, SALK_0200712C plants showed significantly lower bacterial growth in the apoplast one and three days post inoculation for both dip inoculation and vacuum infiltration (Figure 9). Very similar results were obtained for inoculation with Pst DC3118 (Figure 10). In the inoculation with both bacteria, vacuum infiltrated plants show greater bacterial counts than dip inoculated plants. Compared to Col-0, both vacuum infiltrated and dip inoculated plants show lower bacterial counts. Table 1 shows the average bacterial colony counts, standard deviation and standard error that were used to plot the graphs in Figures 9 & 10.

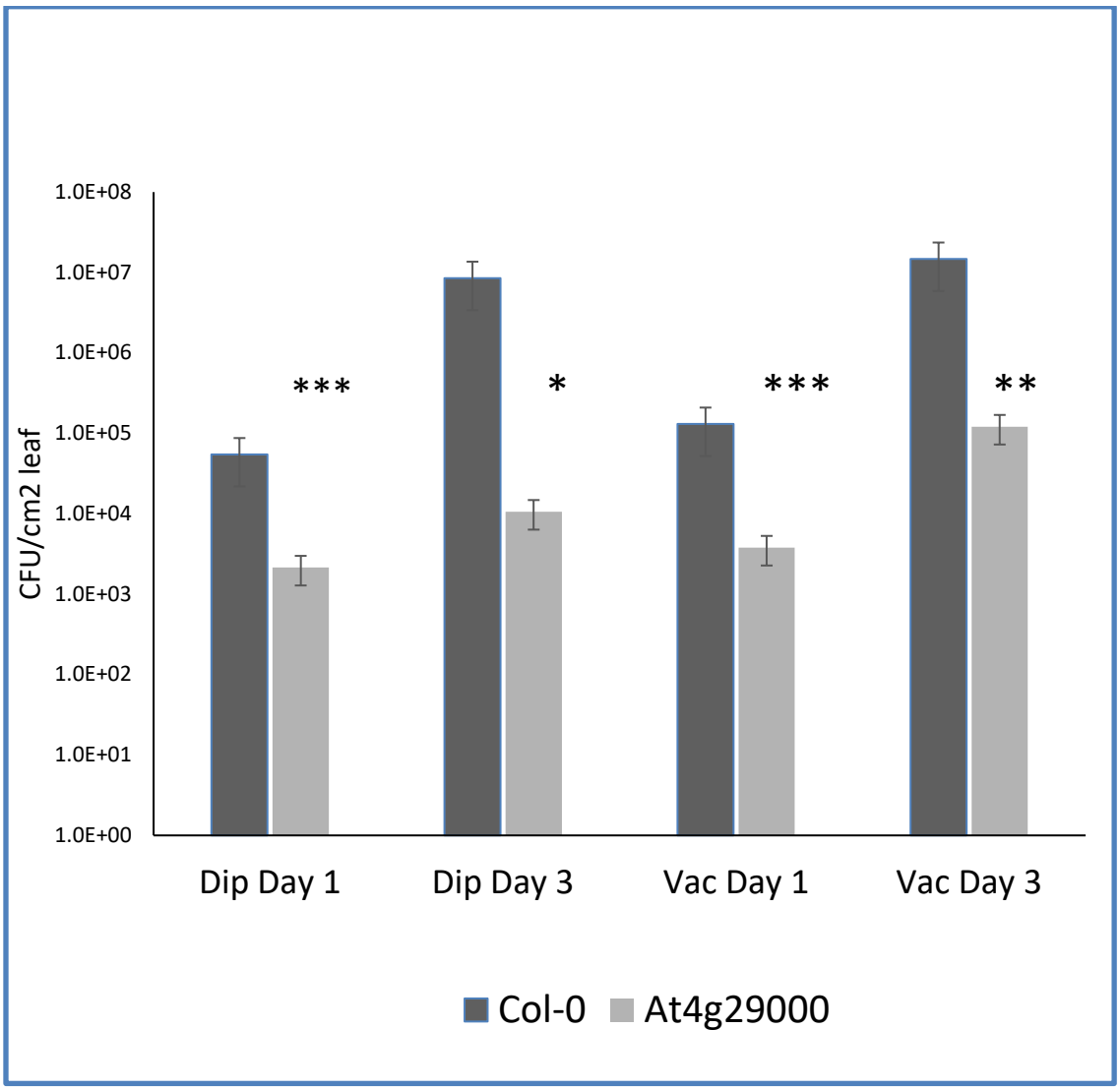

Figure 3.10: Bacterial population in the apoplast of infected plants assessed one and three days post inoculation. A student's t-test was conducted between Col-0 and SALK_020712C plants separately for each timepoint. The graphs show the average bacterial counts for Col-0 and SALK_020712C plants inoculated using dip inoculation and vacuum infiltration with *Pst* DC3000. (*) indicates a P-value less than 0.05, (**) indicates a P-value less than 0.01 and (***) indicates a P-value less than 0.001.

