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1. Microarray Analysis Identifies Potential Genes Associated With Botrytis Cinerea and Hormonal Stresses.

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MICROARRAY ANALYSIS IDENTIFIES POTENTIAL GENES ASSOCIATED WITH *BOTRYTIS CINEREA* AND HORMONAL STRESSES

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This thesis is submitted in partial fulfilment of the requirements for the degree of Master of Science in Molecular Biology and Biotechnology

Under the Supervision of Dr. Synan AbuQamar

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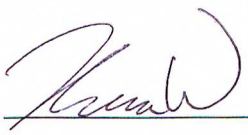
Declaration of Original Work

I, Kenna Marie Whitley, the undersigned, a graduate student at the United Arab Emirates University (UAEU), and the author of this thesis entitled "*Microarray Analysis Identifies Potential Genes Associated with* Botrytis cinerea *and Hormonal Stresses*", hereby, solemnly declare that this thesis is my own original research work that has been done and prepared by me under the supervision of Dr. Synan AbuQamar, in the College of Science at UAEU. This work has not previously been presented or published, or formed the basis for the award of any academic degree, diploma or a similar title at this or any other university. Any materials borrowed from other sources (whether published or unpublished) and relied upon or included in my thesis have been properly cited and acknowledged in accordance with appropriate academic conventions. I further declare that there is no potential conflict of interest with respect to the research, data collection, authorship, presentation and/or publication of this thesis.

Student's Signature:  Date: 12/05/2019

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Abstract

Biotic and abiotic stresses critically influence plant survival and growth; survival depends on the ability to correctly sense and react to their environment. Certain environmental stresses must be overcome through careful manipulation of internal hormone levels, and of the resulting signaling cascades. Hormone signaling networks and the crosstalk among each respective hormone signaling pathway are principle for response mediation via transcriptional reprogramming or altered signaling pathways. The long-term objective is to determine how plants sense biotic stresses, and how hormone signaling networks control and direct plant responses to the fungal pathogen *Botrytis cinerea*. This research aims to identify commonly regulated genes in *Arabidopsis thaliana* that respond to both *B. cinerea* and four selected hormones to ultimately improve the understanding of biotic stress responses and resistances in plants. The specific aims were to: (1) identify regulated genes in response to *B. cinerea* infection, salicylic acid (SA), jasmonic acid (JA), ethylene (ET), and abscisic acid (ABA); and (2) determine common up- and down-regulated genes in response to *B. cinerea* infection and the phytohormones in Arabidopsis. These goals were accomplished by analyzing publicly available microarray data of Arabidopsis differentially-expressed genes (DEGs) in response to *B. cinerea*, SA, methyl jasmonate (MeJA), 1-aminocyclopropane-1-carboxylic acid (ACC; the ET natural precursor), and ABA. This analysis of microarray data revealed 6.9% of genes were up-regulated and 5.3% of genes were down-regulated at 18 hours post-infection with *B. cinerea*. Between 6.1-7.2% of genes were induced upon individual treatments of SA, MeJA, ACC, or ABA, with 9% and 1.2% of genes identified as commonly up-regulated and down-regulated genes, respectively, for all hormone treatments and *B. cinerea* together. Of these DEGs, most belong to biologically functional binding proteins (*i.e.*, transcription factors) or proteins related to cellular transport. Arabidopsis expression profiling of defense regulated genes in response to *B. cinerea* and hormone stresses aims to improve the understanding of how plants cope with biotic stresses on the transcriptional level and will help identify potential up- and down-regulated genes involved in Arabidopsis defense against this pathogen. This study could lead to the introduction of novel defense genes into crops, or to utilize genes already present in the organism to subtly modify the hormonal signaling cascade in response to *B. cinerea*, improving these crops' resistance to *B. cinerea* infections. Changes in hormone regulation through gene expression following exposure to *B. cinerea* would allow a marketable and responsible solution to improving crop resistances to *B. cinerea*.

Keywords: Arabidopsis, *Botrytis cinerea*, abscisic acid, jasmonic acid, ethylene, salicylic acid, hormone signaling pathway.

Title and Abstract (in Arabic)

التعبير الجيني للجينات المرتبطة بعدة اجهادات في نبات الاربيدوبسيس

الملخص

يتأثر بقاء النبات ونموه تأثرًا كبيرا بالضغوطات الحيوية واللاحيوية. للتغلب والتكيف على الإجهادات البيئية، تعتمد النباتات على الهرمونات وذلك لقدرتها على التحكم في التنبهات الداخلية في النباتات. تعتبر الشبكات الهرمونية والتداخل بين هذه المسارات مهمة للاستجابة النبات للاجهادات عن طريق التحكم في إعادة البرمجة عبر النسخ أو تغيير مسارات التأثير. يكمن الهدف في هذا البحث على المدى الطويل هو تحديد كيفية شعور النباتات بالإجهاد الحيوي، وكيف تستثار شبكات التنبهات الهرمونات النباتية للأستجابة لفطر البوترايتس سينيريا. يهدف هذا البحث إلى التعرف على أستجابة الجينات المشتركة المنظمة النباتية لكل من بوترايتس سينيريا وأربعة هرمونات مختارة في نبات الاريبادوبسيس، لتحسين فهم مقاومة الإجهاد الحيوي في النباتات في نهاية المطاف. ولأجل ذلك، كانت الأهداف المحددة على النحو التالي: (1) تحديد الجينات الخاضعة للرقابة استجابة لعدوى البوترايتس سينيريا، وحمض الساليسيليك، وحمض الجاسمونيك، والإثيلين ، وحمض الاباسيك، (2) تحديد الجينات المشتركة استجابة لعدوى البوترايتس سينيريا والهرمونات النباتية في نبات الاريبادوبسيس .لذلك تم تحليل الميكروأري للبيانات المتاحة من الجينات المعبرة بشكل تفاضلي في الاريبادوبسيس استجابا للبوترايتس سينيريا وغيره من الهرمونات النباتية. فقد تم الكشف عن طريق الميكروأري أن 6.9٪ من الجينات كانت منظمة بشكل تصاعدي وأن 5.3٪ من الجينات قد خضعت للتنظيم التنازلي خلال ال18 ساعة بعد الإصابة للبوترايتس سينيريا. هذا وكان ما بين 6.1-7.2٪ من الجينات محرضًا على علاجات فردية لـ حمض الساليسيلك مع 9٪ و 1.2٪ من الجينات التي تم تحديدها على أنها جينات شائعة التنظيم التصاعدي والتنظيم التنازلي، على التوالي، لجميع معاملات الهرمون و للبوترايتس سينيريا معا. ينتمي معظم هؤلاء التنظيمات التصاعدية التنازلية إلى بروتينات الربط الوظيفية البيولوجية (أي عوامل النسخ) أو البروتينات المرتبطة بالنقل الخلوي. يهدف تعبير الاريبادوبسيس عن الجينات الخاضعة للدفاع استجابةً لضغط البوترايتس سينيريا والهرمونات إلى فهم كيفية تعامل النباتات مع الضغوط الحيوية على مستوى النسخ. سيساعد هذا البحث على تحديد الجينات المحتملة التي يتم تنظيمها لأعلى ولأسفل والمشاركة في الدفاع عن العوامل الممرضة. يمكن أن تؤدي هذه الدراسة إلى إدخال جينات دفاعية جديدة في المحاصيل، أو الاستفادة من الجينات الموجودة بالفعل في الكائن الحي لتعديل سلسلة الإشارات الهرمونية بمهارة استجابةً للبوترايتس سينيريا، مما يؤدي إلى تحسين مقاومة هذه المحاصيل لعدوى هذا الفطر. ان التغييرات في تنظيم الهرمونات من خلال التعبير الجيني بعد التعرض البوترايتس سينيريا ستتيح حلاً قابلاً للتسويق والمسؤول لتحسين مقاومة المحاصيل البوترايتس سينيريا بشكل عام.

مفاهيم البحث الرئيسية: بوترايتس سينيريا ، حمض الاباسيك، حمض الجاسمونيك، الاثلين، حمض الساليسيلك، المسار الهرموني.

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Dedication

To my beloved parents and family

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List of Abbreviations

2xV8	Agar made with V8 juice
ABA	Abscisic acid
ACC	1-aminocyclopropane-1-carboxylic acid
AMPs	Antimicrobial peptides
CDPKs	Calcium-dependent protein kinases
CPPs	Cell-penetrating peptides
CWDE	Cell wall degrading enzyme
DAMPs	Damage-associated molecular patterns
DAVID	Database for annotation, visualization, and integrated discovery
DEG	Differentially expressed genes
ET	Ethylene
ETI	Effector-triggered immunity
GC-RMA	GeneChip RMA
HPI	Hours post infection
HR	Hypersensitive response
JA	Jasmonic acid
MAMPs	Microbe-associated molecular patterns
MAPKs	Mitogen-associated protein kinases
MAS5	Microarray Suite 5 normalization
MeJA	Methyl jasmonate
PAMPs	Pathogen-associated molecular patterns
PCC	Pearson’s correlation coefficient

PCD	Programmed cell death
PRRs	Pattern-recognition receptors
PTI	PAMP-triggered immunity
qRT-PCR	Real-time polymerase chain reaction
RMA	Robust multiarray analysis
ROS	Reactive oxygen species
SA	Salicylic acid
sRNA	Small RNA

Chapter 1: Introduction

1.1 Overview

Plants have adapted many various defense mechanisms to respond to and thrive in the face of constantly developing environmental stresses. Each defense response is precisely tailored to protect the organism against abiotic stresses such as temperature, humidity, nutritional changes, or from biotic stresses such as animals, insects, and pathogens. These responses are categorized into two groups: the passive defenses and the active defenses. Passive defenses are the first lines of defense against stresses and are often enough to protect plants sufficiently against stresses. Passive defenses are described as the physical and chemical barriers of the plant (Serrano et al., 2014). Physical barriers are the plants' cuticles and cell walls, whereas the chemical barriers are toxic compounds such as antimicrobial peptides (AMPs) and cell-penetrating peptides (CPPs) possessing antimicrobial properties against pathogens (Nawrot et al., 2014). Certain AMPs are not permanent members of a plant's defenses, however, and must be induced. These AMPs, and all other responses which must be induced, do not belong to the passive defenses but are instead classified as active defenses (Gust et al., 2017).

Active defense responses are not constantly active and instead must be induced upon infection (Gust et al., 2017). First, to mount an effective active defense, the attacking pathogen must be accurately identified. Once a pathogen overcomes the passive defenses of a plant, plant cells identify the pathogen through pattern-recognition receptors (PRRs) located on the cell surface (Couto and Zipfel, 2016). These PRRs identify pathogens via pathogen-associated molecular patterns (PAMPs) or microbe-associated molecular patterns (MAMPs). PRRs can also

recognize host damage through damage-associated molecular patterns (DAMPs), *i.e.*, free-floating cell wall fragments caused by wounding or infection (Monaghan and Zipfel, 2012). PAMPs, MAMPs, and DAMPs activate the active defense responses known as PAMP-triggered immunity (PTI) (Macho and Zipfel, 2015). PTI triggers substantial transcriptional reprogramming through a combination of reactive oxygen species (ROS), calcium, mitogen-associated protein kinases (MAPKs), and calcium-dependent protein kinases (CDPKs) (Cui et al., 2015; AbuQamar et al., 2017).

Although PTI can protect plants against a group of pathogens, pathogens can still surpass this line of defense through effector proteins which either interrupt PAMP signaling, or evade the system entirely (Couto and Zipfel, 2016). For example, *B. cinerea* silences MAPKs through small RNA (sRNAs) to disrupt PAMP signaling, effectively circumventing PTI entirely (Weiberg et al., 2015). In return, some plants have developed resistance (R) proteins that can recognize certain effector proteins, offering a second line of defense known as effector-triggered immunity (ETI) (Liu et al., 2016). ETI often culminates with the hypersensitive response (HR) and programmed cell death (PCD), though the HR response often assists *B. cinerea* and other necrotophic pathogens as opposed to furthering plant defenses (Mengiste, 2012; McCormick, 2017).

On the other hand, certain pathogens do not produce effector proteins, rendering ETI ineffective. Necrotrophic pathogens such as *B. cinerea* actively kill host tissue by producing nonspecific toxins, cell wall degrading enzymes (CWDEs), and defense suppressing enzymes, yet there is little effective resistance against these toxins. This suggests that even if these molecules are detected, these virulence functions may override PTI and ETI processes (Kazan and Lyons, 2014; AbuQamar et al., 2017).

1.2 Statement of the Problem

The necrotophic pathogen *B. cinerea* is an aggressive pathogen that affects over 200 plant species and destroys crops before and after harvest. It is the second most important economical pathogen and poses an immense risk to food crops worldwide (Dean et al., 2012). The lack of effector molecules and disruption of PAMP signaling by *B. cinerea* renders PTI and ETI useless in defending against this pathogen, stressing the need for an alternative solution (Weiberg et al., 2015).

Genetic engineering offers a unique solution to this problem, as plants are not equipped with successful native defenses against *B. cinerea*. Genes that improve plant resistance to *B. cinerea* must be identified and implemented to lower or fully stop the food-loss caused by this aggressive pathogen, saving millions of dollars in cultivation and shipping losses (Dean et al., 2012).

1.3 Relevant Literature

B. cinerea is a fungal pathogen that has ailed agriculture for centuries. *B. cinerea* poses great risk to many crops, including tomatoes, grapes, berries, stone-fruits, etc. (Rosslenbroich and Stuebler, 2000). This fungal pathogen causes gray mold disease and can persist and grow even in cold storage conditions (10°C) (Dean et al., 2012). As a necrotrophic pathogen, this fungus kills its host to absorb the host's nutrients. *B. cinerea* does not produce a host-specific toxin; thus, plant defense against this pathogen consists of a mixture of defense responses orchestrated by the hormones: salicylic acid (SA), jasmonic acid (JA), ethylene (ET), and abscisic acid (ABA), among others.

