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CHARACTERIZATION OF THE INTERACTION BETWEEN S-LOCUS F-BOX PROTEINS AND S-RIBONUCLEASES OF *PETUNIA INFLATA* THROUGH THE USE OF CHIMERIC S-LOCUS F-BOX PROTEINS AND CRISPR/CAS9 KNOCKOUT

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ABSTRACT

Petunia inflata uses a genetic mechanism known as self-incompatibility (SI) to prevent inbreeding and promote outcrossing. SI allows the pistil to reject genetically identical (self) pollen, yet accept genetically dissimilar (non-self) pollen for pollination. Self/non-self recognition is determined by the polymorphic *S*-locus, which houses the female and male determinant genes. Seventeen SLF proteins (SLF1 to SLF17) have been identified in *P. inflata* that constitute the male determinant, and a single S-RNase protein constitutes the female determinant. A current model predicts that at least one of the 17 SLF proteins will recognize any non-self S-RNase taken up into a pollen tube to mediate its ubiquitination and degradation, thus resulting in cross-compatible pollination. However, none of the 17 SLF proteins recognize their self S-RNase, allowing the S-RNase to arrest self-pollen tube growth.

Although the amino acid sequences of S_2-SLF1 (SLF1 of *S_2*-haplotype) and S_3-SLF1 (SLF1 of *S_3*-haplotype) are 88.7% identical, S_2-SLF1, but not S_3-SLF1, interacts with S_3-, S_7-, and S_{13}-RNases. To determine which domain of the protein is involved in recognition between S_2-SLF1 and these three non-self S-RNases, chimeric gene constructs of *S_2-SLF1* and *S_3-SLF1* were created and introduced into *P. inflata* plants via *Agrobacterium*-mediated transformation. Two chimeric proteins, F322 (containing the first domain of S_3-SLF1 and the second and third domains of S_2-SLF1) and F232 (containing the first and third domains of S_2-SLF1 and the second domain of S_3-SLF1) and their interactions with S_3-, S_{6a}-, S_7-, S_{12}-, and S_{13}-RNases will be explored in this project.

Additionally, a current hypothesis predicts that even though there is a suite of SLF proteins that can collectively recognize all non-self S-RNases, there may be some redundancy in the interactions with a particular non-self S-RNase. If each non-self S-RNase were only recognized by one SLF protein, a mutation that abolishes the ability of an SLF protein to interact with the non-self S-RNase it recognizes would result in the pollen being incompatible with normally compatible pistils that produce this non-self S-RNase. Up until this point, S_2-SLF1 has been found to interact with the largest number of S-RNases, including S_3-, S_7-, S_{12}-, and S_{13}-RNases. This hypothesis of redundancy has been explored with the use of the CRISPR/Cas9 genome editing system by knocking out *S_2-SLF1* in *S_2S_3* plants and using that plant to pollinate the pistils of various other *S*-haplotype. It was found that *S_2* pollen lacking *S_2-SLF1* was still compatible with *S_7S_7* plants, suggesting at least one other SLF protein is able to recognize S_7-RNase and mediate its ubiquitination and degradation. This project uses these findings to examine the interactions of S_2-SLF2, S_2-SLF12, S_2-SLF14, and S_2-SLF16 with S_7-RNase.

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Introduction

The flower of an angiosperm has spent thousands of years evolving into a complex structure. Within these flowers, both the pollen-producing organ, the stamen, and the egg containing organ, the pistil, are present. With their close proximity, any sort of outside disturbance, like a gust of wind or an animal running through the plant, can lead to the pollen falling onto the pistil, causing a self-fertilization event to occur. Just like in animals, inbreeding of plants can lead to the accumulation of lethal recessive traits and thereby result in decreased fitness of the plant's progeny. Unlike animals, plants are sessile and are subjected to the whims of nature as to their mate choice. Thankfully, after these years of evolution, plants have evolved certain mechanisms to avoid self-fertilization.

Collectively, these mechanisms are called self-incompatibility (SI) and hinge on a plant's ability to classify pollen as self or non-self (de Nettancourt, 2001). Within SI, there exist two strategies adopted by different plant families. Either the plant can recognize pollen as self and reject it, termed self-recognition SI, or the plant can recognize pollen as non-self and accept it, termed non-self recognition SI (McCubbin and Kao, 2000; Iwano and Takayama, 2012). The determination of self or non-self are controlled by the polymorphic *S*-locus. The haplotypes of the *S*-locus is designated as S_1, S_2, S_3, etc and, due to the diploid nature of plants, each plant has two *S*-haplotypes. If a pollen grain carries one of the *S*-haplotypes also carried by the receiving plant, then the pollen grain is recognized as self. If the *S*-haplotype is different *S*-haplotype from either *S*-haplotypes carried by the pistil, then it is recognized as non-self. As illustrated in **Figure 1**, neither S_1 nor S_2 pollen grains will grow in an S_1S_2 pistil whereas an S_3 pollen grain,

transported via wind or insect from an S_3-haplotype plant, will grow in the same pistil. The Kao lab studies *Petunia inflata*, a representative member of the Solanaceae family, which along with the Rosaceae and Plantaginaceae families uses the non-self recognition SI system to prevent inbreeding (McCubbin and Kao, 2000).

