UNDERSTANDING PLANT CELL DEATH WITH "BAK TO LIFE" SCREEENS

An Undergraduate Research Scholars Thesis

by

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ABSTRACT

Understanding Plant Cell Death with “bak to life” Screens

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As sessile organisms, plants often need to activate a sophisticated immune system to evade pathogen infections. However, uncontrolled activation of defense pathways can have extremely damaging effects to the host. Many elements that control these events are still unknown. Defense receptors like Brassinosteroid Insensitive 1-Associated Receptor Kinase 1(BAK1) and Somatic Embryogenesis Receptor Kinase 4 (SERK4) are a vital part of plant defense, growth and development. However, when they become suppressed, it can lead to defense over-activation and cell death. My research focuses on finding suppressors of cell death caused by silencing *BAK1* and *SERK4* with virus-induced gene silencing (VIGS) on *Arabidopsis* knockout collections. My research will provide insight into the understanding of BAK/SERK4-mediated cell death and how plants activate defense without causing massive cell death. Likewise, this research may impact the future of crop production by genetically modifying the plants for maximizing defense without detrimental defect. I showed that “*bak to life*” (*btl*) mutants have reduced cell death phenotype compared to wild type plants, when BAK1/SERK4 are silenced.

NOMENCLATURE

BAK1	Bassinosteriod Insensitive 1-Associated Receptor Kinase 1
SERK4	Somatic Embryogenesis Receptor Kinase 4
MEKK1/MAP3K	Mitogen-activated Protein Kinase Kinase Kinase 1
DAB	3,3'-Diaminobenzidine
CASPL	Casparian Strip Membrane Domain Protein-Like
SBP	S-Ribonuclease Binding Protein
RNase	Ribonuclease
GPI	Glycosylphosphatidylinositol
ERF	Ethylene Response Factor
SUMM2	Suppressor of MEKK1 MEKK2 2

CHAPTER I

INTRODUCTION

For sessile plants, keeping a highly complex immune system is imperative for warding off pathogen infections. The activation of immunity is due to the recognition of pathogen elicitors, which lead to a robust immune system. However, the uncontrolled activation of defense pathways can have damaging effects to the hosts, which may lead to massive cell death within the host organism. Bassiniosteroid Insensitive 1-Associated Receptor Kinase 1 (BAK1) also named Somatic Embryogenesis Receptor Kinase 3 (SERK3) and its homolog SERK4 are important immune regulators that associate with multiple immune receptors, such as FLS2 recognizing bacterial flagellin (Li, Wen et al. 2002, Nam and Li 2002). In addition, BAK1 and SERK4 are also important regulators in plant growth and development. The combination of multiple SERKs signal many key functions such as root growth, cell expansion and elongation, stomatal patterning, floral organ abscissions, and male gametophyte development (Ma, Xu et al.). Mitogen Associated Protein Kinases (MAPKs) are proteins that start a kinase cascade (MEKK-MKK-MAPK) throughout the cell when pathogens attempt to invade the host. These signals induce a plant's innate immunity to ward off the pathogen. Low levels of *BAK1*/*SERK4* or *MEKK1* in *Arabidopsi*s causes extreme defense activation leading to cell death, as well as an accumulation of defense genes, such as PR1 and PR2. Finding suppressors to BAK1/SERK4-mediated cell death will be crucial in understanding over-activation of immunity-induced cell death.

Dr. He's laboratory has developed an innovative genetic screen for "*bak to life*" (*btl*) mutants that suppress cell death caused by silencing of *BAK1* and *SERK4* with virus-induced

gene silencing (VIGS) on *Arabidopsis* knockout collections (de Oliveira, Xu et al. 2016). VIGS is a new technique used to greatly reduce the expression of genes within a plant genome. This method uses siRNA along with post transcriptional gene silencing in order to shut off a gene of interest (Becker and Lange 2009). The target gene's mRNA degradation prevents translation. By utilizing the bacteria *Agrobacterium tumefaciens* as a vector. To infect the *Arabidopsis*, the T-DNA from the virus is incorporated into the plant genome. Eventually this T-DNA will become a short interfering RNA (siRNA) which is able to find complementary RNAs and signal the cleavage of them (Becker and Lange 2009). In my research, these complementary strands are the mRNAs of BAK1/SERK4. I screened ~200 *Arabidopsis* knockout and identify a new suppressor of BAK1/SERK4 or MEKK1-mediated cell death by VIGS. Possible mutants found are verified using qPCR as well as crossing. I will cross the mutant with *bak1/serk4* mutant (heterozygous *BAK1bak1/serk4serk4* will be used for cross) to isolate *bak1/serk4/btl5* triple mutant and check whether the triple mutant has reduced cell death and growth defect than *bak1/serk4* double mutant. To characterize the any new mutants, I will perform a trypan blue staining to check whether cell death caused by silencing of *BAK1/SERK4* or *MEKK1* is reduced. In addition, I will use the 3,3'-diaminobenzidine (DAB) staining to examine the level of H_2O_2 accumulation and perform RT-PCR to test PR1 and PR2 expression in the wild type and mutant after BAK1/SERR4 VIGS. I will also look for other T-DNA knockout lines in order to check if different insertions will result in the same phenotype.