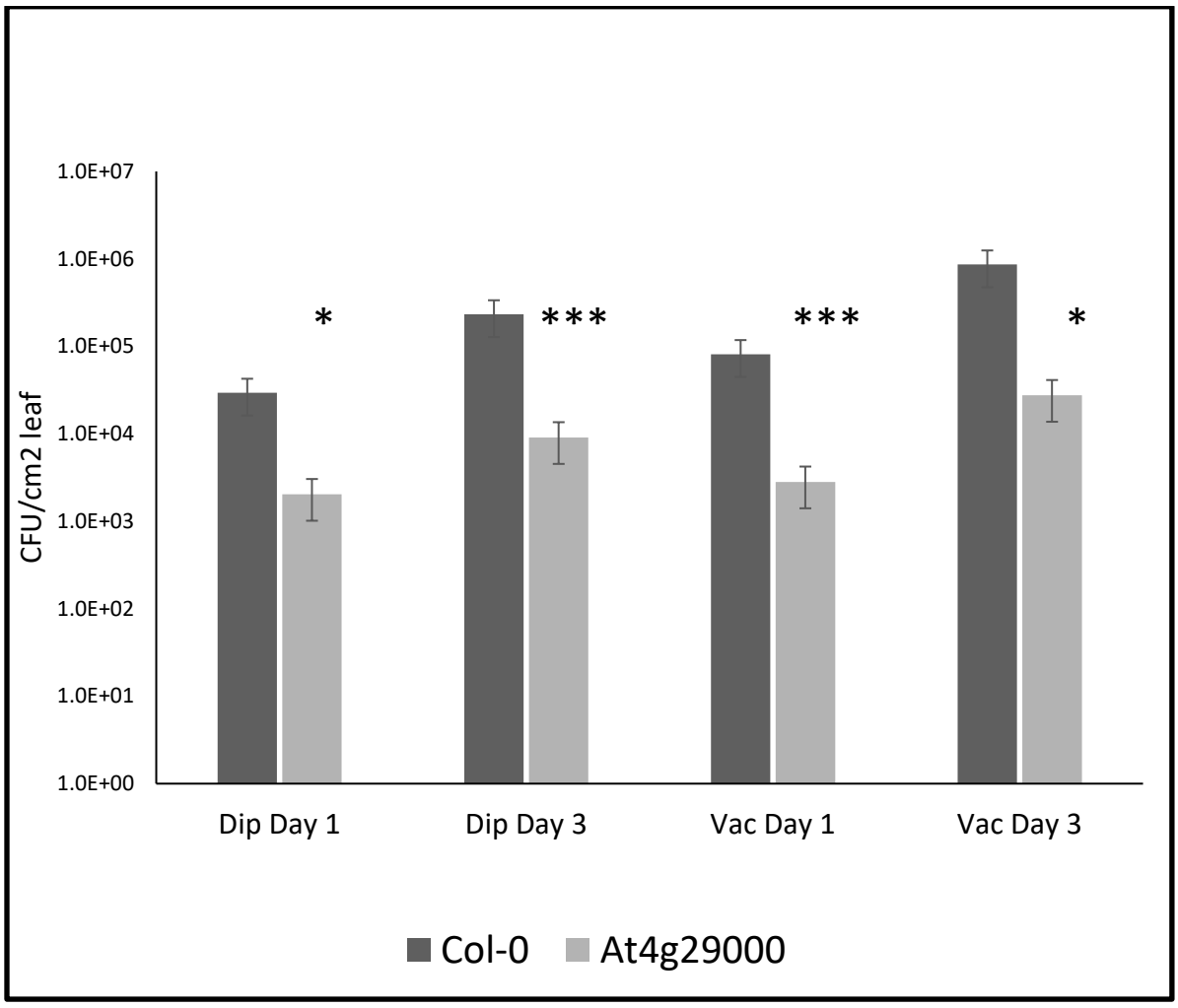

Figure 3.11: Bacterial population in the apoplast of infected plants assessed one and three days post inoculation. A student's t-test was conducted between Col-0 and SALK_020712C plants separately for each timepoint. The graphs show the average bacterial counts for Col-0 and SALK_020712C plants inoculated using dip inoculation and vacuum infiltration with *Pst* DC3118. (*) indicates a P-value less than 0.05, (**) indicates a P-value less than 0.01 and (***) indicates a P-value less than 0.001

Table 3.1: Table displaying the average bacterial counts, standard deviation and standard error for Col-0 and SALK_020712C inoculated with *Pst* DC3118 and *Pst* DC3000 with both using dip inoculation and vacuum infiltration one and three days post inoculation.