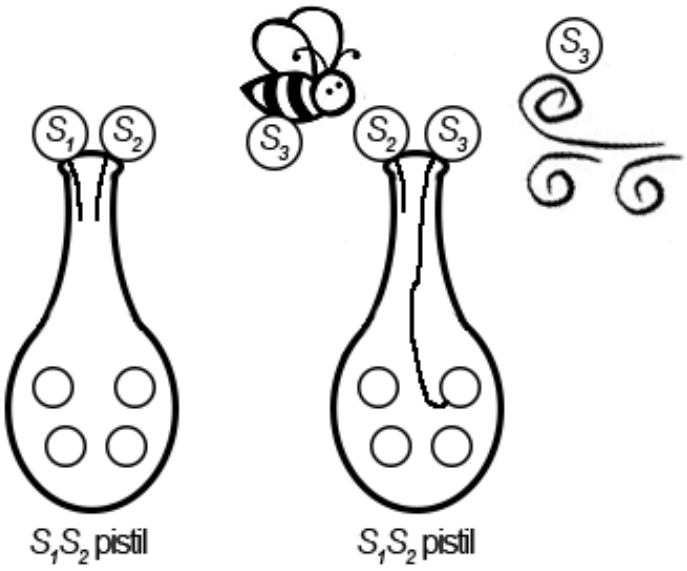

Figure 1. Self-incompatibility in *Petunia inflata*.

In *Petunia inflata*, a collaborative non-self recognition model has been proposed as the mechanism of SI (Kubo et al., 2010). In this model, self/non-self discrimination is determined via the pollen and pistil determinant genes housed at the *S*-locus. First discovered in *Nicotiana alata* (McClure et al., 1989), another member of the Solanaceae family, and subsequently functionally confirmed in *P. inflata* (Lee et al., 1994), the pistil determinant was found to be an S-Ribonuclease (S-RNase) enzyme. The pollen determinant was found to be multiple S-Locus F-box (SLF) proteins in *Petunia* (Sijacic et al., 2004; Kubo et al., 2010). To date, 17 SLF proteins have been discovered in *P. inflata* through a combination of BAC clone screening and transcriptome analysis (Sijacic et al., 2004; Williams et al., 2014). The S-RNase is produced in

the pistil of the plant and is taken up into the growing pollen tube through some mechanism yet unknown and acts to degrade the pollen tube RNAs (McClure et al., 1990), thereby arresting growth of the pollen tube (Huang et al., 1994).

However, to combat the cytotoxic activity of S-RNases, the SLF proteins mediate the ubiquitination and subsequent degradation of the S-RNases (Hua and Kao, 2006). F-box proteins, like the SLF proteins involved in SI, are known to function with Skp1, Cul1, and Rbx1 as a part of an SCF complex, which then functions as a type of E3 ubiquitin ligase. Along with E1 (ubiquitin-activating enzyme) and E2 (ubiquitin-conjugating enzyme), the SCF complex polyubiquitinates whatever the F-box protein recognizes, which is then degraded by the 26S proteasome (Cardozo and Pagano, 2004). The finding that the pollen determinant of SI was a suite of F-box proteins led to the discovery of PiCUL1-P (*Petunia inflata* Cul1), PiSSK1 (*Petunia inflata* SSK1, a type of Skp1), and PiRBX1 (*Petunia inflata* Rbx1), which together function as a non-canonical SCF complex. PiCUL1-P and PiSSK1 were found to be pollen specific, leading to the thought that these components of the SCF complex evolved to function specifically in SI (Li et al., 2014). The proposed structure of the SI SCF complex is shown in **Figure 2**. SLF proteins had been found to interact differentially with non-self RNases where each S-RNase is the substrate of the SCF complex that contains the SLF with which it interacts (Hua et al., 2007). Each SLF in the suite of SLF proteins produced by pollen interacts with a subset of its non-self S-RNases, causing the ubiquitination and degradation of the non-self S-RNases (Entani et al., 2014). Interestingly, it was found that multiple S-RNases can be recognized by one SLF protein (Sun and Kao, 2013). Moreover, it was proposed that more than one SLF may recognize the same non-self S-RNase. Functional redundancy would help ensure

compatible pollination between non-self pollen tubes and the pistil carrying a particular S-RNase (Sun et al., 2014).

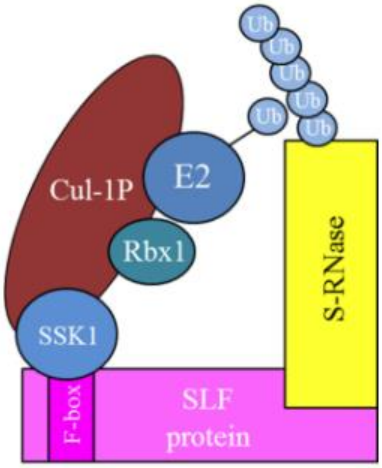

Figure 2. Proposed structure of the *Petunia* SI SCF complex.

Project Goals

As a whole, the Kao lab looks to expand our knowledge of the interactions of SLF proteins and S-RNases in order to fully understand this non-self recognition SI mechanism. As seen in **Table 1**, much work has been done to uncover these interaction relationships. In order to test each and every interaction, a series of steps must be performed. First, the DNA sequence of the *SLF* of interest must be ligated along with the *Late Anther Tomato 52 (LAT52)* promoter, a *GFP* tag, and the *Nopaline Synthase (Nos)* terminator. GFP allows us to easily detect expression of the SLF protein in the pollen grains through fluorescence microscopy and gives us a common sequence to amplify in PCR when confirming presence of a transgene. The *SLF* gene is under the control of the *LAT52* promoter due to the strong and pollen-specific expression of *LAT52* controlled genes (Gerola et al., 2000). This piece of DNA must then be inserted into the pBI101

construct. A typical construct is shown in **Figure** 3. The whole construct is made and inserted into *E. coli* to propagate it, then transformed into *Agrobacterium tumefaciens* for *Agrobacterium*-mediated plant transformation.