1.3.1 The Role of Hormones

The phytohormones, SA, JA, ET and ABA play important roles in controlling Arabidopsis responses to *B. cinerea*. Previous studies on Arabidopsis have shown these hormones mediating resistance/susceptibility to *B. cinerea*, in addition to demonstrating complex crosstalk(s) between hormone-regulated disease response pathways. For example, crosstalk between JA and ET synergistically upregulates expression of plant defensin *PDF1.2*, a gene shown to improve resistance in Arabidopsis against *B. cinerea* infection (Nie et al, 2017). Defects in the JA and ET signaling responses increase Arabidopsis susceptibility to *B. cinerea* (Lu and Yao, 2018; Li et al, 2019). Crosstalk between JA- and SA-regulated pathways may act as positive and negative regulators in defense responses to *B. cinerea*; yet JA alone plays a major role in *B. cinerea* defense through *Botrytis susceptible 1* (*BOS1*) regulation (Grabke et al., 2014; Sham et al., 2015).

An up-regulation of SA-responsive genes showed an increase in susceptibility in Arabidopsis, though it is possible that this up-regulation caused suppression of JA-regulated genes required for *B. cinerea* resistance (Oirdi et al., 2011). In addition, Arabidopsis mutants exhibiting spontaneous cell death phenotypes due to increased SA levels showed an increase in susceptibility to *B. cinerea* (Zhang et al., 2017).

ABA has been implicated in necrotroph-plant defenses and may regulate defense responses based on reactive oxygen intermediates (Segarra et al., 2013). ABA hypersensitivity is accompanied by local susceptibility to *B. cinerea* (AbuQamar et al., 2013). ABA has also been found to play a major role in inducing the *expansin-like A2* (*EXLA2*) gene in Arabidopsis and also plays a role in *B. cinerea* resistance (Sham et al., 2015).

Arabidopsis *coronatine insensitive 1* (c*oi1*) is a JA-insensitive mutant demonstrating increased susceptibility to *B. cinerea*, yet the SA response mutant *npr1-1* increased resistance to *B. cinerea* (Nie et al., 2017). The *ein2-1* mutant, an ET-insensitive mutant, increased susceptibility to *B. cinerea*, but pre-treatments of ET and methyl jasmonate (MeJA) to Arabidopsis plants prior to infection with *B. cinerea* reduced infection in wild-type plants (Thomma et al., 1999). Pre-treatment of ET on *ein2-1* mutants did not reduce infection, though interestingly, pre-treatment of MeJA to *ein2-1* mutants still reduced infection. In addition, MeJA and ET induce the Arabidopsis molecular marker genes *PDF1.2*, *PR-3*, and *PR-4*; indicating a coordination between JA and ET signaling (Li et al, 2019). Both JA and ET signaling pathways are necessary to activate *ETHYLENE RESPONSE FACTOR1* (*ERF1*), a gene encoding a transcription factor implicated in preventing disease progression (Cerrudo et al, 2012).

Crosstalk between ABA, JA, and ET can also exist. Disruption of Arabidopsis *MYC2*, a transcription factor and a positive ABA signaling regulator, induces JA and ET defense genes. Conversely, exogenous application of ABA has the opposite effect, and suppresses transcription of these same genes (Anderson, et al., 2004). The hormones SA, JA, ET and ABA play a complex role in mediating plant defenses against *B. cinerea*. Due to the complex nature of hormonal cross-talk for efficient defenses against the pathogen *B. cinerea*, this study aims to identify common up- and down-regulated genes for the hormone treatments and *B. cinerea* to better understand the relationship between SA, JA, ET, ABA, in plant response to *B. cinerea*.

To identify these genes, microarray technology was utilized. Microarrays are small chips containing gene sequences, referred to as “probes”, that bind to

complementary DNA. During a microarray experiment, DNA from the control and the experimental condition are extracted and labelled using different fluorophores, and then introduced to the microarray. Complementary DNA binds to the probes on the microarray, and the microarray is washed of excess or unbound DNA. The microarray is then scanned, and an image is created and ready for processing (Rueda, 2014). It is in this way previous experiments generated data on gene expression changes in Arabidopsis following individual treatments with *B. cinerea*, SA, JA, ET, and ABA (Ferrari et al., 2003; Goda et al., 2008; Kilian et al., 2007). These microarray experiments provided data identifying genes that are up- or down-regulated following treatment with these stresses, setting the necessary groundwork for comparison in this study.

1.4 Hypothesis and Objectives of the Study

My hypothesis is that common up- and down-regulated genes in response to *B. cinerea* infection and hormonal stresses mediate plant defense against *B. cinerea*. The overall objective of this study is to identify common differentially expressed genes (DEGs) between *B. cinerea* and the hormones SA, JA, ET, and ABA to ultimately improve plant resistance to *B. cinerea*. The specific aims are to: (1) identify regulated genes in response to *B. cinerea*, SA, JA, ET, and ABA individually; and (2) determine common up- and down-regulated genes in response to *B. cinerea* infection and the phytohormones in Arabidopsis.

Chapter 2: Materials & Methods

2.1 Data Source and Microarray Analyses

Publicly available raw experimental microarray data obtained from Nottingham Arabidopsis Stock Centre (www.arabidopsis.info) were analyzed. Three different experiments using Arabidopsis (ecotype *Col-0*) wild-type plants were downloaded from this database: *B. cinerea* (NASCArray-167), the hormones: SA (NASCArray-192), MeJA (NASCArray-174), ACC (NASCArray-172), ABA (NASCArray-176), and the control (NASCArray-137).

The raw data files obtained from each experiment were normalized using MAS5.0, RMA, and GCRMA methods with the software R (https://www.r-project.org/) and R package simpleaffy (https://CRAN.R-project.org/package=simpleaffy). MAS5.0 normalization was selected for downstream analyses based on previous published microarray analyses of similar type (Sham et al., 2015). The expression levels and *P*-values of the top 100 genes induced by *B. cinerea* can be found in Appendix I. RMA and GCRMA methods were also used for comparison purposes with MAS5.0 normalization method. The resulting $\log_2$ values from RMA and GCRMA normalizations were converted to linear values and filtered to remove genes with low variance that would be unlikely to pass statistical testing for differential expression. DEGs were identified for all normalization methods via fold change using a cut-off value of ≥ 2 for up-regulated genes and ≤ 0.5 for down-regulated genes.

The scatterplots, Venn diagrams, and heatmap analyses were generated using R version 3.4.2 with the R package gplots (https://CRAN.R-project.org/package=gplots). Based on the MAS5.0 normalization method, the top

100 DEGs (50 up- and 50 down- regulated) identified from the *B. cinerea* treatment and the corresponding expression values from the hormone treatments were analyzed for the heatmap. The clustering for the heatmap was constructed using Pearson's Correlation Coefficient (PCC) of the selected probes' expression values. The PCC was converted into a measure of distance between correlated points to better visualize the changes in expression patterns over the different treatments.

2.2 Categories of Functional Classification of DEGs

DEGs were functionally classified using the free online software Database for Annotation, Visualization, and Integrated Discovery (DAVID) v6.8 (Huang et al., 2009a; 2009b). The visualization of the gene ontologies of the DEGs was performed using Classification Superviewer (Provart and Zhu, 2003).

2.3 Plant Growth and Disease Assays

The *B. cinerea* experimental plants were grown by Carine Denoux, Fred Ausubel, Julia Dewdney, and Simone Ferrari for their AtGenExpress *B. cinerea* infection experiment (Ferrari et al., 2003). Briefly, adult leaves of four-week-old Col-0 plants (in triplicates) were assayed at 18 hours post inoculation (hpi) with *B. cinerea*. RNA was extracted by the group as described by AtGenExpress (https://www.ebi.ac.uk/arrayexpress/experiments).

The hormonal treatments experimental data was generated using Arabidopsis (*Col-0*) wild-type seedlings grown by Goda et al. (2008). Two replications for each hormone treatment were performed, and whole seedlings were collected at 3 hours post treatment (hpt) (Goda et al., 2008).

Eighteen-day-old Arabidopsis (Col-0) wild-type plants were used for the control (0 hpi/hpt) arrays and were grown and assayed by Kilian et al. (2007).

For our qRT-PCR experiment, Arabidopsis Col-0 plants (4-week-old) were grown for the *B. cinerea* infection according to Sham et al. (2014). The *B. cinerea* strain *BO5-10* was cultured and maintained on 2xV8 agar and 10-day-old cultures were used to harvest conidia (Mengiste et al., 2003). A fungal spore density of $3x10^5$ in 1% Sabouraud Maltose Broth (SMB) buffer was used to inoculate detached leaves, with 3 µL droplets of the spore solution on each leaf. The leaves were kept on moistened filter paper in a sealed transparent container at room temperature (Sham et al., 2017). The treated leaves were retrieved at 18 hpi and stored at -80°C until RNA extraction.

2.4 RNA Extraction and Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

RNA extraction of Arabidopsis (Col-0) wild-type leaf tissues infected with *B. cinerea* at 0 and 18 hpi was performed with the Norgen RNA/DNA Purification Kit (catalog. #48700; Biotek Corp.). The leaves were ground with liquid nitrogen using a mortar and pestle. RNA was extracted and eluted twice with 50 µL of elution solution. Three biological replicates were generated.

The qRT-PCR was performed using the GoTaq 1-Step RT-qPCR System kit (Promega Corp.). Ten genes chosen for qRT-PCR were selected from a pool of the top 50 up- and down-regulated genes identified in common with all treatments (*B. cinerea*, SA, MeJA, ACC, and ABA). These identified genes were then sorted based on greatest differential gene expression in response to *B. cinerea* treatment, and genes mentioned in other studies were excluded. Ultimately, ten promising genes

were selected: six up-regulated genes and four down-regulated genes. The designed primers and their respective sequences can be found in Table 1.

Table 1: Primer sequences used for qRT-PCR (5'-3')

Gene	Forward Primer	Reverse Primer
Actin2	GTCGTACAACCGGTATTGTGCTG	CCTCTCTCTGTAAGGATCTTCATGAG
CCR2	CACGTAAACCGACTCTAAACCTAGAAA	AGATGTGTAGAACGATGTAGACGAAGT
αDOX1	GATTAGAAGCGGATAGGTTTTTCAC	CATCCTTGAGACTCTCTGTAGTATTCA
RBCX1	ATAGTGTCTGCTCAGCTTGAGAGTTAT	CAGAATTTATCACCGTCACTTACAGAG
At1g56300	AAAAACCATTCCCTCTCTCAACTCT	GAAGTGTTTCTCTGTTCTCAGACGTTA
At3g51660	GAGATAGTATTTGGAGGGAACAAAG	GTCGCTATGAGTTCTCTCTTAACTTGT
JAZ1	CGAGTTCTATGGAATGTTCTGAGTT	TTAGATACTGACTCAATCGACTACACG
DIR1-LIKE	CTGACTACACTTGTCTTTGTGGCTA	TAGGTCACACTCTTTAGGGAGACTAGA
At1g65490	CATCAGTATTTGCTTCTTCCAAGTG	GTACTATGGAGATTGATCAGAAACAGG
bHLH	AACTTCTATAAGTCCGGTGGTCTATG	TTGGTAAAAGACGTTCCTACTTCTG
HAD	GTAGGAGATGACCGTAGGAATGATGTA	GAGCAACCTGTTTAAATGACGTAAC

Two biological replicates and three technical replicates were analyzed for the qRT-PCR. The resulting data were pooled for the qRT-PCR analysis.

2.5 Statistical Analysis

Statistical analysis of the qRT-PCR data was done using the relative quantification method. Raw Cq values were taken from all biological replicates and pooled together for the analysis. The averages were calculated for each replicate, and the resulting average for Arabidopsis *Actin* was subtracted from the resulting averages of every primer, giving the ΔCt values for the 0 and 18 hpi experimental time points. The ΔCt control value was subtracted from the respective ΔCt value at 18 hpi for each probe, giving the ΔΔCt value. The expression fold change was then calculated by taking $2^{(-(\Delta\Delta Ct))}$. The graphical representation of the qRT-PCR values utilizes $\log_2(2^{(-(\Delta\Delta Ct))})$ values.

Chapter 3: Results

3.1 Number of Up- and Down-Regulated Genes

For each individual treatment, approximately 5-8% of the 22,810 tested genes were identified as DEGs. In Arabidopsis, this microarray analysis demonstrated 12.2% (2802 genes) were identified as DEGs upon inoculation with *B. cinerea* (Table 2). A total of 1575 (6.9%) genes were up-regulated and 1227 (5.3%) genes were down-regulated after infection with *B. cinerea*. After individual treatments of SA and ACC, 6.1% representing 1382 and 1402 of the total tested genes were up-regulated, respectively. However, SA and ACC treatments repressed 1743 (7.6%) and 1285 (5.6%) of Arabidopsis genes, respectively (Table 2). MeJA treatment caused 1459 (6.4%) genes to be identified as up-regulated, whereas 1545 (6.8%) genes were identified as down-regulated. On the other hand, ABA treatment increased the transcript levels of 1630 (7.1%) genes (the largest number of upregulated genes in all treatments). ABA also reduced the level of transcripts of 1703 (7.5%) of genes in the Arabidopsis genome (Table 2).

Overall, the analysis of plants treated individually with *B. cinerea* and all hormones yielded 211 (0.9%) up- and 269 (1.2%) down-regulated genes (Table 2).

Table 2: Number and percentage of identified up- and down-regulated genes in response to *B. cinerea* and hormone stresses.

Treatment	Up-regulated Genes		Down-regulated Genes		P-value
	Number	Percentage	Number	Percentage	
Botrytis cinerea (*Bc*)	1575	6.9%	1227	5.3%	0.039
Salicylic acid (SA)	1382	6.1%	1743	7.6%	$1.35x10^{-19}$
Methyl jasmonate (MeJA)	1459	6.4%	1545	6.8%	$4.43\ x10^{-19}$
1-aminocyclopropane-1-carboxylic acid (ACC)	1402	6.1%	1285	5.6%	$6.88\ x10^{-34}$
Abscisic acid (ABA)	1630	7.1%	1703	7.5%	$6.28\ x10^{-29}$
B. cinerea and all hormones	211	0.9%	269	1.2%	

Although exogenous ABA treatment induced the greatest number of genes, SA caused a down-regulation of the largest number of genes in Arabidopsis. *B. cinerea* treatment induced the second-highest number of up-regulated genes, and the lowest number of down-regulated genes than all other treatments.