CHAPTER II

METHODS

In order to fully answer the question, each objective must be completed. These objectives are cyclical in nature. When finding another mutant, it must be characterized. After you characterize that mutant, another one must be found. After multiple mutants have been found and characterized, a larger picture develops. This methodical approach helps accurately piece together factors that contribute to over-activation of immune pathways in sessile plants. To achieve objective one, I worked closely with a doctorate student in a laboratory. They guided me through many of the experiments needed to characterize these mutants, as well as provide insight into the biochemical interactions and reasons for each experiment. Every two weeks, I germinated a new set of plants (Table 1). Two weeks after, the plants are inoculated and another round of plants are be germinated. Two weeks later the inoculated plants can be phenotyped and possible candidates logged. The tables at the end of the timeline demonstrate my weekly processes. After week five I germinated, prepped, inoculated, and phenotyped every week. The second semester followed the same methods, but only candidates obtained in the first semester were screened (Table 2). In order to achieve objective two, a general screenings were the first thing that were done order to find candidates for the BAK1/SERK4 or MEKK1 mutants. This will required a laboratory and a growing room for the *Arabidopsis*. For the characterization of and *btl* mutants, I will be utilizing many techniques such as DAB staining, PCR, and DNA extractions. I mainly completed objective two alone, with only some help from a doctoral student for any questions I may have. I used TAIR, to find the location of the T-DNA inserts, as well as begin the characterization of the genes found.

Soil preparation and germination

Arabidopsis accessions Col-0 (WT), and various mutants used in the study were grown in soil (Metro Mix LP5) in a growth room at 23 °C, 60% relative humidity, 70 µE m^{-2} s^{-1} light with a 12-h light/12-h dark photoperiod for 2 weeks before VIGS assay. The *Arabidopsis* T-DNA insertion lines 5490 (SALK_026603C), 5552 (SALK_027664C), 5558 (SALK_027719C), 7137 (SALK_062374C) were obtained from the *Arabidopsis* Biological Resource Center (ABRC). Germinations were completed in a 4x4 pattern in plastic square pots. Pots contained MEKK1 and BAK1/SERK4 in the same pot split in half for the first screen, and separate pots for each gene in the second screen. There was approximately 4 control pots every 44 SALK lines (de Oliveira, Xu et al. 2016).

Plasmid construction

To create VIGS constructs for individual genes fragments of BAK1 (319 bp), SERK4 (310 bp), CLA1 (541 bp), MEKK1 (520 bp) were amplified from Col-0 *Arabidopsis* cDNA. The fragments were digested with EcoR1 and Kpn1, and ligated into *pYL156* vector. Clones were confirmed via sequencing (de Oliveira, Xu et al. 2016).

***Agrobacterium*-mediated VIGS assay**

Plasmids containing *pTRV-RNA1, pYL156-BAK1/SERK4, pYL156-MEKK1, pYL156-GFP* (a vector control), and *pYL156-CLA1* were introducing into Agrobacterium GV3101 cultures via electroporation. Cultures were grown in a small batch of Liquid Broth (LB) medium containing 50 µg ml^{-1} kanamycin and 100 µg ml^{-1} gentamicin overnight at 28°C in a roller drum. After this the cells were subcultured in larger fresh LB containing 50 µg ml^{-1} kanamycin and 100 µg ml^{-1} gentamicin as well as 10 mM MES at pH 5.7 and 20 µM acetosyringone overnight at 28°C. Cells are pelleted by a 3200 rpm certification for 15 minutes and re-suspended in a solution of 10 mM

$MgCl_2$, 10 mM MES at pH 5.7, 200 µM acetosyringone. The solution is adjusted to 1.5 at A_{600} using spectophotometry. The solutions of pTRV-RNA1 and pYL156 plasmids were combined in a 1:1 ratio.

Inoculation and phenotyping

After 14 to 19 days the plants were ready for inoculation. Using a needleless syringe the first two true leaves (a phenotype may not appear if coteledians are inoculated instead) were carefully lifted and the VIGS solution was slowly injected into the back of each leaf (if there are more than two). The leaves were blotted with a Chemwipe to ensure they did not wilt. Once the *pYL156–CLA1* plants began to show an albino phenotype, this was approximately 2 weeks after inoculation, the plants were able to be phenotyped. It should be noted that occasionally the *MEKK1* phenotype did not fully appear until 3-4 days after the albino phenotype for *CLA1*, so phenotyping was often around 18 days to three weeks after inoculation to ensure the strongest phenotype possible (Table 1 and 2).