Figure 3. Generic construct used in SI interaction determination.

Typically, the constructs are transformed into *P. inflata* of S_2S_3 background and then crossed out to the background to be tested. This way, only one plant transformation is necessary per *SLF* gene. To test a specific interaction with the SLF protein, the construct must be in the S_xS_y background, where x would be the *S*-haplotype of the SLF protein and the y is the *S*-haplotype of the S-RNase to be tested. For example, to test the interaction of S_2-SLF2 with S_7-RNase, an S_2S_7 plant must contain the *S_2-SLF2* transgene. To test whether the SLF protein interacts with the S-RNase, a self-cross is performed. If the self-crossed T_1 plant produces fruit, this would suggest that the SLF protein interacts with the S-RNase to allow fertilization to occur. If the self-cross does not produce fruit, this would suggest that the S-RNase is able to degrade pollen tube RNAs (McClure et al., 1990) to inhibit pollen tube growth. This interpretation of the self-cross can be inferred from independent segregation of the transgene during meiosis.

Following the previous example, S_2S_7 plants can produce four genotypes of pollen: S_2 pollen with the transgene *S_2-SLF2*, S_7 pollen with the transgene *S_2-SLF2*, S_2 pollen without the transgene, and S_7 pollen without the transgene. S_2 pollen with the transgene, S_2 pollen without the transgene, and S_7 pollen without the transgene will all essentially act as wild-type pollen and be rejected by the S_2S_7 pistil. Even with the transgene, S_2 pollen with *S_2-SLF2* will not act any

different than S_2 pollen without S_2-*SLF2* since the S_2-*SLF2* sequence is already present in S_2 pollen. The presence of an extra copy of the gene should not affect the pollen grain's ability to withstand S-RNases. However, the S_7 pollen with the S_2-*SLF2* transgene has a novel sequence and its protein product can potentially detoxify S_7-RNase. If the S_2-SLF2 is able to interact with S_7-RNase and mediate its subsequent degradation by the 26S proteasome, then the S_7 pollen with the S_2-*SLF2* transgene will fertilize the eggs of the plant. If S_2-SLF2 cannot interact with S_7-RNase, then the S_7 pollen with the S_2-*SLF2* transgene will be rejected and no fruit will form. This is shown below in **Figure 4.**

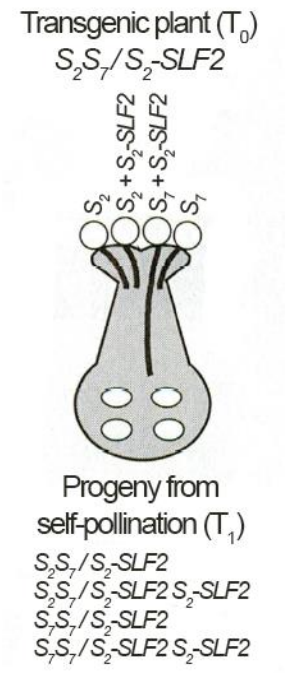

Figure 4. Diagram of the transgenic assay used to ascertain interaction relationships. As shown, only the S_7 pollen with the transgene S_2-*SLF2* is able to bypass the S-RNases in the pistil. S_7 pollen already has an SLF protein to recognize and mediate the degradation of S_2-RNase and the S_2-SLF2 protein expressed from the transgene recognizes and mediates the degradation of S_7-RNase. The eggs themselves will also be either S_2 or S_7 and may or may not contain the S_2-*SLF2* transgene, resulting in the progeny listed. This figure was adapted from Sijacic et al. (2004).

An *in vitro* system, based on co-immunoprecipitation, has been used to confirm the interactions of SLF and S-RNase (Kubo et al., 2010). Testing interactions in yeast would be less time consuming: however it has been found that an 18 amino acid degradation motif exists in S_2-SLF1, reducing the stability of S_2-SLF1 in yeast (Sun et al., 2015). At this time, testing of protein-protein interactions between SLFs and S-RNases requires the *in vivo* system using the transgenic assay as described above.

Chimeric protein analysis of S_2-SLF1 and S_3-SLF1

In our quest to uncover the interactions of SLFs and S-RNases, we have found that two allelic SLF proteins, S_2-SLF1 (SLF1 of the S_2-haplotype) and S_3-SLF1 (SLF1 of S_3-haplotype) to be 88.7% identical in amino acid sequence, yet to interact in opposite manners with three S-RNases. S_2-SLF1 interacts with S_3-RNase, S_7-RNase, S_{12}-RNase, and S_{13}-RNase, while S_3-SLF1 only interacts with S_{12}-RNase. Despite such a similar amino acid sequence that differs by only 44 amino acids, there are three differences in the interaction patterns. Our thought is that a subset of these amino acids must determine the interaction specificity of these SLF proteins with these S-RNases (Wu et al., 2017). To study which domain of the SLF protein, and eventually the specific amino acids, contributes to the interaction specificity of these SLF1 proteins, systematic examination of each portion of the protein is necessary.