3.2 DEGs in Response to *B. cinerea*

DEGs in response to *B. cinerea* were also determined using scatterplot analysis (Figure 1). The majority of DEGs cluster around the trend-line. The farther the point from the line, the stronger induction (above the line) or repression (below the line) of the gene(s) in response to *B. cinerea.* Interestingly, *At1g73260* (probe

260101_at) was the most strongly up-regulated gene in response to the necrotrophic fungus *B. cinerea*. *PDF1.2* gene (*At5g44420*; probe *249052_at*) is another up-regulated gene, and *PYK10* (*At3g09260*; *259009_at*) was highly down regulated in response to *B. cinerea*.

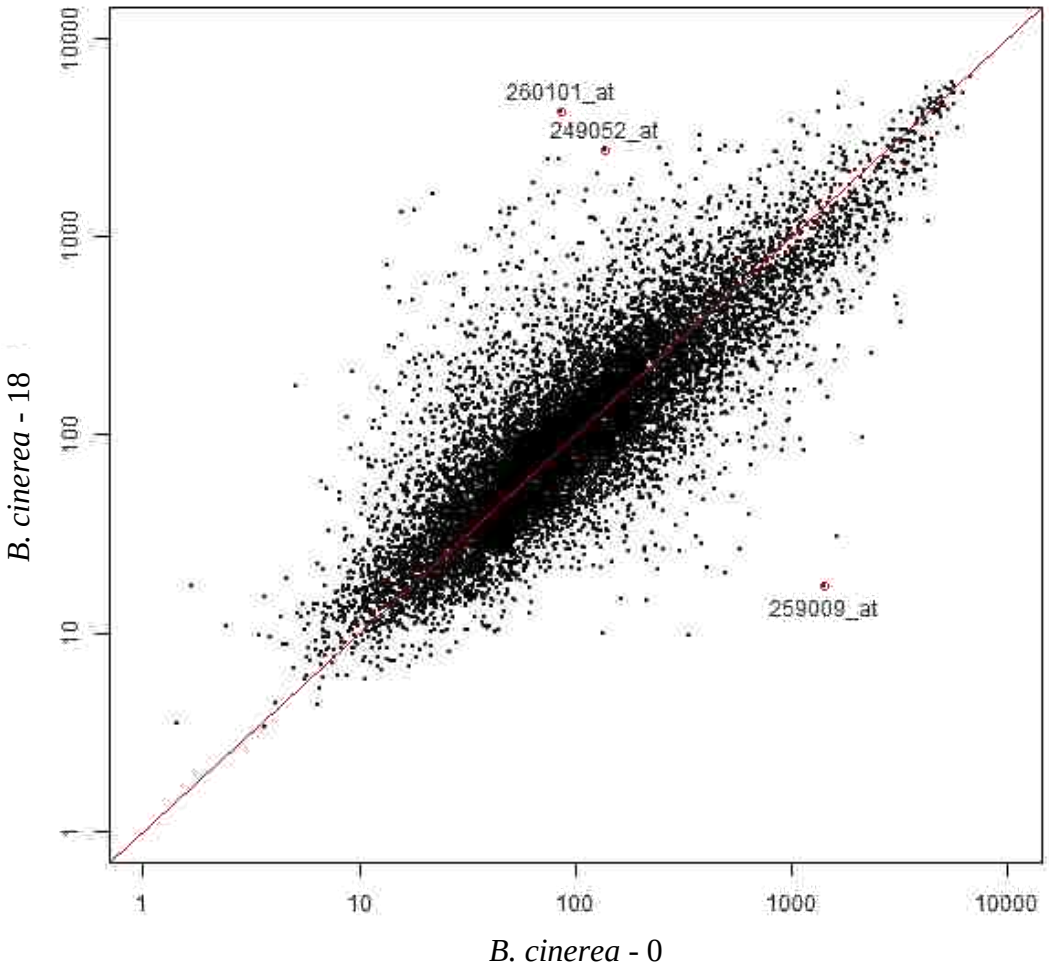

Figure 1: Scatter plots of differentially expressed genes in response to *B. cinerea* and the control.

Log_2 expression values of differentially expressed genes identified as up- or down-regulated by 2-fold following treatment with *B. cinerea*. Plotted along the X-axis are the 0 hpi time-point control expression values. The red line indicates the 2-fold threshold: points above the line signify genes that are up-regulated by two-fold, and those that are below the line are genes that are down-regulated by two-fold.

3.3 DEGs in Response to Hormones

When regulated genes upon hormonal treatments were plotted against the control dataset, each hormone treatment elicits a similar pattern of Arabidopsis gene regulation (Figure 2).

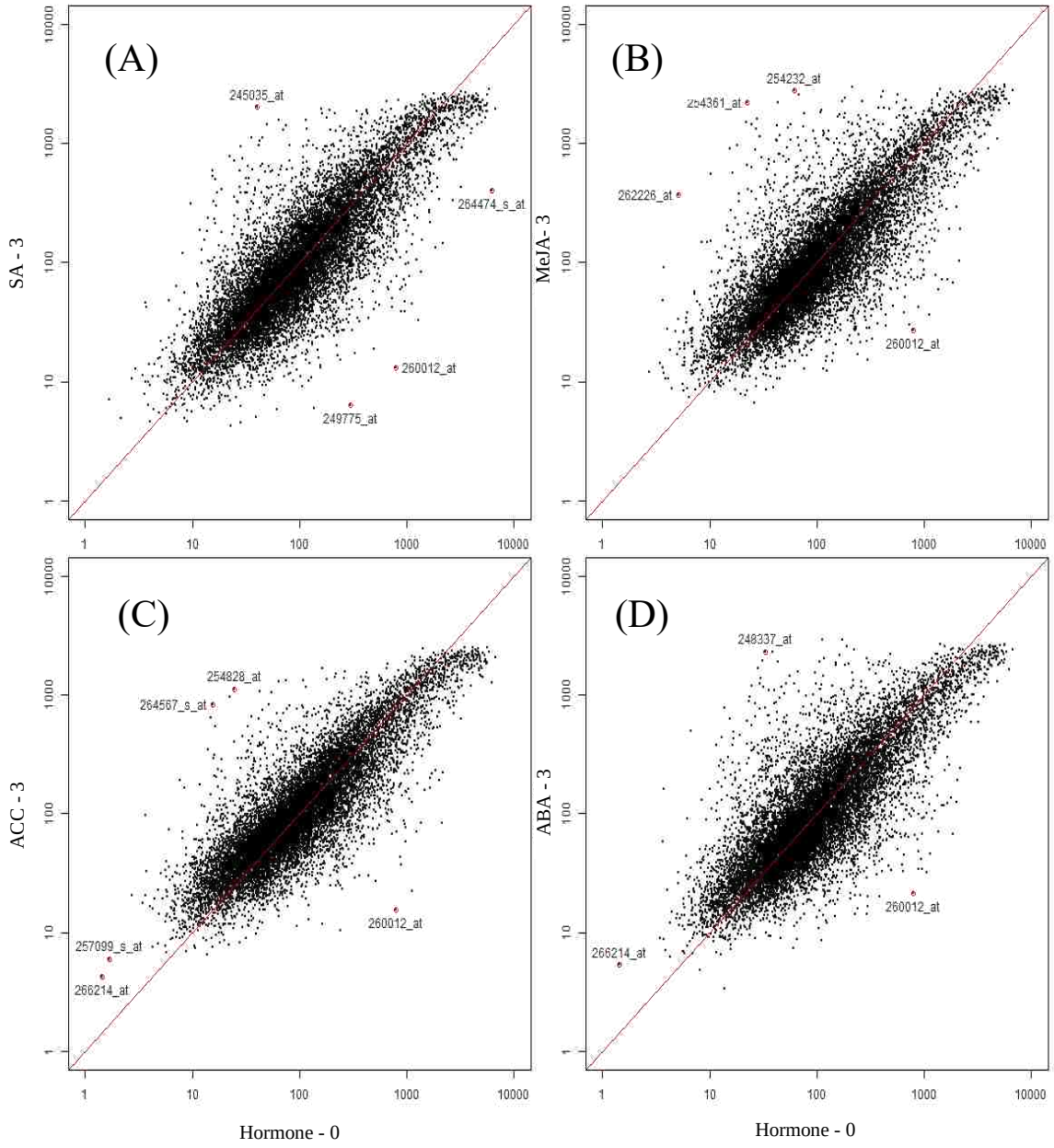

Figure 2: Scatter plots of differentially expressed genes in response to hormone treatments and the control.

Log expression values of DEGs identified as up- or down-regulated by 2-fold following treatment with SA (A); MeJA (B); ACC (C); and ABA (D). Plotted along the X-axis are the 0 hpi time-point control expression values. The red line indicates the 2-fold threshold: points above the line signify genes that are up-regulated by two-fold, and those that are below the line are genes that are down-regulated by two-fold.

The most highly-expressed gene in response to SA was found to be an acireductone dioxygenase (*At2g26400*; *245035_at*) (Figure 2A), though none of the top highly-expressed genes in response to SA were identified in all treatments. The

three most down-regulated genes to SA treatment were identified as: RuBisCo (*At5g38420*; *264474_s_at*), SQP1.2 (*At5g24160; 249775_at*), and the hypothetical protein (*At1g67865; 260012_at*) as the third. *At1g67865* was found to be significantly down-regulated in response to all hormone treatments (Figure 2).

In response to MeJA, three genes were highly induced in Arabidopsis. These genes were the senescence-associated protein *At1g53885* (*262226_at*), *DEFL* (*At4g22212*; *254361_at*), and *CORI3* (*At4g23600*; *254232_at*) (Figure 2B). Two genes were highly up-regulated by ACC (Figure 2C): *At1g05250* (*264567_s_at*) and *AIR1* (*At4g12550; 254828_at*), with two more genes identifiable in the bottom left corner: a transposable element gene (*AT2G06890; 266214_at*) and *AT3G24982* (*257099_s_at*). This transposable element gene, *AT2G06890*, was also identifiable as up-regulated during ABA treatment, along with *COR78/RD29A* (*At5g52310*; *248337_at*) (Figure 2D).

3.4 DEGs in Response to *B. cinerea* and Hormone Treatments

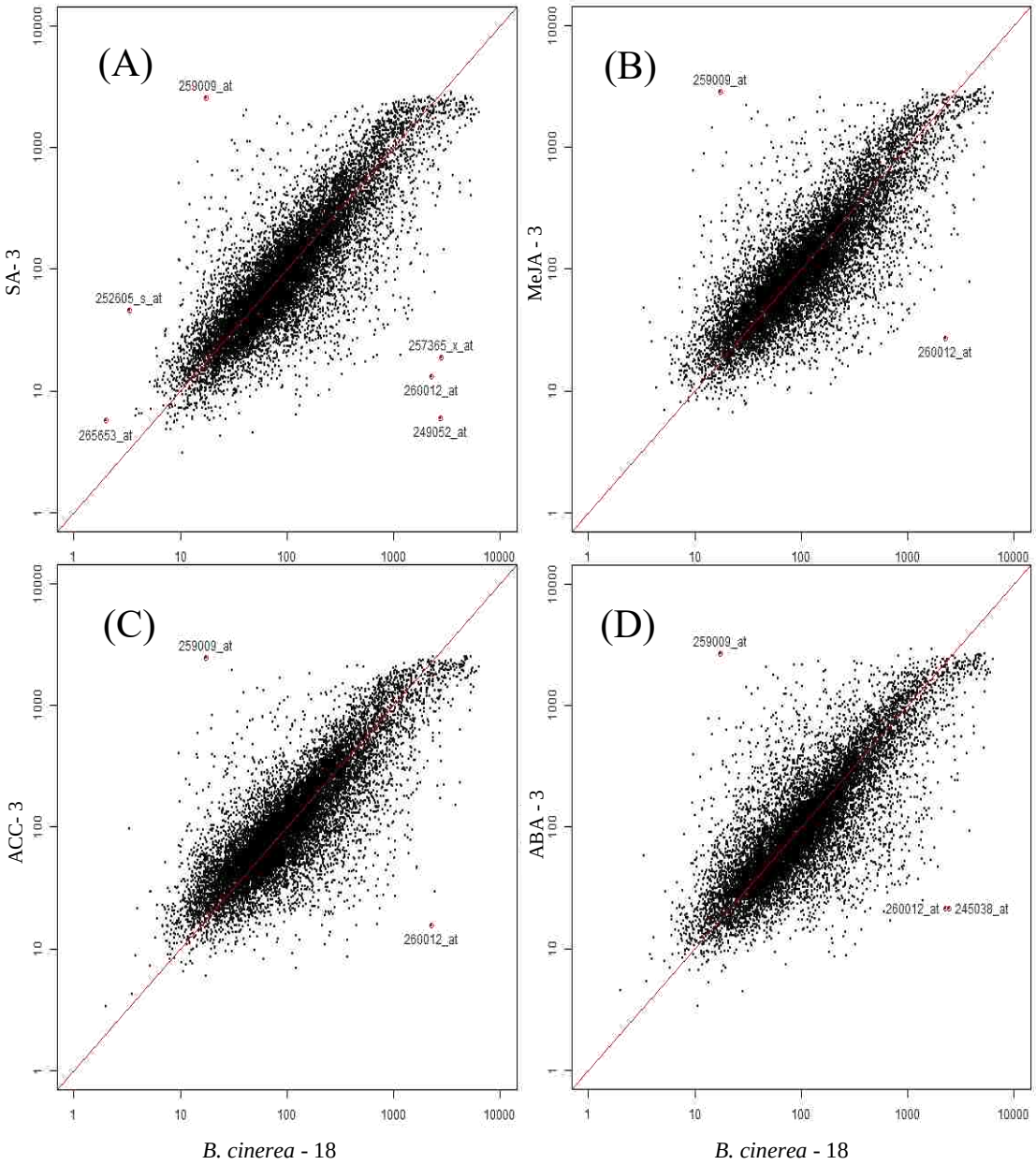

Figure 3: Scatter plots of differentially expressed genes in response to hormone treatments and *B. cinerea*.

Log expression values of differentially expressed genes identified as up- or down-regulated by 2-fold following treatment with SA (A); MeJA (B); ACC (C); and ABA (D). Plotted along the X-axis are the 18h post-infection (hpi) *B. cinerea* expression values. The red line indicates the 2-fold threshold: points above the line signify genes that are up-regulated by two-fold, and those that are below the line are genes that are down-regulated by two-fold.