Plant line	Bacteria	Method of Inoculation	Average Bacterial Enumeration (CFU/cm^2)		Std. Deviation. (SD)		Std. Error (SE)	
			1 DPI	3 DPI	1 DPI	3 DPI	1DPI	3DPI
Col-0	*Pst* DC3000	Dip	$5.5x10^4$	$8.5x10^6$	$3.82x10^4$	$1.2x10^7$	$1.1x10^4$	$3.5x10^6$
SALK_020712C	*Pst* DC3000	Dip	$2.1x10^3$	$1.1x10^4$	$1.61x10^3$	$1.22x10^4$	$4.6x10^2$	$3.5x10^3$
Col-0	*Pst* DC3000	Vac. Infil.	$1.3x10^5$	$1.5x10^7$	$8.2x10^4$	$1.53x10^7$	$2.4x10^4$	$4.4x10^6$
SALK_020712C	*Pst* DC3000	Vac. Infil.	$3.8x10^3$	$1.2x10^5$	$1.5x10^3$	$1.32x10^5$	$4.3x10^2$	$3.8x10^4$
Col-0	*Pst* DC3118	Dip	$2.9x10^4$	$2.3x10^5$	$3.13x10^4$	$1.08x10^5$	$9.0x10^3$	$3.1x10^4$
SALK_020712C	*Pst* DC3118	Dip	$2.0x10^3$	$9.1x10^3$	$1.33x10^3$	$1.26x10^4$	$3.8x10^2$	$3.6x10^3$
Col-0	*Pst* DC3118	Vac. Infil.	$8.1x10^4$	$8.6x10^5$	$3.63x10^4$	$1.37x10^6$	$1.0x10^4$	$4.0x10^5$
SALK_020712C	*Pst* DC3118	Vac. Infil.	$2.8x10^3$	$2.7x10^4$	$2.95x10^3$	$8.5x10^2$	$1.79x10^4$	$5.2x10^3$

3.3 Stomatal Assay

The results obtained from stomatal assay have been primarily divided on which bacteria was used for inoculation. Within each graph, the data has been divided into categories based on the when the stomatal assay was done post inoculation and on whether inoculation was done using bacterial inoculum or water.

The results obtained from the stomatal assay conducted after inoculation with *Pst* DC3000 show that SALK_020712C plants show significantly smaller stomatal aperture size compared to Col-0 two and four hours post inoculation with both water and bacteria (Figure 12). For inoculation with *Pst* DC3118, no significant difference was observed between the sizes of the stomatal aperture of SALK_020712C compared to Col-0. However, it must also be noted that in this case, SALK_020712C plants did not show a similar trend in when the leaves were inoculated with water (Figure 13). Table 2 shows the average size of the stomatal aperture, standard deviation and standard error used to plot the graphs in Figures 12 & 13.