Table 1. **Fall 2017 Schedule** a general outline of the Fall 2017 weekly schedule $X_{\#}$ denotes the round of germinations for that week. The * denotes rounds that are not completed on the table due to Christmas break.

Fall 2017	Germination	Inoculation	Phenotype	Possible Candidates
Week1	X_1			
Week2				
Week3	X_2	X_1		
Week4				
Week5	X_3	X_2		
Week6			X_1	Germination
Week7	X_4	X_3		Germination
Week8			X_2	Inoculation
Week9	X_5	X_4		
Week10			X_3	Phenotype
Week11	$*X_6$	X_5		Picture
Week12			X_4	
Week13	$**X_7$	$*X_6$		
Week14			X_5	

Table 2. **Spring 2018 Schedule** a general outline of the Spring 2018 weekly schedule $X_{\#c}$ denotes rounds where possible candidates will be screened.

Spring 2018	Germination	Inoculation	Phenotype	Pictures	Possible Candidates
Week1	X_{1c},				Germination
Week2					
Week3	X_{2c}	X_{1c}			Germination
Week4					
Week5	X_{3c}	X_{2c}			Germination
Week6			X_{1c}	X_{1c}	
Week7		X_{3c}			
Week8			X_{3c}	X_{2c}	
Week9	Spring Break	Spring Break	Spring Break	Spring Break	Spring Break
Week10					
Week11				X_{3c}	
Week12					DAB/Plant Crosses
Week13					RNA/DNA extraction
Week14					RT-PCR (or other experiments)

CHAPTER III

RESULTS FALL 2017

Three candidates: 5490, 5552 and 5558, passed the first screening

After screening approximately 100 of the 200 total the T-DNA knockout lines. Two possible candidates that were found. The candidates were 5552 (SALK_026603C) and 5558 (SALK_027664C) and displayed medium to medium-weak cell death compared to the control for MEKK1. A separate line, 5490 (SALK_026603), was also repeated due to the line showing no cell death, but there were not enough plants left to accurately determine if it is a possible candidate. All of these mutations are located on the fourth chromosome within three thousand bases of one another. The exact locations of each mutation is shown on Figures 1-3. As each figure shows, there are many T-DNA inserts possible within a single gene. The location of the T-DNA insert provides insight into whether or not a candidate could be a cell death suppressor. Exons are the coding regions of DNA. These regions are coded into proteins. SALK lines with T-DNA inserts in the exon regions are most likely to be cell death suppressors. Introns are in between the exons in genes. These regions are removed from the mRNA by splicing. Introns can provide stability and create new gene products by alternative splicing, but are not likely cell death repressors. The intergenic region is the area between genes and is also known as junk DNA. These areas are the least likely to contain cell death repressors because they do not code for proteins or assist in gene regulation.

Location of T-DNA knockdowns and gene descriptions of 5552, 5558

The two SALK lines found have T-DNA insertions at specific areas in the genome. Knowing where these mutations are can help understand how the mutation is able to resist MEKK1-mediated cell death and assist with characterization of the mutant. Line 5490 is in the intron region of loci At4g37235 (Figure 1). The SALK line of interest is highlighted in red. The gene At4g37235 is a Casparian Strip Membrane Domain Protein homolog (CASP-like protein). CASPL proteins have been found to have multiple areas of expression in Arabidopsis including trichrome, xylem pole pericycle cells, and peripheral root cap cells (Roppolo, Boeckmann et al. 2014). CASPLs have also been found create a wall around the endodermis of plants to prevent extracellular diffusion. This helps the plant's endodermal cells to control the flow of water and nutrients throughout the organism (Naseer, Lee et al. 2012, Geldner 2013). Unfortunately At4g37235 is not a well characterized gene. It is still part of an uncharacterized protein family, although it is still a CASPL5 protein in this gene is part of an unidentified protein family. The exact function and area of expression is still unknown. Most of At4g37235 is a large intron, this is where most of the T-DNA knockdowns are located (Figure 1). SALK_026603C is found in an intron. This will make this protein more difficult to characterize in the future if it found to be a repressor of MEKK1-mediated cell death.