In this study, similar patterns of gene regulation were found when plants were infected with *B. cinerea* and treated with hormones individually (Figure 3). However, when SA and *B. cinerea* are compared, four genes are noticed as outliers: a transposable element gene (*AT2G13890; 265653_at*), and *AT3G45070/AT3G45080* (*252605_s_at*) are both up-regulated, and two plant defensin genes *PDF1.2b* (*AT2G26020; 257365_x_at*) and *PDF1.2* (*AT5G44420; 249052_at*) are down-regulated. *PLP2* (*At2g26560*; *245038_at*) was highly down-regulated when Arabidopsis plants were treated individually by *B. cinerea* and ABA (Figure 3A). *PYK10* (*At3g09260*; *259009_at*), encoding a beta-glucosidase enzyme, was up-regulated in response to all individual phytohormones and *B. cinerea* infection, whereas *At1g67865* (*260012_at*) was down-regulated for all comparisons of the phytohormones and the control as well as the phytohormones and *B. cinerea* (Figure 2; Figure 3).

3.5 Heatmap

Another perspective on the relationship of regulated genes in Arabidopsis plants treated with hormones and infected with *B. cinerea* infection can be observed using the heatmap of all individual treatments (Figure 4). This heatmap indicates the expression of each gene according to treatment, in relation to each other. The top 50 up- and down-regulated genes upon *B. cinerea* infection along with the corresponding expression of these selected genes following hormonal treatments are shown, and the list of genes can be found in Appendix I.

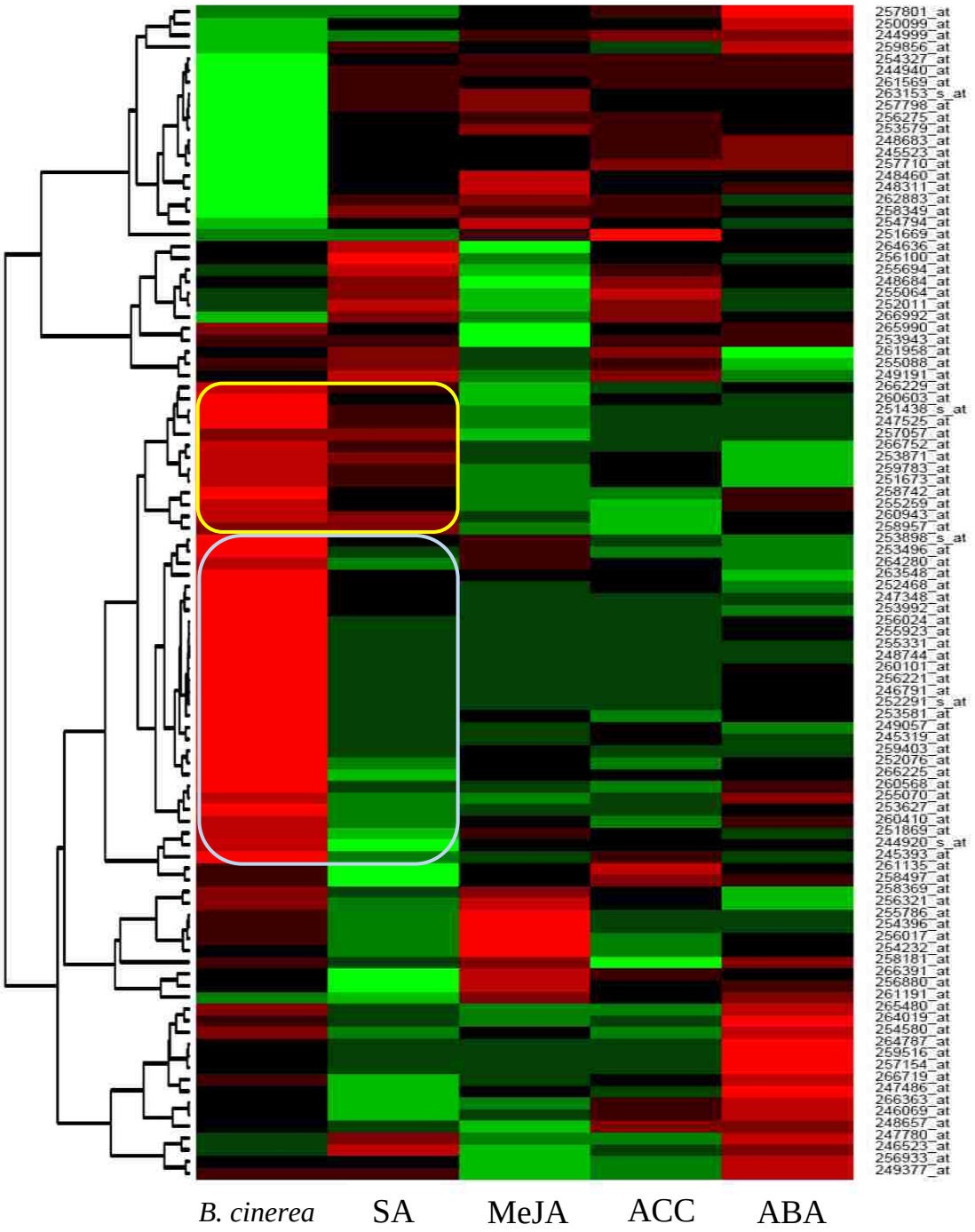

Figure 4: Heatmap representation of the most 50 up- and down-regulated microarray probes in response to *B. cinerea.*

Red and green indicate up- and down-regulated probes, respectively. The area surrounded by the blue rectangle indicates a section of up-regulated probes in response to *B. cinerea* which contrasts with the down-regulation of the same probes following hormone treatments. The area surrounded by the yellow rectangle indicates shared common up-regulation among probes for *B. cinerea* and a hormone.

Most genes/probes appear to have opposing expression levels when comparing *B. cinerea* expression patterns with the hormone treatments. In other words, probes that were up-regulated following treatment with *B. cinerea* were down-regulated following treatment with hormones (Figure 4). This indicates a general antagonism between the most 100 DEGs in response to *B. cinerea* and their corresponding expression values after hormone treatments. However, this antagonism was not observed in all probes/genes, as 18 of the genes were commonly up-regulated in plants treated with *B. cinerea* or SA (Figure 4B). According to DAVID v6.8, eight of these 18 genes were involved in membrane function, and five were involved in the oxidation-reduction process. The remaining genes fell into various other categories, such as fatty acid metabolism, transit peptides, auxin signaling pathway, and NADP.

3.6 Common Genes in Arabidopsis After Hormonal Treatments and *B. cinerea* Infection

Among all treatments (*B. cinerea* and the four hormones), 211 were commonly up-regulated and 269 were identified as commonly down-regulated genes (Table 2). *B. cinerea* and the hormone stresses identified 534, 516, 448, and 571 up-regulated genes in SA, MeJA, ACC, and ABA treated plants, respectively. The highest number of common up-regulated genes were identified between *B. cinerea* and ABA treatments. For common down-regulated genes, 540, 480, 434, and 523 genes were identified from SA, MeJA, ACC, and ABA treatments, respectively, when compared with genes during *B. cinerea* treatment. *B. cinerea* and SA shared the highest number of common down-regulated genes (Figure 5).

It is well-known that plant cells use hormones as signaling molecules to direct metabolism and membrane changes during stress, as well as to alter expression patterns (Verma et al., 2016).

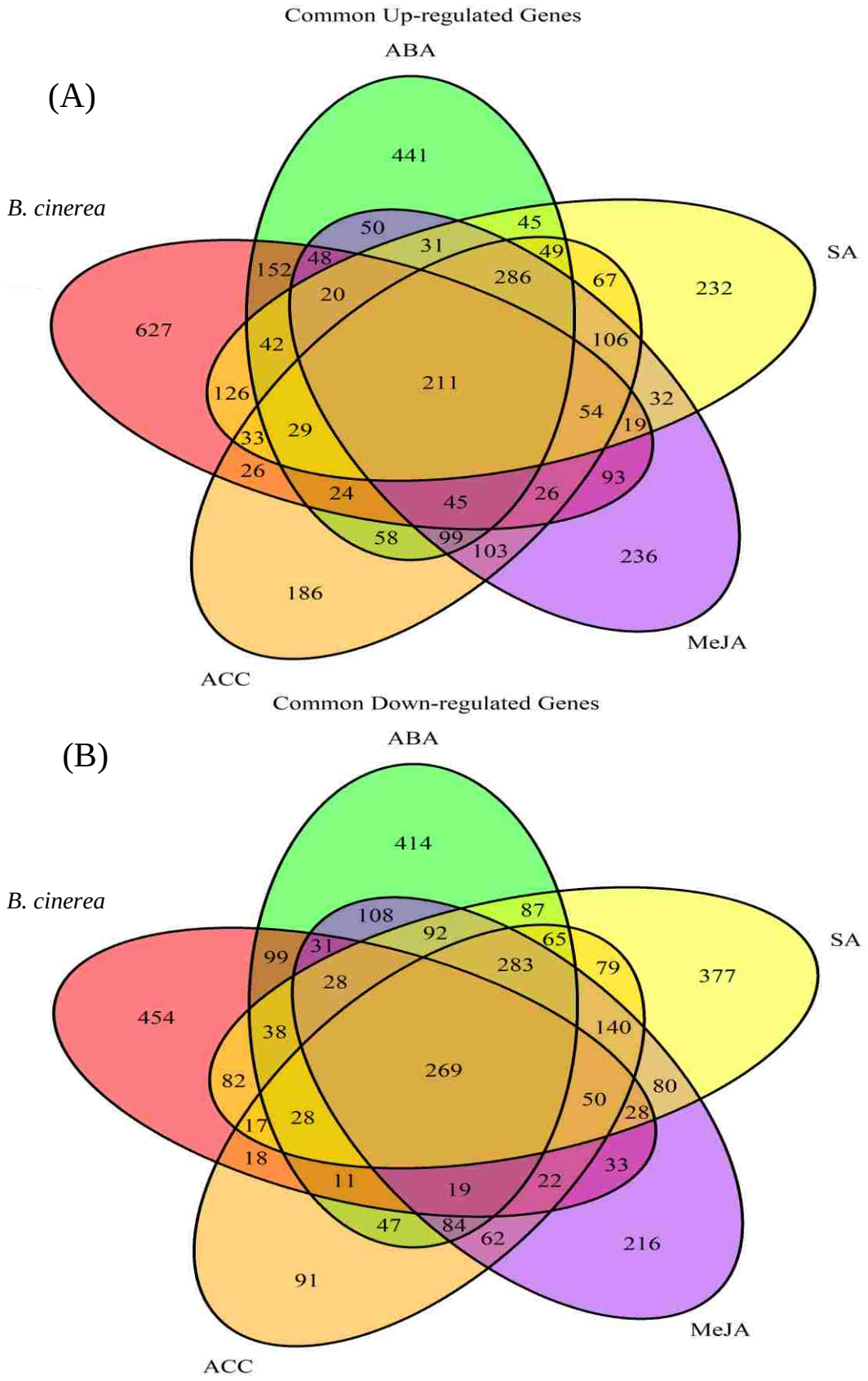

Figure 5: Venn diagrams showing the number of DEGs in response to *B. cinerea* and the hormonal stresses: SA, MeJA, ACC, and ABA.
Overlapping areas depict common DEGs among treatments. (A) Up-regulated genes; and (B) Down-regulated genes.

B. cinerea treated plants shared 152 unique up-regulated genes with ABA: the largest number of unique up-regulated genes when comparing only two treatments. Plants treated with SA shared 126 unique up-regulated genes with *B. cinerea*-treated plants (Figure 5A). ABA and SA shared the largest and second largest number of unique commonly down-regulated genes when compared with only *B. cinerea* treatment, with 99 and 82 genes, respectively (Figure 5B).

After application of MeJA and ACC, Arabidopsis plants shared the highest number (930 genes) of up-regulated genes in common than any other combination of treatments. SA and MeJA shared the highest number of common down-regulated genes than all other combination of treatments, with 970 shared down-regulated genes. Both SA and MeJA have a higher number of common up-regulated genes with ACC than the remaining two treatments, with 835 and 930 common genes, respectively.

Gene ontology (GO) analysis was performed on those genes in common with *B. cinerea* and all hormone stresses (Figure 6; Figure 7). The genes utilized for categorization can be found in the Appendix II. According to Superviewer, most common up-regulated genes fell into abiotic or biotic stimulus response, involving hydrolase activity mostly in the cell wall. As for the down-regulated genes, most were categorized as pertaining to electron transport or the energy pathways, with the second most down-regulated genes occurring in processes related to abiotic or biotic stimulus, with the third most down-regulated genes occurring in relation to stress.