A previous graduate student, Ning Wang, divided the coding sequence of SLF1 into three Functional Domains (FDs). Functional Domain 1 (FD 1) consists of amino acids 1-130 of the 395 amino acids of SLF1, Functional Domain 2 (FD 2) consists of amino acids 131-260, and

Functional Domain 3 (FD 3) consists of amino acids 261-395. According to the general F-box protein structure, the N-terminal domain of the protein contains the F-box motif that interacts with Skp1 in the SCF complex, while the C-terminal domain is responsible for interacting with the substrate of the SCF complex. In this scheme, FD 1 corresponds to the N-terminal F-box domain, while FD 2 and FD 3 are the C-terminal interaction domain. In order to study which domain of S_2-SLF1 and S_3-SLF1 contributes to their interaction specificity, chimeric proteins were built using FD 1, FD 2, and FD 3. Examining how these chimeric proteins differ in their interactions with a variety of S-RNases will give us insight into which domain of the protein contains amino acids that are required for the differential interactions with S-RNases. In this project, the main focus is on chimeric proteins F322 and F232. The naming scheme comes from the arrangement of functional domains in the chimeric protein. For example, F322 designates that FD 1 comes from S_3-SLF1 while FD 2 and FD 3 come from S_2-SLF1. Likewise, F232 designates that FD 1 comes from S_2-SLF1, FD 2 comes from S_3-SLF1, and FD 3 comes from S_2-SLF1. A schematic of F322 and F232 is shown in **Figure 5.** Even though this project focuses mainly on F322 and F232, other chimeric proteins exist. These other chimeric proteins have been studied by another graduate student, Lihua Wu (Wu et al., 2017).

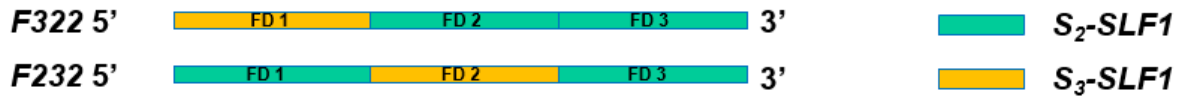

Figure 5. The makeup of the chimeric proteins F232 and F322.

One distinction to make within this project and all experiments using these chimeric SLF proteins is the use of the term "interaction specificity." The goal is to determine which amino acids are used to selectively recognize one S-RNase versus another. However, this information does not tell the whole story. Even though some amino acids are able to discriminate S-RNase molecules, it is likely that some of the amino acids conserved across SLF proteins may also be involved in interactions with S-RNases. The identification of these amino acids is beyond the scope of this project and will not be studied. Determining them necessitates methods other than chimeric protein creation, such as protein crystallization.

CRISPR/Cas9 driven determination of SLF - S-RNase interactions

CRISPR/Cas9 is a technology that has recently revolutionized molecular biology. Through the use of CRISPR/Cas9, editing genomic DNA sequences has become easier than ever before. The CRISPR/Cas9 system was originally found in bacteria and archaea as a type of acquired immunity against bacteriophage infection (Horvath and Barrangou, 2010). The CRISPR-associated endonuclease (Cas) uses a guide RNA to target a specific DNA sequence and introduce a double strand break, which can be repaired in two ways (Hsu et al., 2014). To insert a sequence of interest, the sequence can be supplied through transformation and the double strand break will be repaired through homologous recombination (Hsu et al., 2014). To knock out the gene, nothing extra is necessary since the cell will go through non-homologous end joining, which will induce a frameshift mutation (Hsu et al., 2014). This frameshift mutation will likely knock out the gene of interest, but it is still necessary to check for levels of gene

expression (Hsu et al., 2014). This system has been seen to work well in many organisms, including *Arabidopsis thaliana* (Fauser et al., 2014), zebrafish (Hwang et al., 2013) and human cells (Shalem et al., 2014). CRISPR/Cas9 can work in many different systems across domains of life and has already been documented to perform targeted mutagenesis of *Petunia inflata* (Zhang et al., 2016).

With the CRISPR/Cas9 system, a current graduate student, Linhan Sun, has knocked out *S_2-SLF1* in *S_2S_3* transgenic plants of *P. inflata* (Linhan Sun, unpublished results). Previous experiments had shown that of all SLF proteins tested only S_2-SLF1 interacts with S_7-RNase to allow fertilization of the eggs by the pollen. Note that a small subset of the 17 total SLF proteins of *S_2*-haplotype had been tested against S_7-RNase at this point. However, the *S_2-SLF1* knockout plant produced through the CRISPR/Cas9 system was still able to fertilize *S_2S_7* plants and produce progeny carrying the *S_2*-haplotype. This would suggest that another SLF protein is also responsible for interacting with and mediating the degradation of S_7-RNase. With that prediction, we looked at the activity of S_2-SLF2, S_2-SLF12, S_2-SLF14, and S_2-SLF16 in an *in vivo* system to determine their interactions or lack thereof with S_7-RNase. These were all SLF proteins that had been waiting to be tested, but now it was predicted that one of them likely interacted with S_7-RNase.

Table 1. All known interactions of S_2-SLFx with various S-RNases.

		S-RNase										
		S_2	S_3	S_5	S_{6a}	S_7	S_{11}	S_{12}	S_{13}	S_{16}	S_{22}	S_{24}
SLF	S_2-SLF1	–	+	–	–	+	–	+	+	–	–	N/A
	S_2-SLF2	–	–	N/A	N/A	+	N/A	N/A	N/A	N/A	N/A	N/A
	S_2-SLF3	–	–	–	–	–	–	–	–	–	N/A	N/A
	S_2-SLF4	–	–	+	–	–	–	–	–	–	N/A	–
	S_2-SLF5	–	–	–	–	–	–	+	–	–	–	–
	S_2-SLF6	–	–	–	–	–	–	–	–	–	N/A	–
	S_2-SLF7	–	–	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	S_2-SLF8	–	–	–	+	–	–	–	–	–	N/A	N/A
	S_2-SLF9	–	–	–	–	–	–	–	–	–	–	–
	S_2-SLF10	–	–	–	–	–	–	–	–	–	–	–
	S_2-SLF11	–	–	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	S_2-SLF12	–	–	N/A	N/A	–	N/A	N/A	N/A	N/A	N/A	N/A
	S_2-SLF13	–	–	N/A	N/A	–	N/A	N/A	N/A	N/A	N/A	N/A
	S_2-SLF14	–	–	N/A	N/A	–	N/A	N/A	N/A	N/A	N/A	N/A
	S_2-SLF15	–	–	–	–	–	–	–	–	–	–	N/A
	S_2-SLF16	–	–	N/A	N/A	–	N/A	N/A	N/A	N/A	N/A	N/A
	S_2-SLF17	–	–	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

"+" indicates an interaction, "–" indicates no interaction.