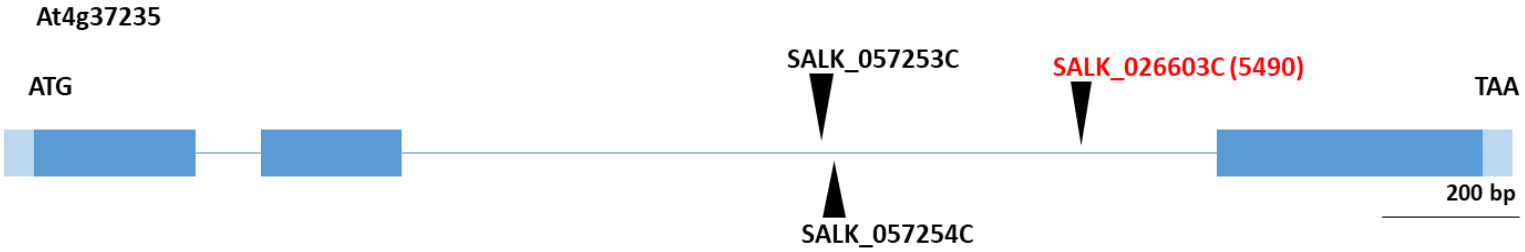

Figure 1. **Location of SALK_026603C (5490).** The T-DNA knockout line SALK_026603C (5490) is located in the first exon region in gene At4g37235.

The line 5552 contained a T-DNA insert in the At4g35070 gene. This is within an exon region in the S-ribonuclease binding protein (SBP) loci. Figure 2 shows the location of 5552 on the gene. The blue regions represent exons, which will be coded into SBP. The function of ribonucleases is to catalyze the degradation of RNA. This can be either ssRNA, dsRNA, or RNA-DNA hybrids. Ribonucleases are involved in numerous cellular functions that are key to the regulation of genes and protecting the host from invasion (Luhtala and Parker 2010). One of the most common uses for RNases is to fight off foreign invaders such as pathogens that have entered the organism. RNase S is a combination of RNase A, a common RNase, and the S-protein. Therefore, RNase S has similar structure and enzymatic activity to RNase A (Nadig, Ratnaparkhi et al. 1996). It has been suggested that RNase S is used to create self-incompatibility in the cell. This ensures the organism does not degrade its own RNA, or RNA that still needs to be used. It is likely that this protein will bind to S-RNase in order to prevent self-deterioration. The T-DNA insert was located in the first exon region. This increases the likelihood that this is a MEKK1-cell death repressor. It also will be easier to characterize in future experiments.

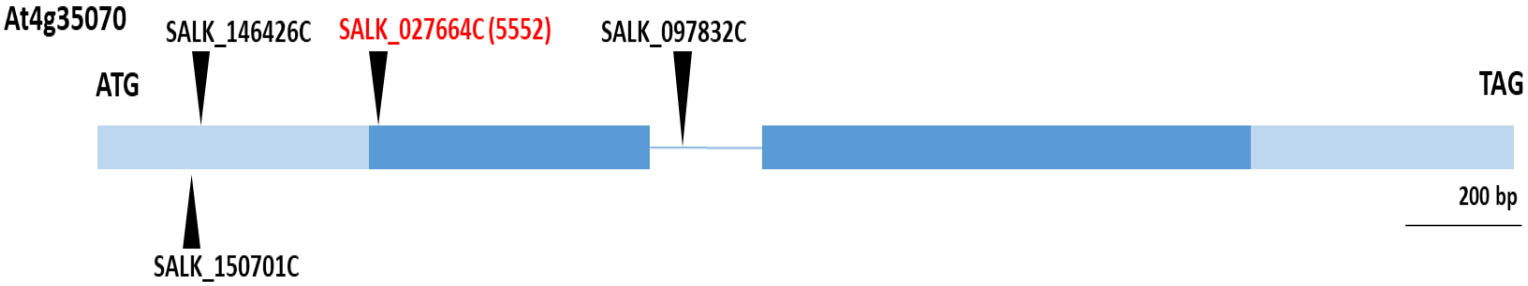

Figure 2. **Location of SALK_027664C (5552).** The T-DNA knockout line SALK_027664C (5552) is located in the first exon region in gene At4g35070.

Unfortunately there in not much information on SALK_027719C. This line is located in an intergenic region between genes At4g36020 and At4g36030. As shown by Figure 3 there is

no coding region where this mutation is located. At4g36020 codes for cold shock proteins. These proteins help the organisms survive under lower than normal temperatures. At4g36030 codes for Armadillo Repeat Only 3 (ARO3). These proteins contain a conserved 40 amino acid repeat called an armadillo repeat (Bob Riggleman 1989). These repeats are found in numerous proteins that are involved in a wide variety of functions (Peifer, Berg et al. 1994). The intergenic regions of DNA do not within the open reading frame of any genes. This makes these areas less likely to be make an impact in the interactions necessary for MEKK1 or BAK1/SERK4 cell death suppression. There is also no listed protein that this mutation affects. This greatly lowers the chance that this is a MEKK1-cell death repressor. It will also be very difficult to characterize due to the lack of information on this area of the genome.