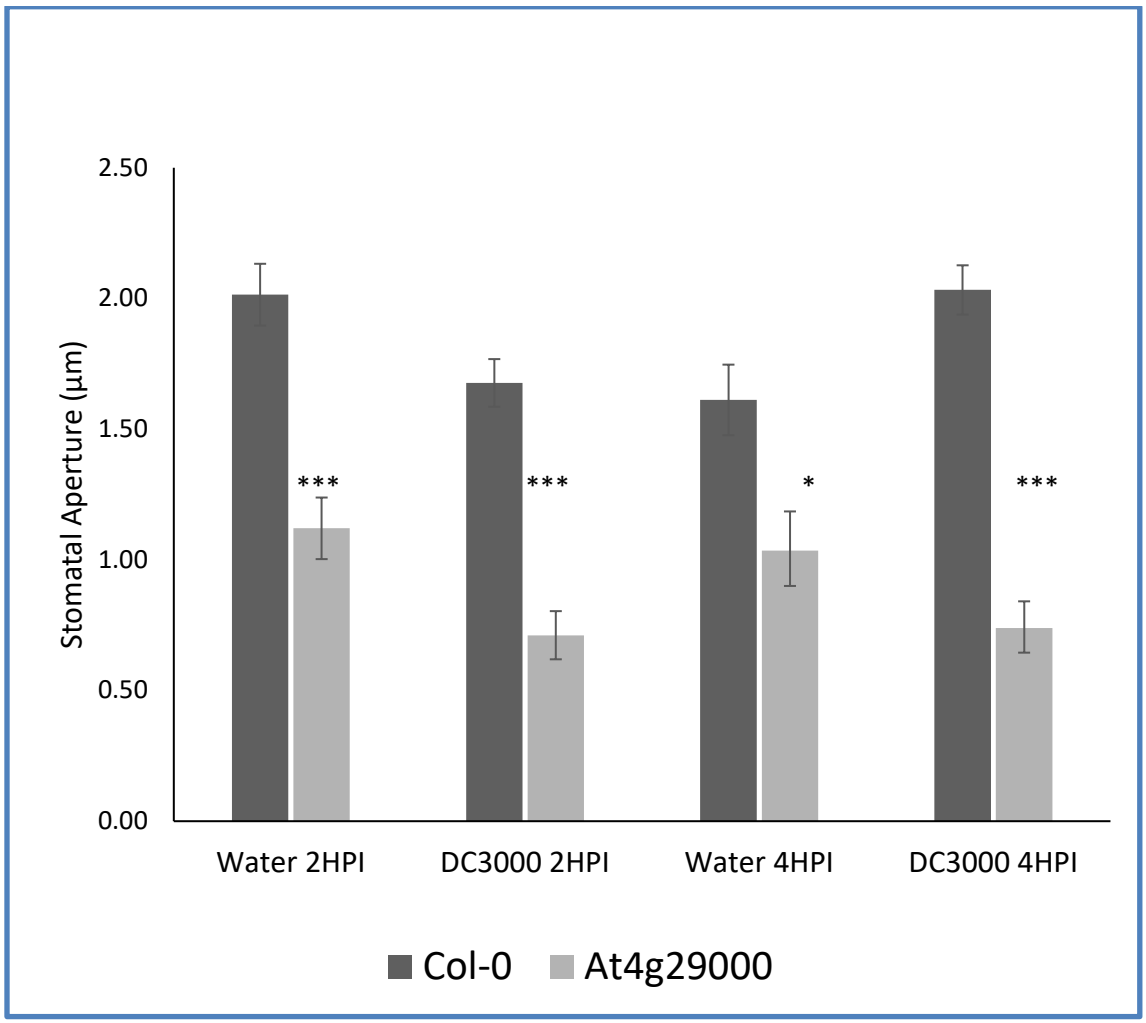

Figure 3.12: Stomatal assay conducted on Col-0 and SALK_020712C plants with water (Mock) and *Pst* DC3000 and *Pst* DC3118 at 2HPI and 4HPI. A student's t-test was conducted between Col-0 and SALK_020712C at each time point. The graph shows the average length of stomatal aperture for plants inoculated with water and *Pst* DC3000. (*) indicates a P-value less than 0.05, (**) indicates a P-value less than 0.01 and (***) indicates a P-value less than 0.001.