Materials and Methods

Transgenic Plant Generation. The constructs used in this experiment were all constructed by Justin Williams and Ning Wang, both previous members of the lab. All transgenic plants were produced through regenerative *Agrobacterium* mediated plant transformation as described in Lee et al. (1994) and were done by Justin Williams, Ning Wang, and Linhan Sun (a current graduate student of the lab).

Seed Germination. Approximately 50 seeds were soaked in 1 mL of 0.005% gibberellic acid solution for 30 minutes. Afterwards, both seeds and solution were spread in a petri dish onto a sheet of coarse pore filter paper. Seeds were spread out so that no two seeds were touching. Extra dH_2O was added until the entire filter paper was dampened and all standing water was removed. The petri dish was sealed with Parafilm and placed for 2 days in a 26°C incubator while covered with aluminum foil, then for another day uncovered. Seeds were then washed off the filter paper onto lightly damp soil and incubated at 30°C until true leaves sprouted. Sprouts with true leaves were moved to individual plots of soil and incubated at 30°C until plant was approximately 15 cm tall. Plants were transferred to a greenhouse kept at 25°C with a 16 hour light cycle.

DNA Isolation. Genomic DNA was isolated from plant leaf tissue using the Plant DNAzol® Reagent by Invitrogen according to manufacturer's protocol. Approximately 0.5 g of leaf tissue was used per plant per reaction. Samples were stored at -20°C.

PCR cocktail. Polymerase chain reaction (PCR) and gel electrophoresis were used to confirm the presence of green fluorescent protein (GFP), our marker for each transgene, in each DNA sample. The gene of interest was amplified according to the protocol described by Meng et al. (2011). Genotyping was accomplished by amplifying the specific *S*-haplotype *S-RNase* gene or *SLF1* gene. PCR conditions and genotyping primer sequences are listed in **Tables 2** and **3** respectively.

Pollen Germination. To validate transgene expression in adult plants, pollen was germinated in pollen germination media (0.07% (w/v) $Ca(NO_3)_2 \cdot 4H_2O$, 0.02% (w/v) $MgSO_4 \cdot 7H_2O$, 0.01% (w/v) KNO_3, 0.01% (w/v) H_3BO_3, 0.2% (w/v) sucrose, 15% (w/v) polyethylene glycol 4000, and 20 mM MES, pH 6.0) and observed under fluorescent light for GFP fluorescence. A JENOPTIK ProgRes C14plus camera on an Olympus S2X16 microscope was used to visualize pollen tubes at 110x magnification.

Table 2. PCR Conditions for transgene verification and genotyping.

S_2-RNase		
Temperature	Time	
95°C	5:00	
95°C	0:30	Repeat 40x
57°C	0:30	
72°C	1:00	
72°C	10:00	
4°C	30:00	

PiSLF2 (S_2-SLF1)		
Temperature	Time	
95°C	5:00	
95°C	0:30	Repeat 40x
59°C	0:30	
72°C	0:40	
72°C	10:00	
4°C	30:00	

PiSLF3 (S_3-SLF1)		
Temperature	Time	
95°C	5:00	
95°C	0:30	Repeat 40x
57°C	0:30	
72°C	0:40	
72°C	10:00	
4°C	30:00	

GFP		
Temperature	Time	
95°C	5:00	
95°C	0:30	Repeat 35x
55°C	0:30	
72°C	0:45	
72°C	10:00	
4°C	30:00	

S_{6a}-RNase		
Temperature	Time	
95°C	5:00	
95°C	0:30	Repeat 35x
57°C	0:30	
72°C	0:30	
72°C	10:00	
4°C	30:00	

S_7-RNase		
Temperature	Time	
95°C	5:00	
95°C	0:30	Repeat 40x
60°C	0:30	
72°C	1:20	
72°C	10:00	
4°C	30:00	

S_{12}-RNase		
Temperature	Time	
95°C	5:00	
95°C	0:30	Repeat 40x
56°C	0:30	
72°C	1:00	
72°C	10:00	
4°C	30:00	

S_{13}-RNase		
Temperature	Time	
95°C	5:00	
95°C	0:30	Repeat 40x
60°C	0:30	
72°C	1:20	
72°C	10:00	
4°C	30:00	

Table 3. DNA Sequences for primers used in PCR.