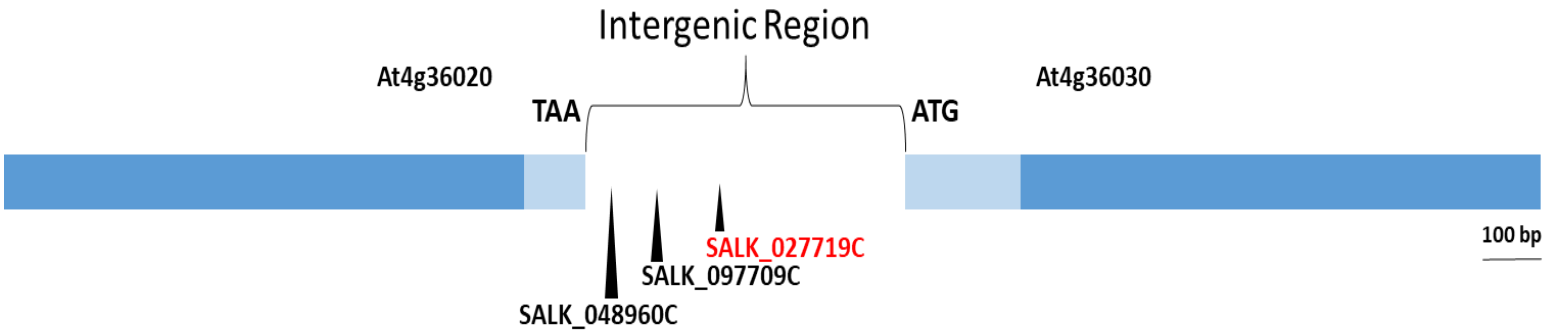

Figure 3. **Location of SALK_027719C (5558).** The T-DNA knockout line SALK_027719C (5558) is located in the intergenic region between genes At4G36020 and At4G36030

Rescreening of lines 5552 and 5558

Lines 5552 and 5558 were re-screened to determine if they were MEKK1 or BAK1/SERK4-mediated cell death repressors. Line 5490 was also re-screened, but this was mainly done because there was not enough plants in the first screen to make any definitive claims. The same methods as before were used to grow and inoculate the lines. The plants were

inoculated at two weeks old and pheotyped two weeks after that. Upon the second screening, it was shown that both of the lines showed strong cell death phenotypes. This includes yellowing in the leaves, wrinkling in the leaves, and undersized plants. The data presented upon the second screening indicates that neither line 5552, nor 5558 were found to be MEKK1 or BAK1/SERK4-mediated cell death repressors. There were no significant differences between 5552 and 5558 and the control plants in either immuno-receptor knockdown (Figure 4). No photos were taken of 5490 because it was also found to have a strong cell death phenotype.