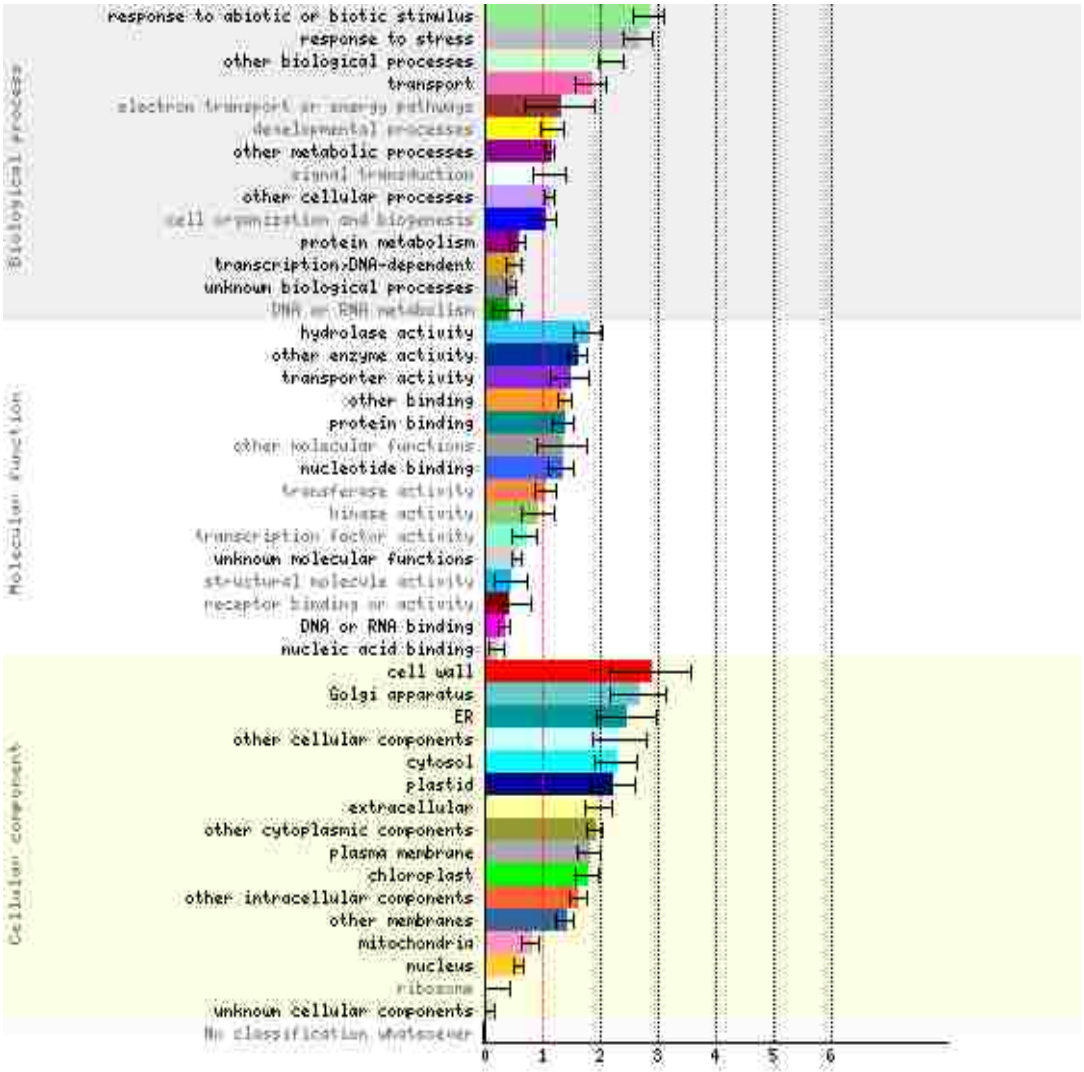

Figure 6: GO analysis categorizing identified up-regulated genes following *B. cinerea* infection and the hormone treatments SA, MeJA, ACC, and ABA. Statistical analysis of commonly up-regulated genes among all treatments. Error bars are the calculated SD, and normalized class score is displayed on the x-axis.

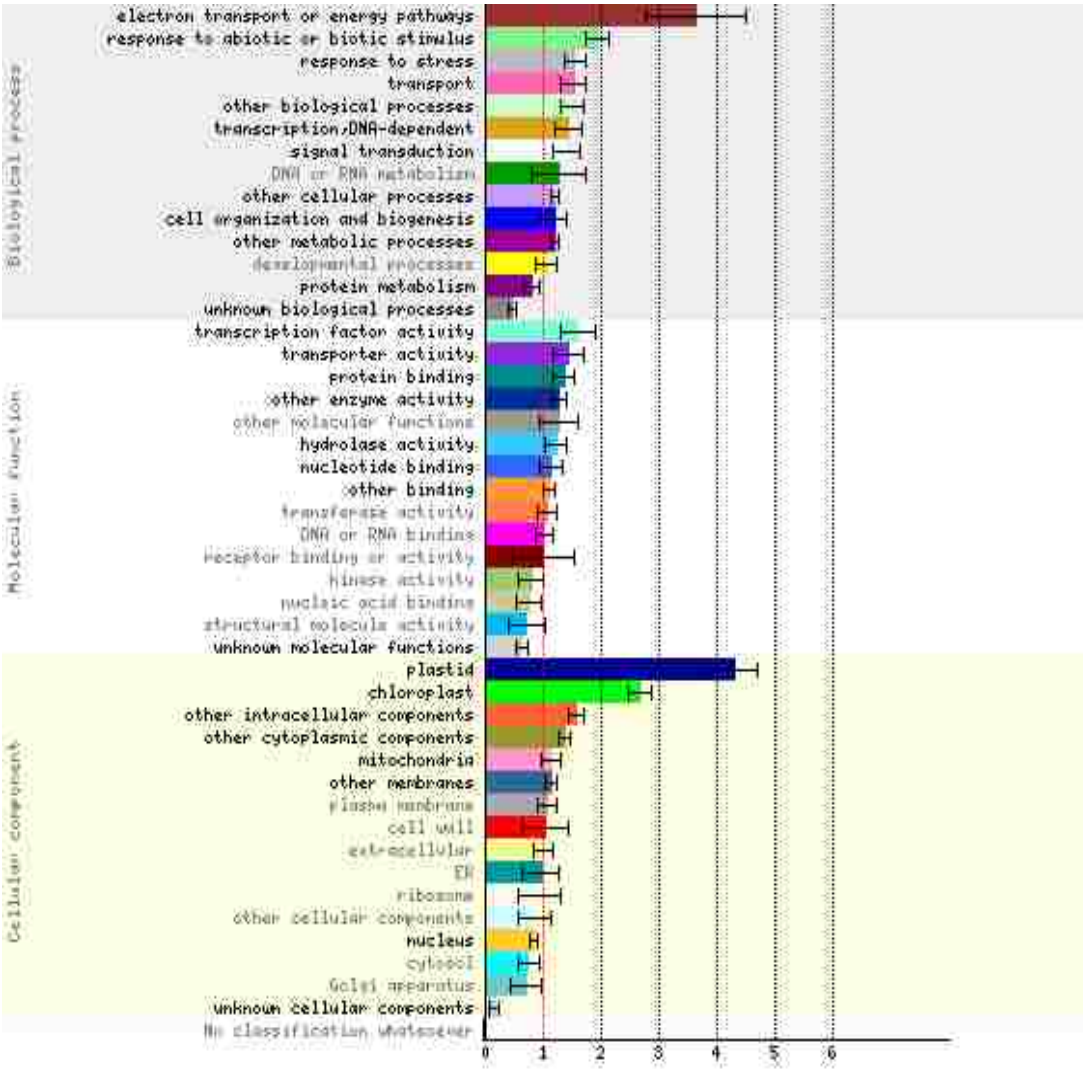

Figure 7: GO analysis categorizing identified down-regulated genes following *B. cinerea* infection and the hormone treatments SA, MeJA, ACC, and ABA.
Statistical analysis of commonly down-regulated genes among all treatments. Error bars are the calculated SD, and normalized class score is displayed on the x-axis.

3.7 Comparisons between Normalization Methods

Data handling and normalization methods play a critical role in experimental analysis, and can change the resulting picture of experiments depending on which analysis method you use. Normalization of microarray data attempts to remove technical variations, i.e. differences between microarray chips or differences in sample hybridization, that are unrelated to the biological variation of the microarray experiment (Wang et al., 2007). However, one normalization method does not suit every project, so normalization methods must be chosen depending on the needs of the project. Several normalization methods have emerged, of which MAS5.0 has been among the very first. Developed by Affymetrix, MAS5.0 normalizes each array independently, and calculates probe signal values based on perfect match (PM) and mismatch (MM) probe intensity (Affymetrix, 2001). Detection calls are generated and are used as filtering critera for probes: Present, Absent, or Marginal, with absent meaning the probe signal is below the calculated detection threshold, and marginal indicating a case of uncertainty.

MAS5.0 differs from both RMA and GCRMA, both in normalization and filtering methods. Unlike MAS5.0, which normalizes each chip independently. RMA and GCRMA use a multi-chip model based on the robust average of $\log_2$ background-corrected PM intensities (Bolstad et al., 2003). Filtering for RMA and GCRMA was performed based on variance, not detection calls (Hackstadt and Hess, 2009).

RMA and GCRMA differ only in the adjustment of PM values: GCRMA takes into account the sequence information, specifically the G/C content of probes

when calculating the background adjustment, whereas RMA does not (Wu et al., 2011). The difference in normalization methods can be observed in Figures (8 and 9).

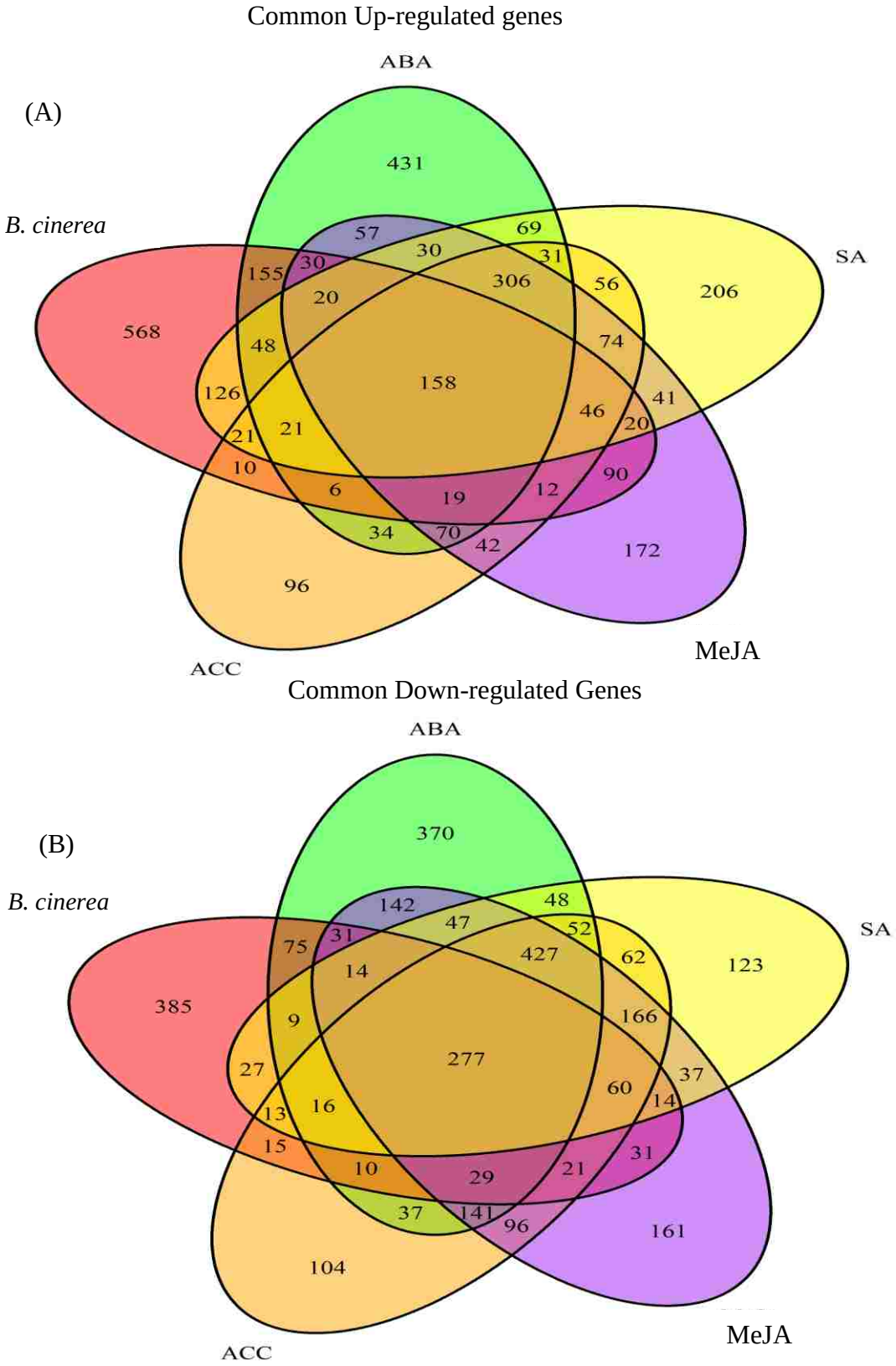

Figure 8: Venn diagrams of up- and down-regulated RMA normalized genes in response to B. cinerea and the hormonal stresses: SA, MeJA, ACC, and ABA. Overlapping areas depict common DEGs among treatments. (A) Up-regulated genes; and (B) Down-regulated genes.

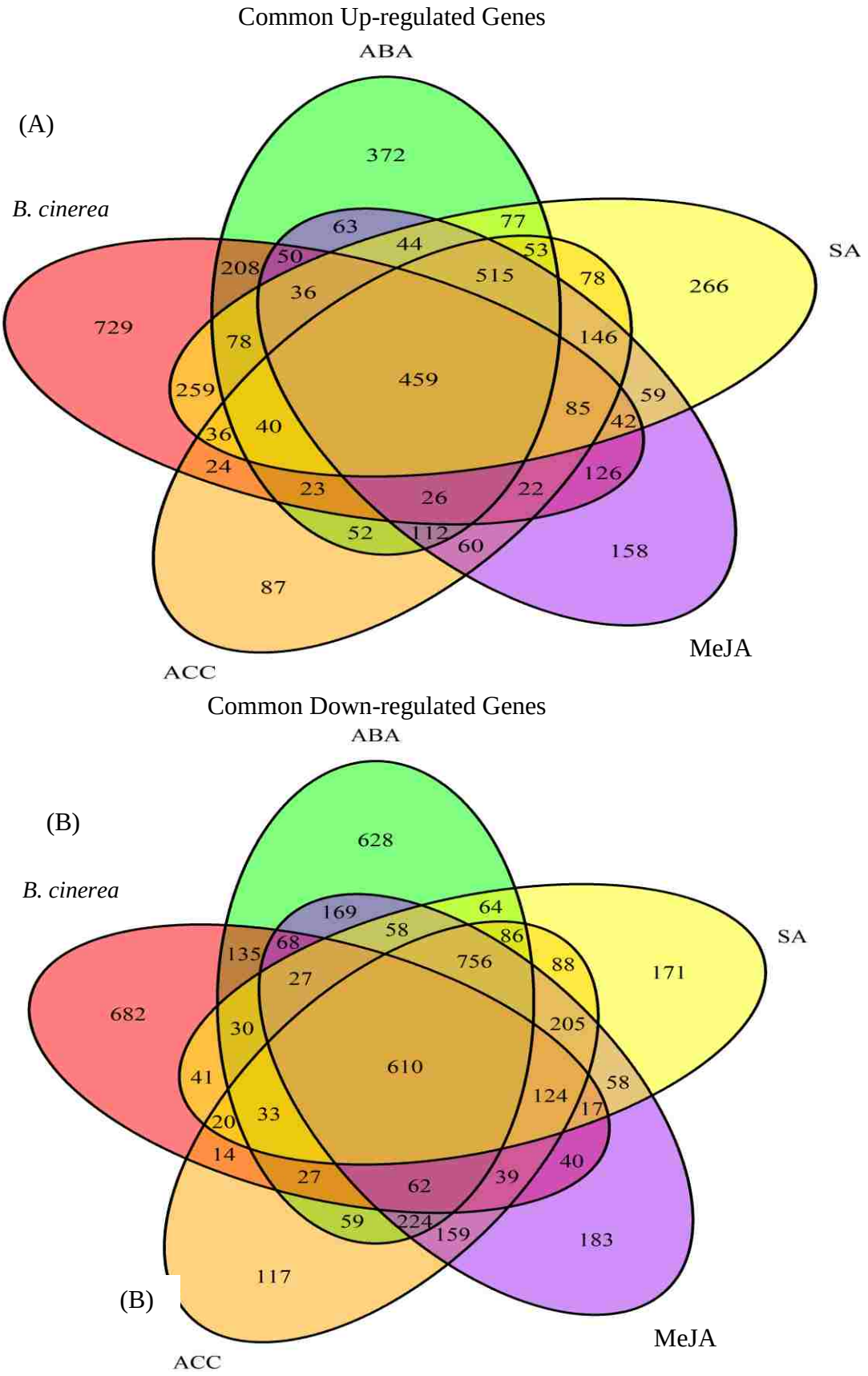

Figure 9: Venn diagrams of up- and down-regulated GCRMA genes in response to *B. cinerea* and the hormonal stresses: SA, MeJA, ACC, and ABA.
Overlapping areas depict common DEGs among treatments. (A) Up-regulated genes; and (B) Down-regulated genes.