Primer Name	Sequence
S_2-RNase FOR	5'-AAGGATCCTGTTTGACTACTTCCAACTCG-3'
S_2-RNase REV	5'-GTCGACTCATCTCCGAAACAGAGTCT-3'
PiSLF2-RT-3For	5'-GTTTGTGATTTGAGTACTGATTCT-3'
PiSLF2-RT-4Rev	5'-AATACAGCTCGTGCGTAATCCTAC-3'
PiSLF3-Copy1-For	5'-GCTTTTGATTTGAGCACTGATTCT-3'
PiSLF3-Copy1-Rev	5'-AATACTGCTTGTGTGTAACAC-3'
GFP001FW	5'-GGCGGAGGTATGGTGAGCAAGGGCG-3'
GFP500REV	5'-ATATAGACGTTGTGGCTGTTGTAG-3'
S_{6a}-RNaseFW	5'-ATGGTTAGATTACAGCTCTTGTCAG-3'
S_{6a}-RNaseREV	5'-TCATCCGCGAAACAGAATCTTCGTG-3'
S_7-RNaseFW	5'-ATGTTTAAACCACAACTCACTTCAG-3'
S_7-RNaseREV	5'-TCATCGCCGAAACAAAATTTTTCCT-3'
S_{12}-RNaseFW	5'-ATGTTTAAATCACAGCTCATGTCTG-3'
S_{12}-RNaseREV	5'-TCATCTTCGAAACAAAATCCTTGTA-3'
S_{13}-RNaseFW	5'-ATGTTTAGATTACAACTCACATCAG-3'
S_{13}-RNaseREV	5'-TCATCTCCGAAACAGAGTCTTCGTG-3'

Results

Analysis of interactions of chimeric protein F322 with non-self S-RNases.

In order to test which domain of S_2-SLF1 interacts with its non-self S-RNases, it is necessary to systematically examine each domain of the protein. Chimeric protein F322 seeks to understand what happens to the interaction pattern of S_2-SLF1 when FD 1 is replaced with FD 1 of S_3-SLF1. Chimeric protein F322 thus contains FD 1 from S_3-SLF1 and both FD 2 and FD 3 from S_2-SLF1. *F322* was put into a pBI101 construct along with the *LAT52* promoter, a *Nos* terminator, and a *GFP* tag. A schematic of this construct is shown in **Figure 6**. Plants with the transgene *F322* in an S_2S_3 background (*F322*/S_2S_3) were previously crossed to S_5, S_{6a}, S_7, S_{11}, S_{12}, S_{13}, and S_{16} backgrounds by Justin Williams. The interaction of F322 with S_7- and S_{12}-RNases has been tested by a previous undergraduate student, Danielle San-Román. However, this experiment looks to increase the number of plants tested to increase the likelihood that these results are true and significant. Transgenic S_2S_7 and S_2S_{12} plants were found from crosses of *F322*/S_2S_3 with S_7S_{13} and $S_{12}S_{12}$ plants after genotyping of the progeny. This has previously been accomplished by Danielle San-Román. Transgenic S_2S_{6a} plants were found by genotyping of seeds produced from a cross of *F322*/S_2S_3 and $S_{6a}S_{6a}$ plants. Each plant was checked for the presence of the transgene by verifying the presence of the GFP tag. PCR was used to identify *GFP* in the genomic DNA and fluorescent microscopy was used to observe the expression of GFP in mature pollen, which is represented in **Figures 7** and **8**.

Each plant was self-crossed in order to determine whether or not F322 interacts with S_{6a}-, S_7-, or S_{12}-RNases. Afterwards, the plants were subjected to an SI behavior confirmation cross to determine whether or not the presence of the transgene broke down SI all together. For example, *F322/S_2S_7* plants were found to produce a large fruit set when self-crossed. A known incompatible cross was then performed where pollen from an *S_2S_7* plant was used to fertilize the *F322/S_2S_7* plant. If the plant somehow started accepting all pollen, then this cross would produce a fruit set. If it behaves as expected and the SI had not broken down, then there would be no fruit set. When self-crossed, *F322/S_2S_{6a}* plants did not produce a fruit set while *F322/S_2S_{12}* plants did produce a fruit set. Additionally, in the confirmation crosses, *F322/S_2S_{6a}* produced a fruit set when pollinated with *S_5S_5* WT pollen, and *F322/S_2S_{12}* did not produce a fruit set when pollinated with *S_2S_2* WT pollen. Individual plant data for the *F322/S_2S_{6a}*, *F322/S_2S_7*, and *F322/S_2S_{12}* is shown in **Table 4**. Additionally, the progeny of the *F322/S_2S_{12}* self-cross were all genotyped to determine which pollen grains were allowed to fertilize the eggs of the transgenic plant. There were no *S_2S_2* plants in the progeny, revealing that all *S_2* pollen was rejected. The presence of the transgene in all the progeny indicates that only transgenic pollen was allowed to fertilize the eggs of the plant. This data can be seen in **Table 5**. The summary of interactions of chimeric proteins with the S-RNases is shown in **Table 8**.

Table 4. Individual plant data for F322 interactions.

Transgene	Genotype	Plant Number	PCR GFP Check	Pollen GFP Check	Self-Cross	Confirmation Cross
F322	S_2S_{6a}	17	Yes	Yes	3x SI	N/A
	S_2S_{6a}	28	Yes	Yes	3x SI	3x SC
	S_2S_7	18	Yes	Yes	3x SC	N/A
	S_2S_7	22	Yes	Yes	3x SC	2x SI
	S_2S_{12}	15	Yes	Yes	3x SC	N/A
	S_2S_{12}	16	Yes	Yes	3x SC	N/A

Table 5. Progeny Analysis of *F322/S_2S_{12}*

Plant Strain	T_1 Progeny Ratio (S_2S_{12}:$S_{12}S_{12}$)	Expected Ratio	χ^2 (1:1)	*P*-value (1:1)	χ^2 (1:2:1) (S_2S_2:S_2S_{12}:$S_{12}S_{12}$)	*P*-value (1:2:1)
F322/S_2S_{12}	11:11	1:1	0	1.00	11.00	0.0041

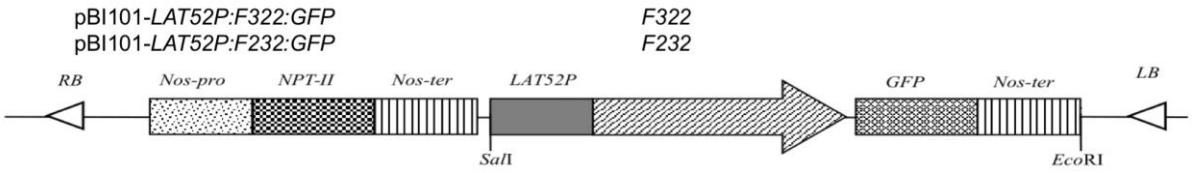

Figure 6. Schematic of F322 and F232 constructs.