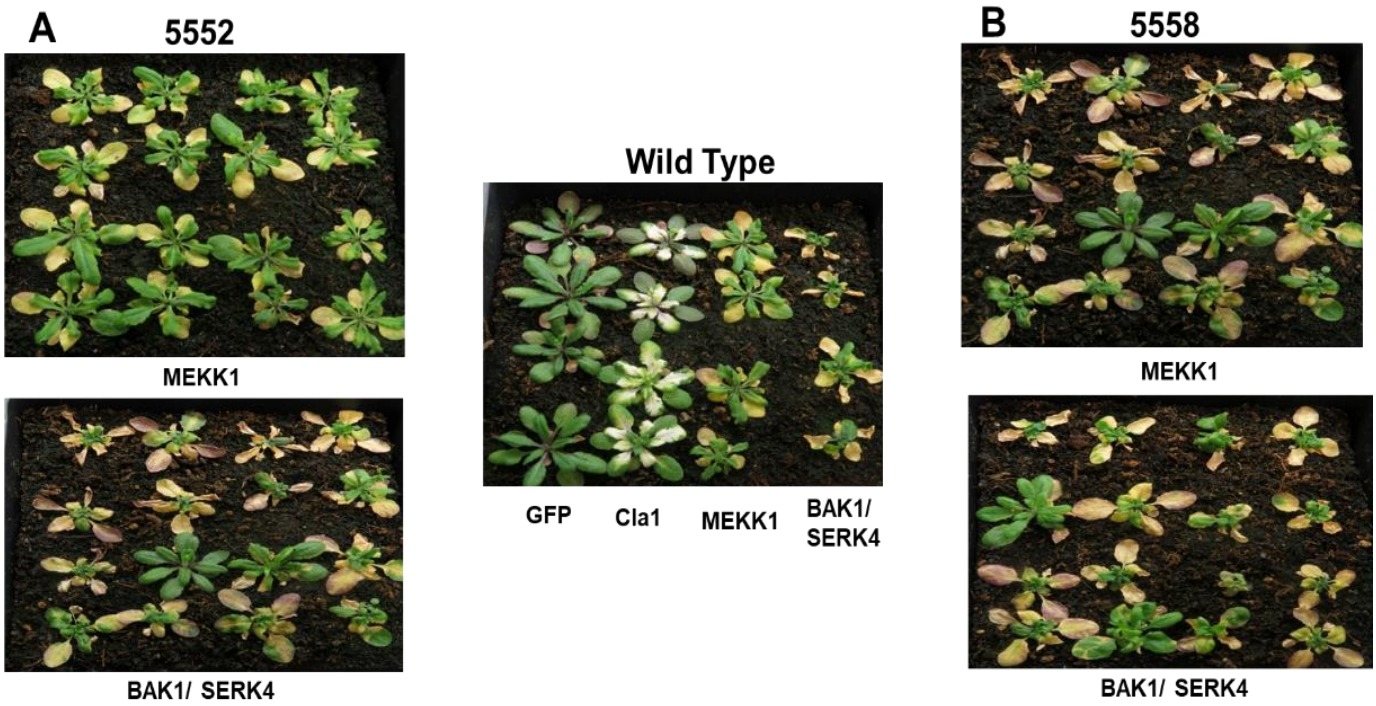

Figure 4. **Lines 5552 and 5558 are not suppressors of BAK1/SERK4 or MEKK1- mediated cell death. A.)** 5552 (SALK_ 026603C) is not an MEKK1 or BAK1/SERK4-mediated cell death repressor. Both 5552 and shows high amounts of cell death in almost all plants. This indicates that this line is incapable of suppressing immune receptor-mediated cell death. **B.)** 5558 shows high amounts of cell death in almost all plants. This indicates these lines are not suppressors MEKK1 or BAK1/SERK4-mediated cell This indicates that these lines are incapable of suppressing BAK1/SERK4 or MEKK1-mediated cell death. Purple leaves on 5552 and 5558 indicate extreme stress to the plant

There are many reasons these lines passed the original screening. It is very likely that the plants were not inoculated well enough to cause a full knockout of BAK1/SERK4 and MEKK1. It is also possible the lines where pheontyped too soon. This would lead to a weaker phenotype for cell death than shown in Figure 4. Regardless of the reason these lines passed the original screening, it is clear now that these lines are not BAK1/SERK4 or MEKK1-mediated cell death repressors. Due to the lack of data in line 5490, it had to be screened again, as expected, this line did not produce a cell death mediator. This is not uncommon for cell lines that need be re-screened due to lack of growth during the original screening.

CHAPTER IV

RESULTS SPRING 2018

Line 7137 (SALK_062374C) is a new cell death suppressor candidate

While 5490, 5552, and 5558 were being re-screened, several new lines were found to suppress cell death upon first screening. Twenty of these lines were re-screened to determine if they are BAK1/SERK4 or MEKK1-mediated cell death suppressors (Table 3). In addition, another 24 lines are currently being grown to obtain seeds. In total, this provides an extra 44 lines of candidates that may be cell-death repressors. Just like 5552 and 5558, these lines have been re-screened. Of the 20 lines that were re-screened, one was found to be a MEKK1 cell death suppressor (Figure 6). Line 7137 (SALK_062374C) showed no cell death when MEKK1 suppressed with VIGS. Line 7137 has a T-DNA knockdown inserted at At3g15720 as shown in Figure 5. This is the exon region of a pectin lyase-like super family gene. In fruits like strawberries and bananas, pectin lysases have been found to contribute to cell ripening and cell wall degradation (Marín-Rodríguez, Orchard et al. 2002). A study of all pectin lyase genes in *Arabidopsis* indicated that this lyase, At3g15720, has no phylogenetic relationship with other pectin-lyases.