Among all three normalization methods, 101 up-regulated and 148 down-regulated genes are common to all MAS5.0, RMA, and GCRMA normalizations. Of these commonly identified genes, ten were used for the qRT-PCR analysis. These genes and expected expressions are found below in Table 3:

Table 3: qRT-PCR genes common to all normalization methods.
Expected fold changes taken from normalized *B. cinerea* infection data.

Array Element	**Locus Identifier**	**Annotation**	**MAS5.0 Fold Change**	**RMA Fold Change**	**GCRMA Fold Change**
263548_at	*At2g21660*	CCR2	32.6	24	34.9
258957_at	*At3g01420*	αDOX1	27.9	7.7	17.7
255331_at	*AT4G04330*	RBCX1	39.9	14.6	82.6
256221_at	*At1g56300*	Chaperone Heat Shock Protein	26.7	27.8	649.2
252076_at	*At3g51660*	Tautomerase	17.8	18.5	68.4
256017_at	*At1g19180*	JAZ1	15.9	10.8	18
248683_at	*At5g48490*	DIR-LIKE	-13.7	-14.3	-25
264636_at	*At1g65490*	Transmembrane Protein	-9.7	-11.1	-14.3
248460_at	*At5g50915*	BHLH	-9.4	-10	-100
266363_at	*At2g41250*	HAD	-7.9	-7.1	-11.1

GCRMA normalization produced significantly different fold changes for genes *At1g56300* and *At5g50915* than both MAS5.0 and RMA normalization. Although the numerical values vary greatly between certain genes and the

normalization methods, there are no unexpected sign changes. This indicates qRT-PCR validation should reveal induction of proposed up- and down-regulated genes, respectively.

3.8 Validation of Microarray Analysis Using qRT-PCR

To verify the findings of the microarray analysis, qRT-PCR was performed on Arabidopsis wild-type plants infected with *B. cinerea*. The selected genes were nominated for qRT-PCR to validate their regulation in response to *B. cinerea* as potential targets for molecular marker consideration and future functional analysis in Arabidopsis. Genes showing similar induction patterns (up/down) between the microarray analysis and qRT-PCR indicate potential molecular markers for *B. cinerea* and the hormonal stresses, and/or genes that effect the phenotype/response of mutants or overexpression transgenic Arabidopsis lines in response to these stresses. The expression fold change of the qRT-PCR is found below in Figure (10), and the expected fold changes from the microarray analysis are listed below in Table (4).

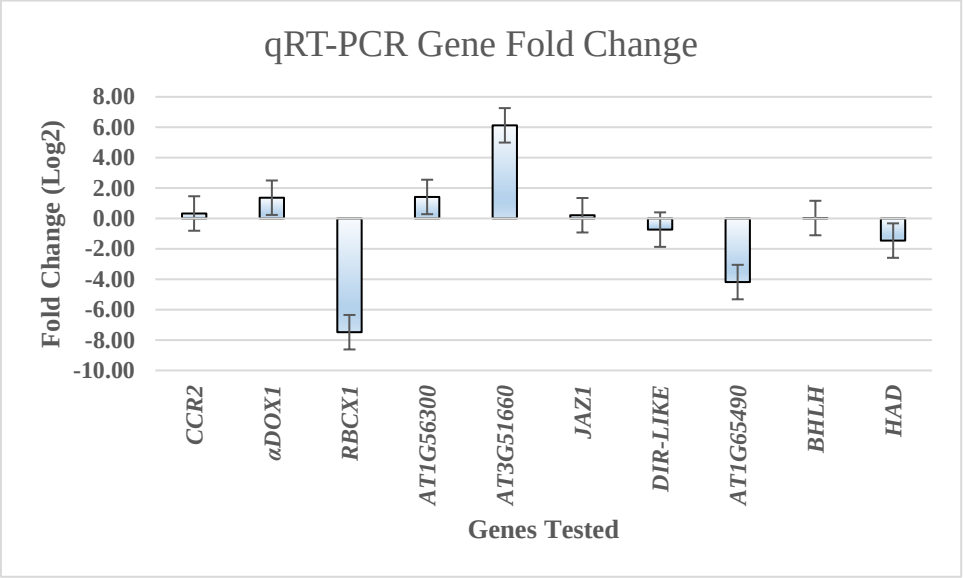

Figure 10: qRT-PCR fold change of ten selected DEGs in response to *B. cinerea*. qRT-PCR log_2 fold changes for the selected DEGs quantifying induction or repression based on RNA extracted at 18 hpi from 4-week-old Col-0 Arabidopsis plants.

Table 4: Calculated fold changes from microarray analysis of Arabidopsis plants infected with *B. cinerea*.

Gene	Probe	MAS5.0 Fold Change (log_2)	RMA Fold Change (log_2)	GCRMA Fold Change (log_2)	qRT-PCR ExpresionValues
CCR2	*263548_at*	5.0	4.6	5.1	0.3
αDOX1	*258957_at*	4.8	2.9	4.1	1.4
RBCX1	255331_at	5.3	3.9	6.4	-7.5
At1g56300	*256221_at*	4.7	4.8	9.3	1.4
At3g51660	*252076_at*	4.1	4.2	6.1	6.1
JAZ1	*256017_at*	3.9	3.4	4.2	0.2
DIR-LIKE	*248683_at*	-3.8	-3.8	-4.6	-0.7
At1g65490	*264636_at*	-3.3	-3.5	-3.8	-4.2
BHLH	*248460_at*	-3.2	-3.3	-6.6	0.03
HAD	*266363_at*	-2.9	-2.8	-3.5	-1.5

According to the expected fold changes from the microarray analysis and the qRT-PCR results, four genes appear to be excellent candidates for future experiments: *αDOX1*, *At3g51660*, *At1g65490*, and *HAD*. Of the ten selected genes, these four genes are most similar when comparing the calculated qRT-PCR values to

the actual qRT-PCR values. These four genes' calculated qRT-PCR values from the microarray data are validated by the resulting qRT-PCR data, and display the lowest discrepency between calculated and expected values, indicating these genes are promising candidates for future *in vivo* studies. Of these four genes, *At1g65490* has been implicated as a potential susceptibility factor for necrotrophic fungi in Arabidopsis (Dobon et al., 2015). This gene and the three other genes should be used for future studies to elucidate the functional characteristics of their gene products, to determine their role in *B. cinerea* defense responses, and to determine whether these genes can be used as molecular marker genes for *B. cinerea*. These are possible molecular markers and are likely to effect Arabidopsis response (susceptibility/resistance) to these stresses. However, some discrepancies do exist between the expected fold change determined by the microarray data and the qRT-PCR results., not only between the magnitudes of fold change but also in the direction (up-/down-regulation) of two genes. *RBCX1* is expected to be up-regulated by 5.3-fold according to Table 4, but the opposite is observed for the qRT-PCR data: *RBCX1* is down-regulated by 7-fold, the greatest magnitude of all qRT-PCR results. *BHLH* also appears to have the opposite expression pattern than expected, with an insignificant increase in expression of 0.03-fold as opposed to the expected decrease of expression by 3.2-fold as seen in Table 4. These two genes are unlikely candidates, though the remaining genes follow the expected induction and repression patterns reaffirming their effectiveness as potential molecular markers or functionality in protecting Arabidopsis to the tested stresses.

Chapter 4: Discussion

There is a complex relationship between plant response to *B. cinerea* and the initiation/maintenance of that response by plant hormones. SA has been shown to negatively affect Arabidopsis resistance to *B. cinerea*, while JA and ET have been shown to have a positive effect (Nie et al., 2017; Thomma et al., 1999; Li et al, 2019; Zhang et al., 2017; Cerrudo et al, 2012; AbuQamar et al., 2017). The common DEGs upon infection with *B. cinerea* and treatment of SA reinforce the idea of a relationship between the pathogen and this hormone. There is evidence that *B. cinerea* manipulates the accumulation of SA in the host plant to promote pathogen growth, so it is possible the manipulation of these common genes may result in the accumulation of SA, helping the pathogen develop and flourish (Oirdi et al., 2011). *NPR1* plays a central role in the regulation of SA-induced defense genes was found to be up-regulated by more than 2-fold in Arabidopsis.

Many common genes are also down-regulated when comparing *B. cinerea* and SA stress. Certain *B. cinerea* strains have been shown to produce ABA, which, in Arabidopsis, has in turn been demonstrated to promote SA biosynthesis (Seo and Park, 2010). It may be the production of ABA from *B. cinerea* which causes SA biosynthesis in Arabidopsis that produces the similar expression profiles when Arabidopsis is exposed to these two stresses. The genes identified by this study pave the direction for future studies to generate a better understanding of the interaction between *B. cinerea* and SA. If *B. cinerea* does manipulate the regulation of SA-repressed genes for its benefit, plant resistance to *B. cinerea* may be improved in mutants or through RNAi silencing of these genes to prevent an increase in SA over

a specific threshold. This could provide a practical solution which may improve crop resistance to *B. cinerea*.

In contrast to the expression patterns caused by *B. cinerea* and SA, an antagonistic relationship is revealed when comparing *B. cinerea* expression patterns to those of MeJA and ACC. Among these three treatments, only 45 up-regulated and 19 down-regulated genes were identified in common. Few genes are commonly regulated between these two hormonal stresses and *B. cinerea*. This is expected as the ET/JA signaling pathway has been documented to assist in the defense against *B. cinerea*. Similarly expressed genes to both *B. cinerea* and MeJA/ACC would be unlikely, as the former stress attacks the organism while the latter two stresses are implicated in its defense (Gautam et al., 2018).

Similar to *B. cinerea* and SA stresses, ABA also shares many DEGs in common with treatment with the necrotrophic fungi. This is unsurprising, as *B. cinerea* produces ABA. The presence of ABA produced by *B. cinerea* should elicit similar expression patterns *in planta* (Ding et al., 2016). Though, there are discrepancies concerning the effectiveness of ABA in increasing resistance to *B. cinerea* among plant species *i.e.*, negative regulation of ABA has been found to be critical for *B. cinerea* resistance in Arabidopsis (Liu et al., 2015).

Several DEGs were identified following *B. cinerea* and the hormone treatments, in general, the common DEGs encode signaling, membrane, metabolic, nucleus-related or chloroplast-related proteins. This suggests that hormonal changes and pathogen infection lead to changes in gene expression, which in turn leads to changes in membrane and metabolic proteins when defensive measures are taken to protect the plant cell (Chapman, 2000; Foyer and Noctor, 2005). Identifying common

genes that are up- or down-regulated between hormonal treatments and treatment with *B. cinerea* provides insights into the pathways that confer *B. cinerea* resistance in Arabidopsis. Future direction towards the characterization of the function the main DEGs/biomarkers identified in this study will lead to possible contributions to resistance against the pathogen in question. The commonly-regulated genes identified in this work should be functionally characterized, and such mutants must be assayed for disease response to determine the strength of these genes on defending Arabidopsis and other plants against *B. cinerea* infection.

The genes of interest identified from the scatterplot analyses of the hormonal treatments and treatment with *B. cinerea* further support evidence of connectivity between *B. cinerea* and the four hormones SA, JA, ET, and ABA. *At1g73260* (probe *260101_at*) was strongly up-regulated in response to *B. cinerea* infection (Figure 1). This gene encodes Kunitz Trypsin Inhibitor 1, a protein likely involved in controlling programmed cell death (PCD) during plant-pathogen interactions and is induced by SA (Jing et al., 2008). The observation that the strongly up-regulated gene in response to *B. cinerea* is also inducible by SA highlights the importance of SA regulation in *B. cinerea* defense and identifies this gene as an important player in understanding hormone-*B. cinerea* interactions. *PYK10* (At3g09260; 259009_at) was another identified down-regulated gene when Arabidopsis wild type plants were inoculated with *B. cinerea*. *PYK10* is known to restrict root colonization by an endophytic fungus and has also been demonstrated to be inversely regulated with *PDF1.2* (Sherameti et al., 2008). Further investigations on testing *At1g73260* and *PYK10* mutants for disease responses against *B. cinerea* infection have to be established.

Three highly expressed genes were identified in response to MeJA: the senescence-associated protein *At1g53885* (*262226_at*), *DEFL* (*At4g22212*; *254361_at*) and *CORI3* (*At4g23600*; *254232_at*). *At1g53885* and *At4g22212* can be considered as excellent candidates for future research as these two genes have not been characterized upon infection to *B. cinerea*. On the other hand, *CORI3* (*At4g23600*) is known to play an important role in JA signaling and defense responses (Devoto et al., 2005). It is likely these three genes play an important role in Arabidopsis defense against *B. cinerea*.