This figure was adapted from Wu et al. (2017).

Analysis of interactions of chimeric protein F232 with non-self S-RNases.

As a part of the systematic determination of which domain of S_2-SLF1 interacts with its non-self S-RNases, FD 2 was examined by the same method as FD 1. Chimeric protein F232 was used in this pursuit, which contains FD 1 from S_2-SLF1, FD 2 from S_3-SLF1, and FD 3 from S_2-SLF1. Just like *F322*, this gene was put into a pBI101 construct along with the *LAT52* promoter, a *Nos* terminator, and a *GFP* tag. A schematic of this construct is shown in **Figure 6**. Each plant was checked for the presence of the transgene by verifying the presence of the GFP tag. PCR was used to identify *GFP* in the genomic DNA and fluorescent microscopy was used to observe the expression of GFP in mature pollen, which is represented in **Figures 7** and **8**. Plants with the transgene *F232* in an S_2S_3 background ($F232/S_2S_3$) were previously crossed to S_5, S_{6a}, S_7, S_{11}, S_{12}, S_{13}, and S_{16} backgrounds by Justin Williams (Wu et al., 2017). Danielle San-Román has previously determined the interactions of F232 with S_5-, S_{6a}-, S_{12}-, and S_{16}-RNases (Wu et al., 2017). This part of the project aims to complete testing the plants that she had generated for the interaction of F232 with S_{12}-RNase and add to our knowledge by testing the interaction of F232 with S_7- and S_{13}-RNases. $F232/S_2S_3$ was previously crossed into S_7S_{13} background by Justin Williams and those seeds were germinated for this experiment (Wu et al., 2017). The desired S_2S_7 and S_2S_{13} genotypes were found via genotyping of genomic DNA of seedlings.

Determination of the interaction of F232 with various S-RNases was carried out in the same pipeline as previously described for F322. When self-crossed, neither $F232/S_2S_7$ nor $F232/S_2S_{13}$ plants produced a fruit set while $F232/S_2S_{12}$ did produce a large fruit set. In the confirmation crosses, $F232/S_2S_7$ produced a fruit set when pollinated with S_5S_5 WT pollen, $F232/S_2S_{13}$ produced a fruit set when pollinated with S_5S_5 WT pollen, and $F232/S_2S_{12}$ did not produce a fruit set when pollinated with S_2S_2 WT pollen, which is all as expected. Individual

plant data for the *F232/S_2S_7*, *F232/S_2S_{12}*, and *F232/S_2S_{13}* is shown in **Table 6**. Additionally, the progeny of the *F232/S_2S_{12}* self-cross were genotyped and analyzed in the same fashion as was the *F322/S_2S_{12}* self-cross. The results indicated that only transgenic S_{12} pollen was able to fertilize the eggs of the plant. This data is shown in **Table 7**. The summary of the interactions of the chimeric proteins with the S-RNases is shown in **Table 8**.

Table 6. Individual plant data for F232 interactions.

Transgene	Genotype	Plant Number	PCR GFP Check	Pollen GFP Check	Self-Cross	Confirmation Cross
F232	*S_2S_7*	11	Yes	Yes	3x SI	3x SC
	S_2S_7	24	Yes	Yes	3x SI	2x SC
	S_2S_7	29	Yes	Yes	3x SI	3x SC
	S_2S_{12}	21	Yes	Yes	3x SC	3x SI
	S_2S_{12}	29	Yes	Yes	3x SC	3x SI
	S_2S_{13}	7	Yes	Yes	3x SI	3x SI
	S_2S_{13}	19	Yes	Yes	3x SI	3x SC

***F232/S_2S_{13}* plant 7 had a different confirmation cross than previously explained. The pollen from this transgenic plant was used to pollinate *S_2S_{13}* WT plants. If F232 was truly unable to interact with the S_{13}-RNase, then it would not interact with it in a wild type plant.**

Table 7. Progeny Analysis of *F232/S_2S_{12}*

Plant Strain	T_1 Progeny Ratio (S_2S_{12}:$S_{12}S_{12}$)	Expected Ratio	χ^2 (1:1)	*P*-value (1:1)	χ^2 (1:2:1) (S_2S_2:S_2S_{12}:$S_{12}S_{12}$)	*P*-value (1:2:1)
F232/S_2S_{12}	12:11	1:1	0.043	0.8348	10.565	0.0051

Table 8. Summary table of F322 and F232 interactions with 8 S-RNases.

		SLF			
		S_2-SLF1	S_3-SLF1	F322	F232
S-RNase	S_2	–	–	–	–
	S_3	+	–	+	+
	S_5	–	–	N/A	–
	S_{6a}	–	–	–	–
	S_7	+	–	+	–
	S_{12}	+	+	+	+
	S_{13}	+	–	N/A	–
	S_{16}	–	–	N/A	–

"+" indicates an interaction, "–" indicates no interaction.