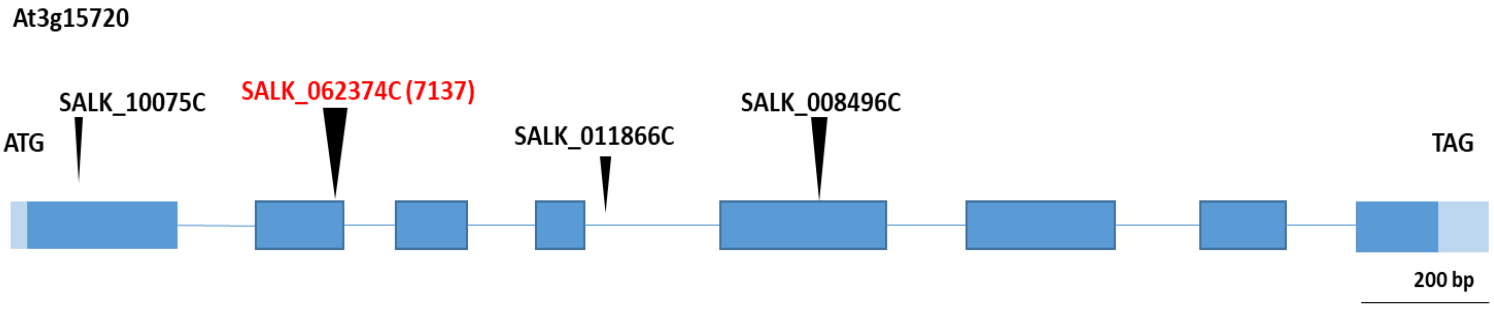

Figure 5. **Location of SALK_062374C (7137).** The T-DNA knockout, SALK_062374 is located on the second exon of the At3g1570 gene.

Table 3. Plant ID #, SALK line and gene location of twenty new candidates found during Fall 2017

LINE	SALK line	GENE LOCATION
6622	SALK_050182C	At3g42052
6625	SALK_050239C	At3g42052
6647	SALK_050974C	At1g04200
6657	SALK_051158C	At2g29480
6658	SALK_051162C	At1g63800
6833	SALK_055942C	At3g54480
6834	SALK_055957C	At3g21215
6853	SALK_056403C	At2g13130
6942	SALK_058204C	At3g48159
7137	SALK_062374C	At3g15720
7250	SALK_064531C	At3g26800
7252	SALK_064646C	At1g14970
7472	SALK_069633C	At3g26260
7495	SALK_070160C	At3g09760
7710	SALK_075519C	At5g61210
7815	SALK_078514C	At5g16790
7838	SALK_079083C	At5g35602
8139	SALK_086894C	At1g06250
8144	SALK_086938C	At1g55160
8176	SALK_087652C	At1g55930

This gene is located in a region of chromosome III that is paralogus (identical) to chromosome I. Although this gene has not yet been identified in chromosome I (Cao 2012).

Figure 6. **Line 7137 (SALK_067423C) is a MEKK1 cell death repressor. A.)** Line 7137 suppresses MEKK1 cell death, but not BAK1/SERK4 compared to control plants. **B.)** MEKK1 cell death is dramatically decreased in 7137 when compared to control, GFP serves as a positive control, Cla1 is a loading control.

At3g15720 is a polygalacturonase within glycosyl hydrolase family 28 (Bayer, Bottrill et al. 2006). This is a family of enzymes with numerous functions. At3g15720 is a polygalacturonase, these enzymes can hydrolyze glyosidic bonds between two carbohydrates or a

carbohydrate and non-carbohydrate. This protein is able to randomly hydrolyze (1→4)-α-D-galactosiduronic linkages in pectate, a polymer polygalactornic acid product in the degradation of plants (Ruttkowski, Labitzke et al. 1990). Atg315720 is located on the cell wall of Arabidopsis and is liked via a Glycosylphosphatidylinositol (GPI) anchor (Felix Elortza 2003, Nawy, Lee et al. 2005). This is one of the 44 known GPI anchored proteins in Arabidopsis. GPIs are long fatty acids that tether proteins to the membrane of a cell; many of the GPI proteins contain a hydrophobic end on the C-terminal side as well as a ω-site. GPIs have been shown to serve many purposes including pathogen defense (Felix Elortza 2003).

This gene's function is not well characterized, its role within the cell is not well cited. It is possible one of its functions within the cell is involved in mediating the suppression of MEKK1 cell death. One study involving a separate gene (At5g13330), a member of the ethylene response factor (ERF) subfamily B-4 within the transcription factor gene family ERF/*APETALA2.* This gene has been found to increase shoot regeneration. When this gene was down regulated by T-DNA knockdowns, At3g15720 expression levels were reduced along with 25 other genes (Che, Lall et al. 2006). This indicates At3g15720 may be involved in a much larger gene network involving At5g13330 and several other genes.