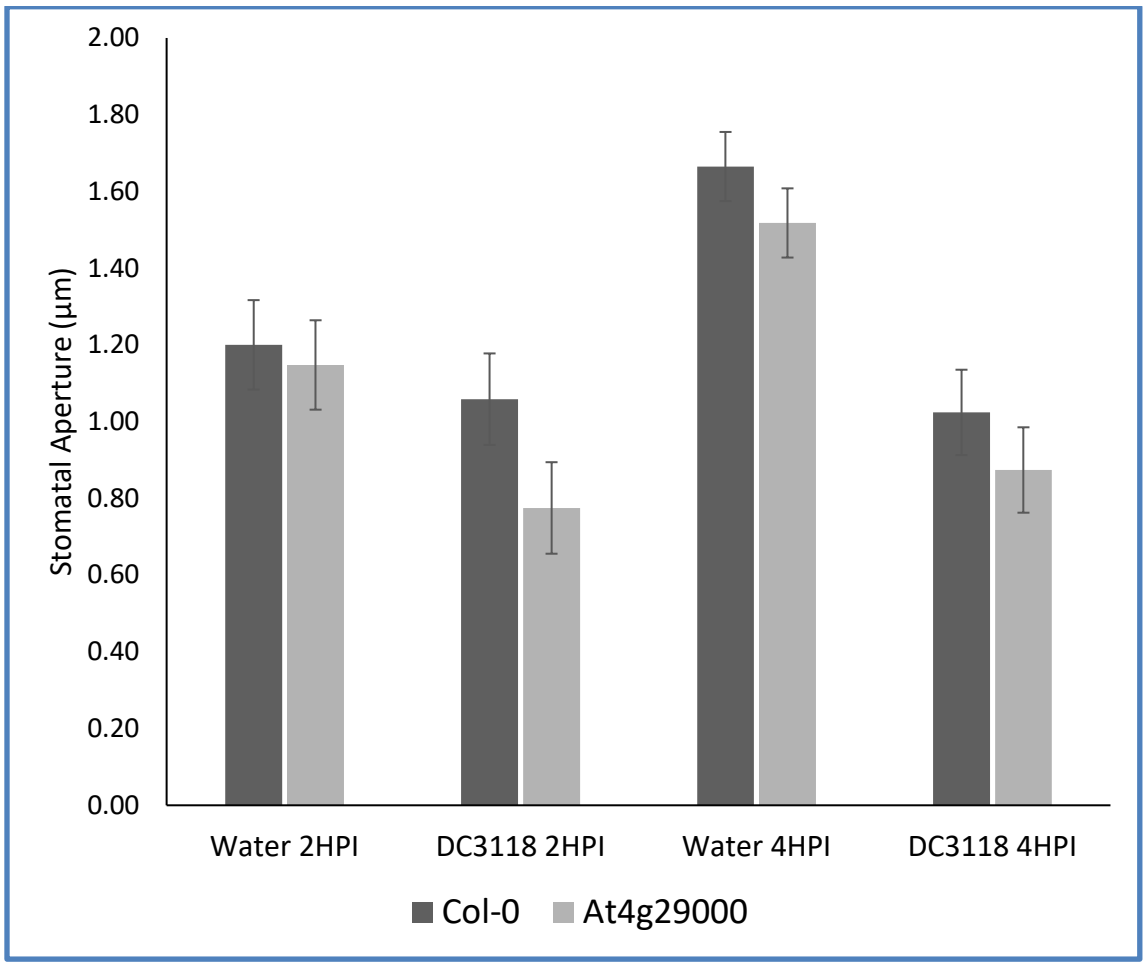

Figure 3.13: Stomatal assay conducted on Col-0 and SALK_020712C plants with water (Mock) and *Pst* DC3000 and *Pst* DC3118 at 2HPI and 4HPI. A student's t-test was conducted between Col-0 and SALK_020712C at each time point. The graph shows the average length of stomatal aperture for plants inoculated with water and *Pst* DC3118.

Table 3.2: Table showing the averages and standard deviation for the stomatal aperture size for Col-0 and SALK_020712C inoculated with both water and bacteria (*Pst* DC3118 and *Pst* DC3000)

Plant Line	Inoculation Type	Average Stomatal Aperture Size (µm)		Std. Deviation (SD) (µm)	
		2HPI	4HPI	2HPI	4HPI
Col-0	Mock(Water)	2.01	1.61	0.12	0.14
Col-0	*Pst* DC3000	1.68	2.03	0.09	0.09
SALK_020712C	Mock(Water)	1.12	1.03	0.12	0.15
SALK_020712C	*Pst* DC3000	0.71	0.73	0.09	0.10
Col-0	Mock(Water)	1.20	1.66	0.12	0.09
Col-0	Pst DC3118	1.06	1.02	0.12	0.11
SALK_020712C	Mock(Water)	1.15	1.52	0.13	0.12
SALK_020712C	*Pst* DC3118	0.78	0.87	0.10	0.11

3.4 Symptoms

Normal images were taken to monitor the visual symptoms visible after infection. The images have been organized according to the duration after inoculation when the pictures were taken, which bacteria was used for inoculation and the method of inoculation used.

From the results obtained from pictures taken three and seven days post inoculation, there are a few cases where very clear differences are observed in the level of symptoms of infection between SALK_020712C and Col-0 plants. For dip inoculation with *Pst* DC3000, three days post inoculation (Figure 14), Col-0 plants show more chlorosis and necrosis compared to SALK_020712C plant, and however, with vacuum infiltration (Figure 15) no differences were observed. Seven days post dip inoculation (Figure 16) with *Pst* DC3000 Col-0 plants showed more severe signs of chlorosis and necrosis compared to SALK_020712C plants, however, for vacuum infiltration (Figure 17) under same conditions, no such differences were observed in the symptoms. For

inoculation with *Pst* DC3118, three days post dip inoculation (Figure 18), slightly greater chlorosis was seen in Col-0 compared to SALK_020712C, however for vacuum infiltration (Figure 19) with the same conditions, no such differences were observed. Seven days post dip inoculation (Figure 20) and vacuum infiltration (Figure 21) with *Pst* DC3118, Col-0 showed considerably greater levels of chlorosis and necrosis compared to SALK_020712C plants.

Figure 3.14: Image displaying symptoms of infection for Col-0 (Left) and SALK_020712C (Right) plants inoculated with *Pst* DC3000 using dip inoculation taken three days post inoculation.

Figure 3.15: Image displaying symptoms of infection for Col-0 (Left) and SALK_020712C (Right) plants inoculated with *Pst* DC3000 using vacuum infiltration taken three days post inoculation.

Figure 3.16: Image displaying symptoms of infection for Col-0 (Left) and SALK_020712C (Right) plants inoculated with *Pst* DC3000 using dip inoculation taken seven days post inoculation.