Following ACC treatment, *At1g05250* (*264567_s_at*) and *AIR1* (*At4g12550*; *254828_at*) were highly induced. *At1g05250* encodes a peroxidase involved in the lignification of cell walls (Shigeto et al., 2013) and *AIR1* (*At4g12550; 254828_at*) is a membrane-spanning protein shown to be induced by auxin-rich callus induction medium (Che et al., 2002). Lignification of cell walls occurs during multiple stresses, but in relation to pathogen infection, lignification attempts to provide a barrier preventing the infection from spreading. Less is known about *AIR1*, however, but an assumption can be made as both are highly expressed during ACC stress: it is possible *AIR1* plays a role in pathogen/stress detection, or signal transduction from the extracellular matrix. Future studies should garner more information on *AIR1* and the role it plays during infection with *B. cinerea*. Two more genes of interest were identified following ACC treatment: *AT2G06890*; *266214_at* and *AT3G24982*; *257099_s_at*. The first gene, *AT2G06890*, is a transposable gene that was also identified as up-regulated during ABA treatment, along with *COR78/RD29A (AT5G52310*; *248337_at*) (Figure 2D). The up-regulation of *COR78/RD29A* follows as expected, as this gene is known to be up-regulated by the phytohormone ABA (Shinozaki and Yamaguchi-Shinozaki, 1999).

Interestingly, *PYK10* and *At1g67865* (*260012_at*) were found to be highly up- and down-regulated, respectively, in response to *B. cinerea* infection and all individual hormone treatment. *PYK10* is involved in plant defenses against pathogens (Sherameti, *et al.*, 2008), as mentioned above, though it has not yet been demonstrated to be effective in increasing Arabidopsis resistance to *B. cinerea*. So far, there is no information about *At1g67865*'s role in plant defense. More research on *PYK10 and At1g67865* is recommended to determine their role in Arabidopsis response to *B. cinerea* and the hormones used in this study.

Hormone defense networks are incredibly complex, and detailed genetic studies of these networks are incredibly valuable when elucidating the mechanics of plant hormone defenses. A thorough understanding of hormonal signaling networks in plants and a complete understanding of the genetic underpinnings of these signaling networks is crucial to engineering appropriate defense strategies against *B. cinerea* or other pathogens. Just as the interactions between the hormones and *B. cinerea* are important, the interactions between the hormones themselves must not be neglected. The mutual antagonistic relationship between SA and JA is well known (Thaler et al., 2012), but the finer details of the crosstalk between these two must be elucidated in future studies. However, two genes have been identified to play a role in this antagonistic relationship: *GLYI4* and *ARR11* (Proietti et al., 2018). A *glyI4* Arabidopsis mutant was found to prevent SA-mediated suppression of *PDF1.2* (a JA-induced gene whose protein is involved in activating Arabidopsis defense responses against necrotophic pathogens (Veronese et al., 2004)), which in turn increased resistance to *B. cinerea*. *ARR11* mutants however displayed an opposite effect to *GLYI4* mutants, as SA-mediated repression of *PDF1.2* was promoted instead of prevented. Interestingly, microarray analysis revealed *GLYI4* was down-

regulated upon treatment with SA and ABA, and up-regulated after treatment with *B. cinerea* and MeJA. It is peculiar SA and *B. cinerea* have opposing regulatory effects on *GLYI4*, considering *B. cinerea* infection results in the biosynthesis of SA in Arabidopsis. *ARR11* was not identified as a DEG in any treatment.

JA and ET are known to be synergistic in Arabidopsis defense against *B. cinerea*, but the large number of shared up-regulated genes between SA and ET hint at a possible link between these two hormones as well. Though mostly implicated in herbivorous insect defense, ABA has also been demonstrated to be linked with SA in interacting with the JA pathway, and must not be neglected (Pieterse et al., 2012). In combination with JA, ABA has been demonstrated to suppress *PDF1.2* expression (a JA-induced gene), leading to compromised resistance to necrotrophs (Anderson et al., 2004). The ability for *B. cinerea* strains to produce ABA results in increased susceptibility in the host organism based on this single gene change, and likely is compounded with other untested ABA signaling pathway interactions.

Based on the microarray analysis, 0.9% and 1.2% of the genes in the Arabidopsis genome were found to be up- and 1.2% down-regulated genes, respectively, to all tested stresses. Many of these common up-regulated genes encode plasma membrane proteins or signaling molecules, whereas many common down-regulated genes were shown to be sublocalized to the chloroplast. This is not completely unexpected, as chloroplasts are known to be involved in the synthesis of the phytohormones SA, JA, and ABA (Serrano et al., 2016). It would be interesting for a future study to focus on the changes in the chloroplast and differences in biosynthesis of these hormones upon infection with *B. cinerea.* The crosstalk of plant response to *B. cinerea* and SA, MeJA, ACC, and ABA as signaling molecules as

well as the common DEGs related to the chloroplast will open a new era for future molecular plant-microbe interaction research to investigate the intricacies of plant hormone biosynthesis in response to *B. cinerea*.

Following identification of genes of interest from microarray data, *in vitro* and *in vivo* verification is the next important step in understanding the information gathered *in silico*. This study utilized qRT-PCR to validate the microarray analysis performed, as opposed to *in vivo* verification. The importance of verifying data generated from *in silico* can be recognized by comparing the microarray analysis in this report with that of the results published by Goda et al. (2008). Both studies used the same raw microarray data for MeJA, ACC, and ABA treatments, yet different genes of importance were gleaned from both studies. The differences in genes may be attributed to the post-processing analysis of the normalized data, the different controls, and the different growth conditions. Personal and technical variability (known as "batch effects") can also be added as another major variable (Johnson, *et al*., 2007).

Considering the great amount of possible interpretations during data processing, it is not so surprising that people cannot agree on normalization methods: a normalization method is a statistical model that tries to explain what happens on the gene chips. Different models have different assumptions; some methods measure the effects within the array, while others measure the effects between arrays. Thus, the normalization methods MAS5.0, RMA, and GCRMA are all viable techniques when one analyzes DEGs of a particular microarray. Many studies have tested the efficiencies of MAS5.0, RMA, and GCRMA normalization methods with different results (Millenaar et al., 2006; Lim et al., 2007).

Consistent with previous publications, microarray data analyses generated in this study comparing different normalization techniques, confirmed what other researchers have previously concluded on different numbers of DEGs. 480 probes (2%) were identified with MAS5.0, and 249 probes (1%) were found to share similar expression patterns among all three normalization methods. It was proposed that DEGs identified in common with multiple normalization techniques can offer more precision when selecting genes of interest from microarray data, meaning these genes identified in common to all three normalization methods could be excellent candidates to elucidate their role in pathogen resistance (Millenaar et al., 2006). In general, the identified probes/genes of each individual normalization method, and all three methods combined, represent DEGs with great potential in coordinating plant hormone response and *B. cinerea* resistance/phenotype.

Following GO analysis of the identified genes, it seems the classified up-regulated genes were involved in abiotic or biotic stimulus responses involving hydrolase activity mostly in the cell wall, whereas the down-regulated genes effected transcription factor and transporter activity within the plastids and chloroplasts. This agrees with the DAVID v6.8 analysis as the majority of genes were classified into membrane and oxidation-reduction functions, along with signaling molecules involved in stress response and cellular metabolism. This follows as the cell walls, plastids, and chloroplasts have membranes, and play roles in stress response, signaling molecules, and cellular metabolism. The chloroplasts are also involved in oxidation-reduction functions.

Ten DEGs identified from the microarray analysis were verified by performing qRT-PCR analysis. Most genes displayed different magnitudes of

expression from the normalized data, and *RBCX1* and *BHLH* were observed with opposite than expected expression levels when comparing between the microarray data and the qRT-PCR analysis. It is noteworthy to mention that the fold-difference in gene expression between the two RNA expression techniques could be due to different plant growth conditions, pathogen inoculation procedures or the stringency conditions in both techniques. Previous literatures have pinpointed the general discrepancies in gene expression between microarray and qRT-PCR techniques for particular stresses indicating the results obtained in this study are still dependable even with these minor discrepancies in microarray and qRT-PCR analyses (Gyorffy et al., 2009).

The amplification efficiency or housekeeping gene of the qRT-PCR could also explain the discrepancies between the microarray and qRT-PCR methods (Gyorffy et al., 2009). Regardless, the accuracy of the results identified by this study are reinforced when considering that other DEGs identified from the microarray and used for qRT-PCR were found to be near the calculated pRT-PCR values from the microarray results. As such, the differences in up- and down-regulation for the two aforementioned probes may stem from the control used for the microarray analysis as opposed to simply originating from either the qRT-PCR data or from the data normalization itself. Reproducing the microarray experiment to eliminate differences in growth conditions is recommended to better understand the discrepancies between the analyzed microarray data and the qRT-PCR data, though this does not mean the data generated in this study is inaccurate. The results generated by this study are valuable and must be considered as such.

This work identified candidate genes that could be used to determine Arabidopsis' response to hormones and to an economically important pathogen. This research is the first step in determining the expression of potential genes effective in increasing plant resistance to *B. cinerea*. Moreover, universal regulation of particular genes will help plant pathologists in breeding programs to confer resistance to necrotrophic pathogens including *B. cinerea*. Future research should aim at elucidating the function of the identified genes from this study and investigating their expression patterns in response to other pathogens and abiotic stresses in Arabidopsis and other important plants, such as Solanaceae and other crops. In addition, these genes can be used as molecular markers upon *B. cinerea* infection. Molecular markers for *B. cinerea* infection in crops enables easy detection during early stages of infection, as well as the possibility to identify between *B. cinerea* strains. Due to *B. cinerea*'s tendency for quick genetic and morphological diversity among sequential generations, molecular markers provide researcher's with easy distinguishability between rapidly evolving strains (Zhao et al., 2009). Expression of the identified genes at the protein level (proteomics) can also be investigated to find out the role of the protein translationally and post-translationally. The identified genes in this work have the potential to improve resistance to *B. cinerea* in plants through the manipulation of hormone levels by genetic means, opening the door for finding a genetic solution to a problem that plagues food importation without necessitating additional costs for added preventative growth/transport measures or chemical preservatives. This would be an excellent solution for the UAE and other countries whom grow and import crops susceptible to *B. cinerea*. Future studies are urged to functionally verify the effectiveness of the identified genes in defending multiple plant species against *B. cinerea*.

Chapter 5: Conclusion

In this work, common genes in response to *B. cinerea*, SA, MeJA, ACC and ABA treatments in Arabidopsis were identified. Such identified genes, *αDOX1*, *At3g51660*, *At1g65490*, and *HAD* are of importance based on microarray data and qRT-PCR results. These genes may be used as molecular markers of hormonal stresses and of *B. cinerea* infection. This work paves the way for further research to characterize the functions of identified common DEGs to determine their ability to confer resistance to *B. cinerea* in Arabidopsis. The identified common genes may serve as potential candidates for their disease resistance against *B. cinerea*.

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Appendix

Table 5: The top 100 most DEGs after *B. cinerea* infection

Gene	Expression Value	Fold Change	P-Value
AT2G07751;ATMG00990	80.35945	-4.34	< 0.0001
ATCG01230;ATCG00905	495.2843	-6.12	0.0022
ATCG00190	40.75962	-6.22	0.0045
AT4G16146	242.0306	13.37	0.2025
AT4G16260	1841.18	28.98	0.2522
AT5G20220	33.73721	-4.9	0.003
AT5G15850	93.26201	-6.16	0.0022
AT5G27280	541.4935	11.12	0.0701
AT5G63810	170.7898	6.69	0.4469
AT5G62140	51.35618	-4.97	0.0102
AT5G61380	230.8281	8.18	0.3054
AT5G58770	66.64031	-7.44	0.0003
AT5G52570	48.6381	-11.1	0.0018
AT5G50915	22.9682	-9.38	0.0186
AT5G48570	26.70439	-7.81	0.0017

Table 5: The top 100 most DEGs after *B. cinerea* infection (Continued)

Gene	Expression Value	Fold Change	P-Value
AT5G48490	83.03767	-13.73	0.0003
AT5G48485	125.1526	-6.05	0.0009
AT5G48250	186.0698	7.08	0.3379
AT5G44480	244.3172	10.17	0.4388
AT5G42760	26.39117	-9.7	0.0003
AT5G40690	196.1816	11.91	0.5117
AT5G17300	30.56133	-5.7	0.0147
AT3G59930;AT5G33355	371.397	8.35	0.5887
no_match	148.7752	-4.3	0.0222
AT3G57180	22.66203	-4.59	0.002
AT3G57240	171.3452	14.1	0.0418
AT3G54500	242.0027	-4.38	0.0077
AT3G52720	60.38016	-6.45	0.0264
AT3G51660	540.832	17.78	0.1589
AT3G49120;AT3G49110	2073.541	7.56	0.213
AT3G46970	915.6175	8.85	0.2092

Table 5: The top 100 most DEGs after *B. cinerea* infection (Continued)

Gene	Expression Value	Fold Change	P-Value
AT4G31870	59.04578	-6.12	0.017
AT4G30610	35.65821	-6.32	0.0074
AT4G30660	450.7269	12.95	0.1946
AT4G27440	1806.809	10.11	0.1635
AT5G54810;AT4G27070	2762.436	11.17	0.0599
AT4G27030	67.94348	-5.4	0.0057
AT4G26060	120.1385	6.16	0.2215
AT4G23600	1538.448	24.81	0.164
AT4G22490	25.72446	-11.84	0.0001
AT4G21680	347.3142	15.47	0.49
AT4G19390	266.8831	7.38	0.0995
AT4G12970	46.96045	-6.51	0.0027
AT4G08950	86.38642	-8.78	0.002
AT4G09020	193.5122	10.4	0.228
AT4G09350	52.6354	-4.66	0.0058
AT4G05020	526.927	7.4	0.2591

Table 5: The top 100 most DEGs after *B. cinerea* infection (Continued)