CHAPTER VI

CONCLUSION

SALK line SALK_067423C, line 7137 may contain an extra T-DNA insert or polymorphism in the Suppressor of MEKK1 MEKK2 2 (SUMM2) gene. This gene is a known suppressor of MEKK1, and thus would not be a novel suppressor of MEKK1-mediated cell death. In order to determine if 7137 is a novel suppressor, it must be verified that there is no polymorphism or insert in *SUMM2* before moving forward in future characterization experiments. The DNA of the plants will be extracted and a primer will be developed. This primer will span the region *SUMM2* encodes. This sequence will undergo PCR to amplify the SUMM2 region. An agarose gel will be run to determine if there is an insert in the SUMM2 sequence. This portion of the genome will be sequenced and compared to a known sequence to determine if there have been any modifications to *SUMM2.*

If the line has been shown to not contain a SUMM2 polymorphism or insert. Further verification of line 7137 as well as characterization is necessary to fully understand its role in MEKK1 cell death. In order to ensure the line is a cell death suppressor, the line will be crossed with a plant line that is recessive for MEKK1. Normally this would immediately result in death for the plant, but the At3g15720 mutant will counteract the low levels of MEKK1. If 7137 is an MEKK1-mediated cell death suppressor, the experiment will yield plants that are still resistant to MEKK1-mediated cell death, while the plant without the At3g15720 will not survive past infancy. Crosses are used to confirm the proteins ability to suppress cell death in *vivo*. It will also confirm the cause of the MEKK1 cell death suppression was At3g15720, and not any other gene.

Along with these test qPCR, western, and northern blots will be done to confirm this protein is involved in suppressing MEKK1-mediated cell death.

To characterize 7137, the mutant's genome will be used to identify the mutation within the gene. This polymorphism and T-DNA insert information can be found on The Arabidopsis Information Resource (TAIR, https://www.arabidopsis.org/index.jsp). These polymorphisms can provide information about how the gene was modified. The amino acid sequence of the wild type gene is already known. The T-DNA insertion may cause a loss or gain in function for At3g15720. Various experiments can be done to determine how At3g15720 is able to repress cell death. Analyzing the relative mRNA levels of pathogenesis-related protein 1 and 2 (PR1 and PR2) with qPCR. PR1 and PR2 accumulate in plants undergoing cell death. Lower levels of PR1 and PR2 indicate the cell lines are suppressing cell death. Experiments with leaf tissues will also be done. I will use the DAB staining to exam the level of H_2O_2 accumulation to determine if the plant can repress peroxide H_2O_2 production as well as cell death. To further characterize the mutant the mutant will be paired other components it is associated with. This may be difficult for line 7137 due to the lack of information about the gene and what it associates with (de Oliveira, Xu et al. 2016). It should be noted that a past BAK1/SERK4-meidated cell death repressor was involved in protein glycoslylation. This could provide some sort of connection between two genes as At3g15720 is a polygalacturonase, which hydrolyzes pectate, a special kind of glucose.

The mechanisms that cause cell death are still not very well understood in plants. The BAK1/SERK4 and MEKK1 families can provide insight into the immune pathways of plant cells as well as reveal how this death can be repressed. BAK1/SERK4 and MEKK1 genes provide the first line of defense for the plant cell. When these genes are not present it allows pathogens to enter the cell undetected. MEKK1 and other Mitogen Associated Protein Kinases (MAPKs) have

been noted as a key first line of defense against pathogens by starting the cascade of defense signals across the cell. The tight regulation of these kinases has been as an important part of preventing plant death (Pitzschke, Schikora et al. 2009, Kong, Qu et al. 2012). BAK1/SERK4 plays an important role in regulation of root growth, light response, cell elongation, and stress response (Ma, Xu et al.). The ability to live without either of these genes could provide key information about the mechanisms involved in cell death.

This method of screening provides a fast and efficient way to determine possible candidates of BAK1/SERK4 and MEKK1 mediated cell death and characterize them. Using this method, human error can be tightly regulated by numerous screenings. Possible candidates such as 5552 and 5558 are found, but quickly eliminated after the second test. While line 7137 (SALK_ 062374C) can be determined and continue for even more rigorous testing. This step-wise method also ensures the best results for the characterizing mutants. Determining the PR1 and PR2 levels, then continuing on to test H_2O_2 accumulation to verify if the gene is suppressing cell death as well as obtaining baseline information for characterization.

My research will provide insight into the understanding of BAK1/SERK4 and MEKK1-mediated cell death and how plants activate defense without causing massive cell death. This research can impact the future of crop production by genetically modifying the plants for maximizing defense without detrimental defect. Increased knowledge of BAK1/SERK4-mediated cell death could be applied to increase crop production. Many bacteria and viruses target BAK1/SERK4 and MEKK1 in order to prevent the signal cascade that leads to an immune response (Hiscott, Kwon et al. 2001, Shan, He et al. 2008). Once mutations that can prevent cell death due to the suppression of BAK1/SERK4 and MEKK1 are found, it may be possible to modify crops in order to make them more resistant to bacteria and viruses. This could

dramatically increase crop yield, while lowering the amount of environmentally harmful pesticides needed. The findings from my research lead to a better understanding of plant life as well as pave the way for novel approaches to crop modifications.

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