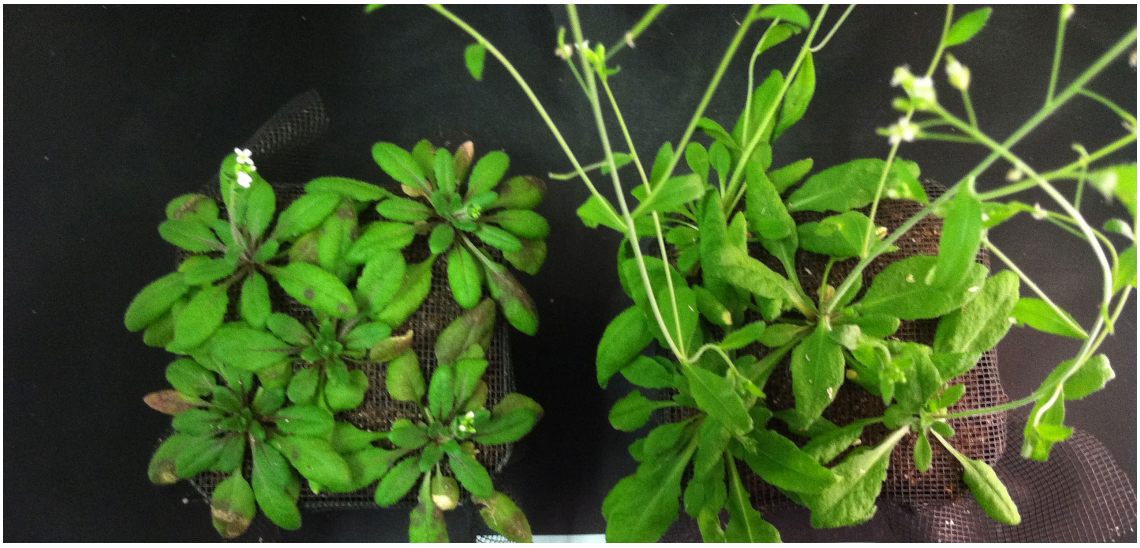

Figure 3.17: Image displaying symptoms of infection for Col-0 (Left) and SALK_020712C (Right) plants inoculated with *Pst* DC3000 using vacuum infiltration taken seven days post inoculation.

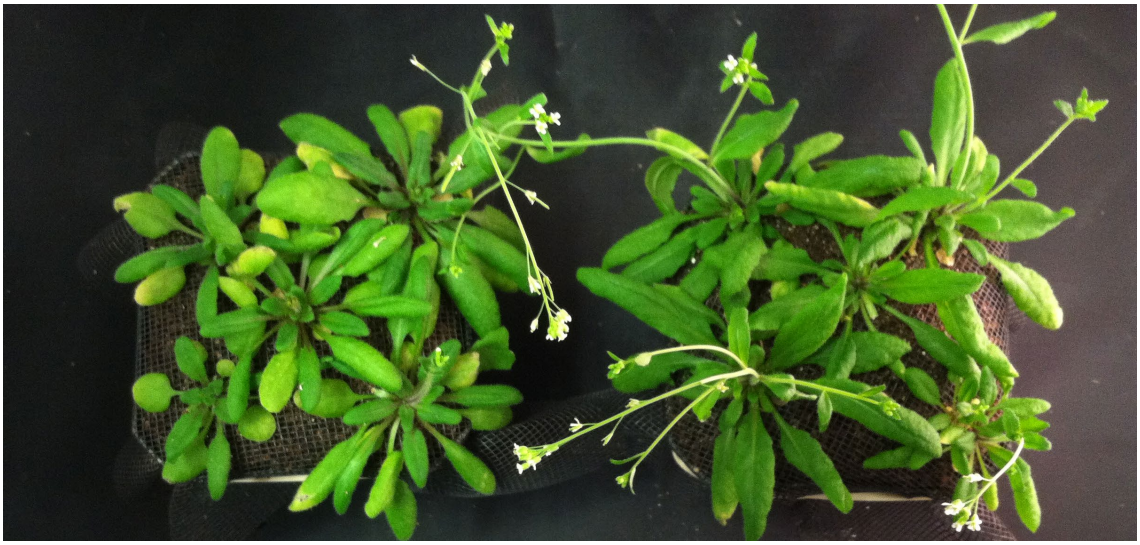

Figure 3.18: Image displaying symptoms of infection for Col-0 (Left) and SALK_020712C (Right) plants inoculated with *Pst* DC3118 using dip inoculation taken three days post inoculation.

Figure 3.19: Image displaying symptoms of infection for Col-0 (Left) and SALK_020712C (Right) plants inoculated with *Pst* DC3118 using vacuum infiltration taken three days post inoculation.