Gene	Expression Value	Fold Change	P-Value
AT4G04330	551.0545	39.91	0.3405
AT4G00050	64.08497	-6.13	0.0026
AT1G22180	125.5514	9.52	0.1828
AT1G19180	711.4195	15.92	0.2099
AT1G58340	216.2794	10.58	0.4954
AT1G13750	196.7348	7.05	0.0007
AT1G56300	476.0626	26.74	0.1913
AT3G12320	171.0102	-4.29	0.0195
AT3G12110	40.37265	-4.2	0.0364
AT1G55020	309.4859	6.26	0.476
AT3G26450	168.1453	-6.11	0.0002
AT3G22600	1238.845	10.49	0.1947
AT3G15310	21.76381	-5.55	0.0017
AT3G27210	73.25531	6.12	0.1838
AT3G27350	30.54429	-5.69	0.0074
AT3G21670	223.9476	-5.47	0.0042

Table 5: The top 100 most DEGs after *B. cinerea* infection (Continued)

Gene	Expression Value	Fold Change	P-Value
AT3G17609	25.84681	-8.11	0.0006
AT3G14310	2313.873	8.7	0.0034
AT3G02380	203.3274	-4.8	0.0144
AT3G05800	113.5182	6.54	0.4759
AT3G01420	1391.776	27.9	0.5299
AT1G17745	1216.521	7.19	0.2847
AT1G20450	1075.235	6.27	0.0573
AT1G29510	54.26837	-4.43	0.0621
AT1G68440	96.1777	-6.93	0.0184
AT1G73260	4247.215	49.96	0.5155
AT1G69870	653.9339	8.08	0.1687
AT2G43570	810.8535	8.23	0.3998
AT1G55960	182.5542	-7.52	0.0063
AT1G45145	1050.396	7.87	0.3409
AT1G19610	27.03258	-4.85	0.0528
AT1G32900	110.8076	-6.67	0.0072

Table 5: The top 100 most DEGs after *B. cinerea* infection (Continued)

Gene	Expression Value	Fold Change	P-Value
AT1G01060	94.59484	-9.76	0.006
AT1G64500	60.61129	-5.66	0.0022
AT1G64780	19.80084	-25.15	0.0057
AT2G21660	2426.205	32.59	0.2516
AT2G21130	215.3299	6.24	0.2726
AT1G61820	67.96889	6.18	0.6158
AT1G65490	152.6022	-9.69	0.0018
AT2G17840	285.7472	7.18	0.1801
AT2G20670	225.7693	-4.33	0.006
AT2G15970	1527.388	7.78	0.2382
AT2G24280	87.64476	-4.38	0.0014
AT2G28900	963.1797	6.72	0.3851
AT2G28840	929.1705	8.63	0.0798
AT2G41250	85.02591	-7.92	0.0006
AT2G41290	37.34616	-4.93	0.0026
AT2G46830	131.5324	-5.41	0.0066

Table 5: The top 100 most DEGs after *B. cinerea* infection (Continued)

Gene	Expression Value	Fold Change	P-Value
AT2G47000	282.9038	6.68	0.5115
AT2G39200	28.02112	-4.56	0.405
AT2G34810	271.0515	6.06	0.397

Table 6: DEGs used in gene ontology

Up-Regulated Genes in Common with *B. cinerea,* SA, MeJA, ACC, and ABA.	Down-Regulated Genes in Common with *B. cinerea,* SA, MeJA, ACC, and ABA.
AT2G39725	AT2G07751
AT4G16146	ATMG00990
AT4G16260	ATMG01000
AT4G17100	ATCG01230
AT4G17140	ATCG00905
AT4G17140	ATCG00600
AT4G15910	ATCG00860
AT1G28290	ATCG01280
AT5G04170	ATCG00190
AT5G11110	ATCG00330
AT5G19440	AT2G45340
AT5G08380	AT1G44446
AT4G37010	AT4G16890
AT4G36760	AT4G15430
AT1G31850	AT5G28750
AT1G16560	AT5G19850
AT3G56720	AT5G13090
AT5G27280	AT5G20220
AT5G26570	AT5G20230
AT5G24910	AT5G20380
AT5G67500	AT4G36730
AT5G65640	AT4G37080
AT5G65020	AT1G31920
AT5G64860	AT1G57770
AT5G63810	AT5G16140
AT5G62900	AT5G15850
AT5G62530	AT1G50730
AT5G62720	AT5G26820

Table 6: DEGs used in gene ontology (Continued)

Up-Regulated Genes in Common with *B. cinerea,* SA, MeJA, ACC, and ABA.	Down-Regulated Genes in Common with *B. cinerea,* SA, MeJA, ACC, and ABA.
AT5G61760	AT5G25630
AT5G61380	AT5G67440
AT5G60460	AT5G66470
AT5G58600	AT5G65730
AT5G57110	AT5G64850
AT5G56630	AT5G64940
AT5G56350	AT5G63480
AT5G54970	AT5G62140
AT5G48250	AT5G61590
AT5G46230	AT5G59750
AT5G45840	AT5G58770
AT5G44480	AT5G58120
AT5G43780	AT5G57560
AT5G43100	AT5G56600
AT5G42890	AT5G56850
AT5G42570	AT5G55300
AT5G42420	AT5G54130
AT5G41670	AT5G53900
AT5G40760	AT5G52900
AT5G40690	AT5G52570
AT5G38020	AT5G52640
AT5G37780	AT5G51720
AT5G23820	AT5G51460
AT5G22630	AT5G50915
AT5G17760	AT5G49970
AT5G10960	AT5G49450
AT5G11150	AT5G49448
AT5G07960	AT5G48570
AT5G07820	AT5G48490
AT5G05960	AT5G48485
AT5G02100	AT5G46800
AT5G01800	AT5G46390
AT5G01050	AT5G44400
AT5G01040	AT5G42760
AT3G63010	AT5G41790
AT3G61200	AT5G37770
AT3G60540	AT5G24210
AT3G59930	AT5G23760
AT5G33355	AT5G19140
AT3G58570	AT5G19120
AT3G57240	AT5G17170
AT3G54960	AT5G17310

Table 6: DEGs used in gene ontology (Continued)

Up-Regulated Genes in Common with *B. cinerea,* SA, MeJA, ACC, and ABA.	Down-Regulated Genes in Common with *B. cinerea,* SA, MeJA, ACC, and ABA.
AT3G54040	AT5G17670
AT3G53990	AT5G16650
AT3G51660	AT5G17230
AT3G50970	AT5G17300
AT3G51430	AT5G13650
AT3G50500	AT5G13770
AT3G50210	AT5G11690
AT3G49930	AT5G10380
AT3G49120	AT5G07240
AT3G49110	AT5G06980
AT3G47860	AT5G02830
AT3G46970	AT5G02120
AT3G46060	AT3G61210
AT3G46440	AT3G57180
AT3G43670	AT3G56400
AT4G39030	AT3G56290
AT4G39090	AT3G55630
AT4G39270	AT3G54500
AT4G35790	AT3G52720
AT4G30660	AT3G51920
AT4G30650	AT3G50060
AT4G27440	AT3G49140
AT5G54810	AT3G48200
AT4G27070	AT3G47430
AT4G26670	AT3G44630
AT4G26130	AT4G38430
AT4G26060	AT4G38280
AT4G23600	AT4G38330
AT4G23680	AT2G45250
AT4G21850	AT4G37550
AT4G21680	AT4G37560
AT4G21105	AT4G37330
AT4G19185	AT4G35090
AT4G19120	AT4G35250
AT4G19390	AT4G34730
AT4G18430	AT4G34090
AT4G12470	AT4G32190
AT4G09020	AT4G31870
AT4G05020	AT4G30610
AT4G04330	AT4G30825
AT4G03960	AT4G28260
AT4G02370	AT4G28270

Table 6: DEGs used in gene ontology (Continued)

Up-Regulated Genes in Common with *B. cinerea,* SA, MeJA, ACC, and ABA.	Down-Regulated Genes in Common with *B. cinerea,* SA, MeJA, ACC, and ABA.
AT4G01480	AT4G28290
AT4G01610	AT4G28080
AT4G00700	AT4G26850
AT1G22180	AT4G27030
AT1G19180	AT4G26860
AT1G58340	AT4G26555
AT1G13750	AT4G22490
AT1G56300	AT4G20170
AT3G12490	AT4G19830
AT1G69450	AT4G18970
AT1G55020	AT4G18740
AT1G42580	AT4G18370
AT3G12030	AT4G17880
AT3G22850	AT4G12970
AT3G26440	AT4G11175
AT3G22600	AT4G10120
AT3G22620	AT4G10330
AT3G19260	AT4G08950
AT3G25010	AT4G09350
AT3G27300	AT4G04850
AT3G27210	AT4G04350
AT3G13175	AT4G01250
AT3G27870	AT4G01080
AT3G27880	AT4G00050
AT3G14430	AT1G19670
AT3G15950	AT2G33250
AT3G17020	AT1G22190
AT3G20810	AT1G58370
AT3G19490	AT1G07010
AT3G18080	AT1G07180
AT3G17790	AT1G18060
AT3G17810	AT1G51820
AT3G14310	AT3G12150
AT3G16740	AT3G12320
AT3G04080	AT3G12110
AT3G07990	AT1G72060
AT3G08720	AT3G10940
AT3G08660	AT1G66130
AT3G05880	AT1G42550
AT3G05800	AT3G14770
AT3G09940	AT3G29290
AT3G01420	AT3G22231

Table 6: DEGs used in gene ontology (Continued)

Up-Regulated Genes in Common with *B. cinerea,* SA, MeJA, ACC, and ABA.	Down-Regulated Genes in Common with *B. cinerea,* SA, MeJA, ACC, and ABA.
AT3G10410	AT3G22150
AT3G01800	AT3G21390
AT3G10300	AT3G26450
AT1G17745	AT3G15310
AT1G13830	AT3G23700
AT1G20450	AT3G26570
AT1G20440	AT1G80850
AT1G29390	AT1G29440
AT1G76790	AT3G10840
AT1G73260	AT3G27350
AT1G66350	AT3G27050
AT1G74020	AT3G18750
AT1G69870	AT3G19480
AT2G43535	AT3G21250
AT2G43570	AT3G23590
AT1G53310	AT3G23530
AT1G45145	AT3G23510
AT1G04990	AT3G21670
AT1G20010	AT3G15850
AT1G35720	AT3G16000
AT1G53035	AT3G17609
AT1G71730	AT3G17650
AT1G63460	AT5G45450
AT1G50480	AT3G06080
AT2G12280	AT3G02690
AT2G12200	AT3G02380
AT1G80130	AT3G09600
AT1G47710	AT3G09600
AT1G21750	AT3G10525
AT1G62730	AT3G01550
AT1G20840	AT3G01060
AT1G79440	AT3G03770
AT1G79500	AT1G44000
AT1G75780	AT1G19000
AT2G27190	AT1G29530
AT1G54000	AT1G29510
AT1G54010	AT1G29430
AT1G05620	AT5G27780
AT2G31360	AT1G66260
AT2G31390	AT1G68440
AT2G22240	AT1G71340
AT2G12400	AT1G76570

Table 6: DEGs used in gene ontology (Continued)

Up-Regulated Genes in Common with *B. cinerea,* SA, MeJA, ACC, and ABA.	Down-Regulated Genes in Common with *B. cinerea,* SA, MeJA, ACC, and ABA.
AT2G21620	AT1G76450
AT2G21660	AT1G67865
AT2G22500	AT1G73870
AT2G21130	AT1G74070
AT1G61820	AT1G72430
AT1G10200	AT1G55960
AT1G08940	AT1G32520
AT1G09100	AT1G49010
AT1G09740	AT1G78290
AT2G17840	AT1G43790
AT1G27030	AT1G12250
AT1G05000	AT1G26560
AT2G28360	AT1G17360
AT2G20370	AT1G62960
AT2G18350	AT1G19715
AT2G22660	AT1G19610
AT2G37130	AT1G19700
AT2G15970	AT1G04770
AT2G05630	AT1G32900
AT2G25730	AT1G18660
AT2G28900	AT1G18730
AT2G28840	AT1G14345
AT2G41380	AT1G71720
AT2G06925	AT1G01060
AT2G46140	AT1G15980
AT2G29630	AT1G30520
AT2G47000	AT1G50450
AT2G25930	AT1G22430
AT2G38360	AT1G22590
AT2G37750	AT1G64500
AT2G19450	AT1G63980
AT2G39900	AT1G50250
AT2G26230	AT1G17050
AT2G34810	AT1G17140
AT2G32690	AT1G15180
	AT1G62750
	AT1G20850
	AT1G64860
	AT1G64780
	AT1G75750
	AT4G13500
	AT2G05310

Table 6: DEGs used in gene ontology (Continued)

Up-Regulated Genes in Common with *B. cinerea,* SA, MeJA, ACC, and ABA.	Down-Regulated Genes in Common with *B. cinerea,* SA, MeJA, ACC, and ABA.
	AT1G78510
	AT2G31380
	AT2G04039
	AT1G04350
	AT1G09340
	AT2G21385
	AT2G36835
	AT2G36990
	AT2G21190
	AT4G38790
	AT1G22630
	AT1G55805
	AT1G04550
	AT1G65490
	AT1G70000
	AT1G70250
	AT1G03630
	AT1G03440
	AT1G61190
	AT1G61180
	AT1G61300
	AT1G61310
	AT1G60550
	AT1G55480
	AT1G55460
	AT1G23480
	AT1G23740
	AT2G20670
	AT2G28305
	AT2G40100
	AT2G24280
	AT2G24270
	ATMG00410
	AT2G07741
	ATMG01170
	AT2G07699
	AT2G29290
	AT2G32450
	AT2G41250
	AT2G41290
	AT1G32380
	AT2G35390

Table 6: DEGs used in gene ontology (Continued)

Up-Regulated Genes in Common with *B. cinerea,* SA, MeJA, ACC, and ABA.	Down-Regulated Genes in Common with *B. cinerea,* SA, MeJA, ACC, and ABA.
	AT2G46735
	AT2G46830
	AT2G26080
	AT2G39200
	AT2G34460
	AT2G41120
	AT2G41040
	AT2G23390
	AT2G23430
	AT2G38230
	AT2G38210
	AT2G22980
	AT2G23670
	AT2G30150
	AT2G40000
	AT2G30520
	AT2G32950
	AT2G32860