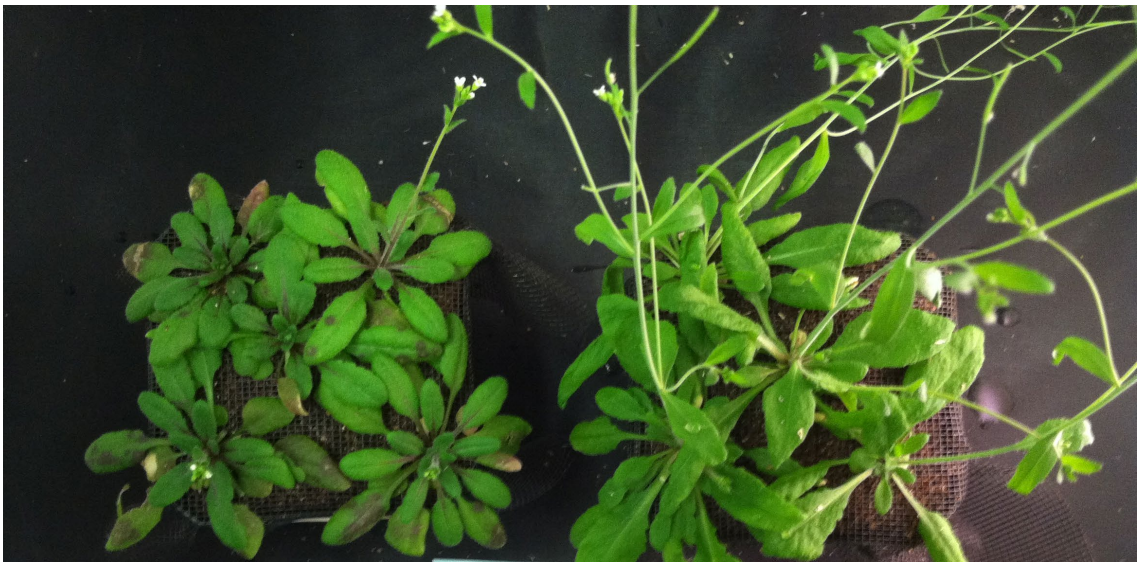

Figure 3.20: Image displaying symptoms of infection for Col-0 (Left) and SALK_020712C (Right) plants inoculated with *Pst* DC3118 using vacuum infiltration taken seven days post inoculation.

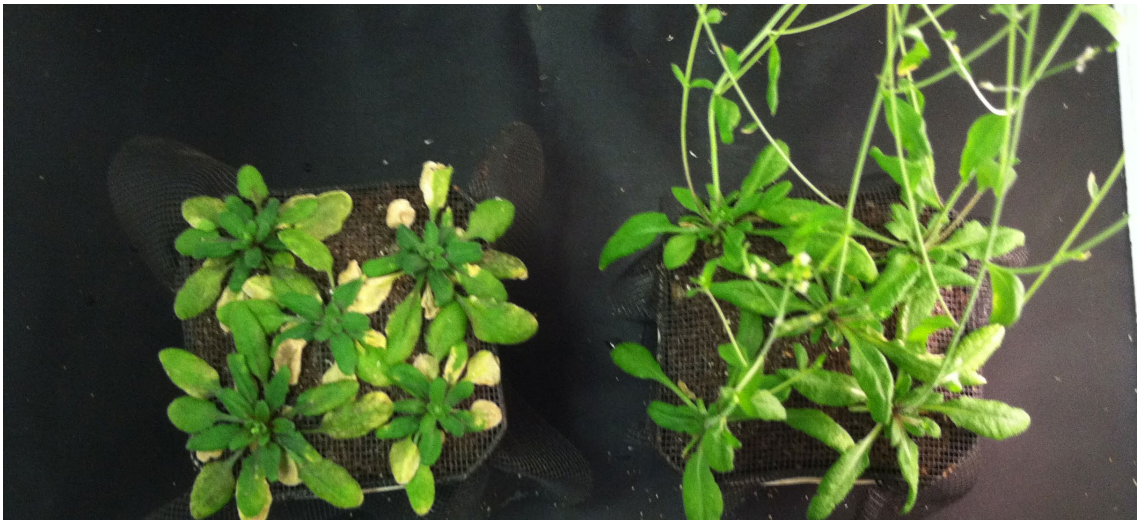

Figure 3.21: Image displaying symptoms of infection for Col-0 (Left) and SALK_020712C (Right) plants inoculated with *Pst* DC3118 using dip inoculation taken seven days post inoculation.

CHAPTER 4

DISCUSSION

Plant-pathogen interaction is a very complex process involving many different factors. Thus, it is necessary to separately examine the effect of each characteristic to better understand the process as a whole. This in turn makes it even more important to improve the methodology of the experiments to eliminate any errors that might occur due to the large number of variables. Our research attempts to do so by using different ways to measure the level of stomatal immunity.

Thermal imaging of the leaf surface after inoculation is useful in relatively estimating the level of stomatal opening and closure. A higher leaf surface temperature compared to the control indicates a higher level of stomatal closure (Mustilli *et al*. 2002). Counter to this, a lower leaf surface temperature indicates a higher level of stomatal opening. The results obtained from thermal imaging indicate that SALK_020712C plants show increased stomatal closure upon inoculation with *Pst* DC3118 and *Pst* DC3000 across both methods of inoculation, two and four hours post inoculation. Although some variation was observed in regard to the exact conditions in which increased stomatal closure was seen, the results were fairly evenly spread across all factors (Figures 2-9). Thus, the mutant shows a positive indication of increased levels of stomatal immunity. It is also important to understand that all these results have to be replicated multiple times in order to make strong conclusions regarding the levels of stomatal immunity seen in the

mutant. It will also be important to distinguish the effects of *Pst* DC3118 and *Pst* DC3000 based on the presence of coronatine and how that affects the stomatal immunity in SALK_0200712C plants. From the current experiments, no substantial conclusions can be made.

The results obtained from the thermal images were supported by the results obtained from the bacterial plating done to analyze the bacterial counts in the apoplast. Overall, with both the methods of inoculation, comparatively lesser bacteria entered and grew in the leaf apoplast of SALK_020712C one and three days post inoculation. Vacuum infiltration is an inoculation method which surpasses the stomatal barrier for the entry of bacteria. SALK_020712C showed significantly lower bacterial growth compared to Col-0. However, this leads to the question of the mechanism in operation in SALK_0200712C plants that makes increases their immunity towards the bacteria (Figures 10 & 11). Thus, the immunity of the SALK_0200712C plants towards both bacteria doesn't seem to be affected entirely by the action of the stomata (Table 1). Thus, further experimentation is necessary to completely understand all the factors that affect the immunity of the SALK_0200712C plant. But, the preliminary trends seen here are promising.

The results obtained from the stomatal assay are more significant for inoculation with *Pst* DC3000. Significantly smaller stomatal aperture size was observed two and four hours post inoculation in SALK_020712C with both water and bacteria. This indicates that when the stomata of the SALK_020712C close in response to both moisture and bacteria and remain closed over both the time points. This indicates that the stomata of the mutant are very responsive to external factors. But, this doesn't help us make any

conclusions about the specificity of the stomata in responding to just moisture or to a combination of moisture and pathogens (Table 2). The inconsistencies observed in the stomatal aperture size observed for both Col-0 and SALK_020712C inoculated with *Pst* DC3118 make it difficult to draw any conclusions about the effect of coronatine on the stomata (Figures 12 & 13). Further experimentation and replication of results is necessary to arrive at any conclusions about the stomatal behavior of SALK_020712C in response to bacteria.

The appearance of physical symptoms of infection and bacterial growth are important indicators of the level and intensity of infection in the plant. Chlorosis and necrosis are the common indicators of infection (Katagiri *et al.* 2002). In most cases SALK_020712C showed fewer symptoms of infection compared to Col-0. In general, the symptoms observed were more intense for inoculation with *Pst* DC3000. This is fairly consistent with what we know about the characteristics of the two bacteria. *Pst* DC3000 shows stronger visible symptoms of infection compared to *Pst* DC3118 due to its ability to produce coronatine (Figures 14-21). However, the symptoms observed for vacuum infiltration were less intense compared to dip inoculation. This counters what is generally observed with this experiment. Thus, it will be necessary to examine the methodology of vacuum infiltration and improve on it in order to see some consistency in the results. Hence, although SALK_020712C plants show some indications of increased immunity towards both bacteria, further experimentation is necessary to make any strong conclusions.

The results obtained from our research are promising positive indications that the gene At4g29000 might be involved in the process of stomatal immunity in Arabidopsis.

Due to the large number of variables involved in the process of plant pathogen interaction such as humidity, type of pathogen involved and external conditions. It is very important to have several replicates showing consistent results to arrive at any conclusions. It will also be important to have proper results obtained for the controls in order to compare the results obtained for the mutant. Also, in order to confirm the results, experimentation with another homozygous line is also necessary. In order to confirm the of these plant lines, it will be necessary to conduct further tests such as RNA extraction to determine the mutation leading to gene disruption and RT-PCR to determine the lack of At4g29000 transcript.

Research with stomatal immunity is extremely valuable in developing plant lines that are innately immune to certain pathogens and this can in turn have applications reducing the amount of pesticides used in the agricultural process. However, in the immediate future, further research is important in order to gain a clear picture of the topic.

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BIOGRAPHICAL INFORMATION

Roshni joined the University of Texas at Arlington because of the extensive scholarship and merit opportunities extended to her. Following her path of wanting to build a career in research, she joined the Melotto lab in the summer of 2012 and started working under Dr. Maeli Melotto. Her first project was to assist in evaluating the levels of stomatal immunity in *Arabidopsis thaliana* mutants. Her work resulted in an ACES 2013 oral presentation at UTA. She worked on learning the research protocols and in the summer of 2013, she worked on the project of evaluating the levels of stomatal immunity in At4g29000 mutants of *Arabidopsis thaliana*. She then worked on her honors senior thesis with the same project. Her project also led to a poster presentation at ACES in 2014. She also worked with Monsanto® in the summer of 2014 in an internship as a protein bio analyst. She aspires to get her doctorate in Plant Pathology and fulfill her dream of becoming a collegiate professor. She is grateful for all the opportunities provided to her by her mentors and the University of Texas at Arlington and she hopes for a bright future in the field of plant biotechnology.