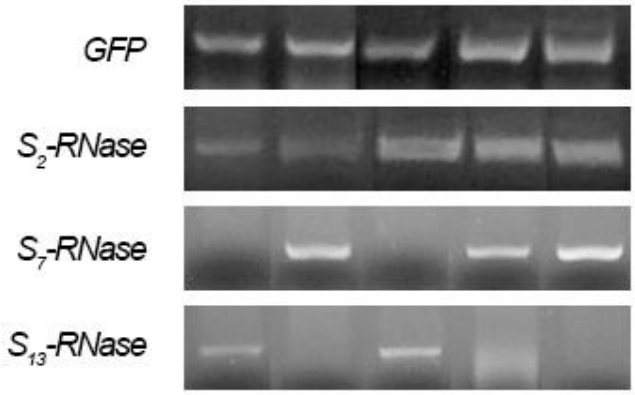

Figure 7. PCR gene verification of *F232/F322* plants

Each plant was genotyped and checked for the presence of the transgene through PCR. The presence of *GFP* indicates that the plants contain the transgene. The presence of *S_2-RNase, S_7-RNase,* or *S_{13}-RNase* indicates the plant carries *S_2-*, *S_7-*, or *S_{13}*-haplotype. Since these plants originated from *S_7S_{13}* plants, the progeny will carry either *S_7-* or *S_{13}*-haplotype, but not both.

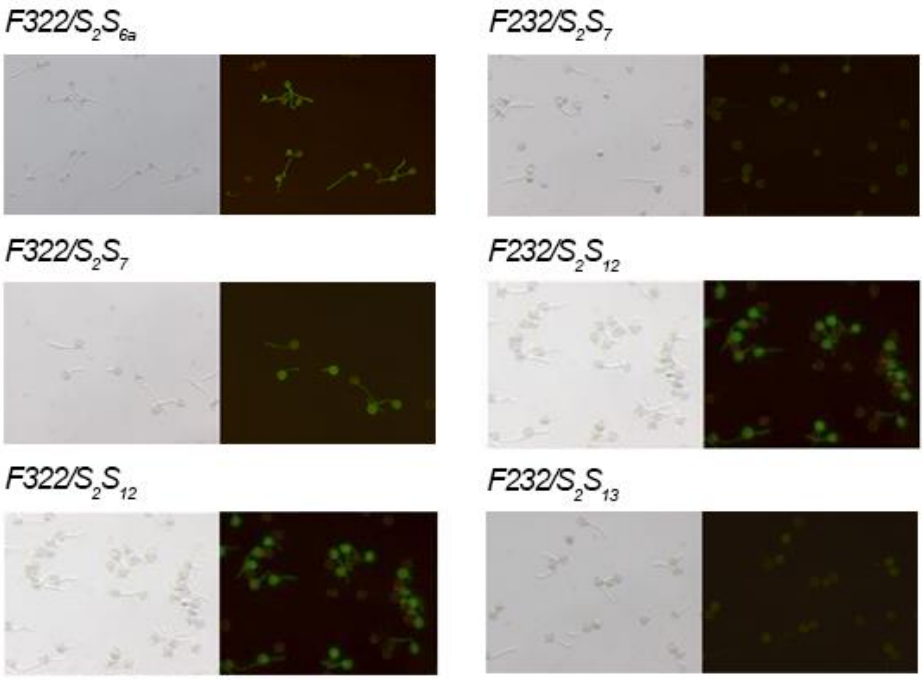

Figure 8. Expression of chimeric proteins in pollen tubes.

Pollen was induced to grow and further observed under a fluorescent microscope. Fluorescence of these pollen grains and tubes indicates expression of our transgene. Both pollen under visible light and fluorescent light are shown.

Analysis of interactions of S_2-SLF2, 12, 14, and 16 with S_7-RNase.

After knocking out *S_2-SLF1* in *S_2S_3* plants using the CRISPR/Cas9 system, our experiment determined that the pollen from these plants were still able to produce *S_2*-haplotype progeny when pollinating *S_2S_7* WT plants, indicating that another SLF protein can recognize S_7-RNase. At this point, there were a few SLF proteins that were readily testable. That list of proteins included S_2-SLF2, S_2-SLF12, S_2-SLF14, and S_2-SLF16. The coding sequence of each

protein was inserted into a pBI101 plasmid along with the *LAT52* promoter, a *GFP* tag, and the *Nos* terminator. A schematic of these constructs is shown in **Figure 9**. Transformation of these constructs into S_2S_3 background and crossing them with S_7S_7 plants was previously accomplished by a current graduate student, Linhan Sun (Linhan Sun, unpublished results). Seedlings were then germinated and the desired S_2S_7 genotype was found by genotyping the progeny. Both PCR and fluorescent microscopy was used to identify the presence and expression of the transgene through the GFP tag, which is shown in **Figures 10** and **11**.

S_2-SLF2/S_2S_7, *S_2-SLF12/S_2S_7*, *S_2-SLF14/S_2S_7*, and *S_2-SLF16/S_2S_7* plants were then self-crossed to determine whether or not a fruit set would be produced. Of these plant lines, only *S_2-SLF2/S_2S_7* plants produced a fruit set when self-crossed. *S_2-SLF12/S_2S_7*, *S_2-SLF14/S_2S_7*, and *S_2-SLF16/S_2S_7* plants did not produce any fruit when self-crossed. The plants are now scheduled to be used to pollinate wild type S_2S_7 plants to determine if the presence of the transgene interferes with normal SI functioning, which is a different confirmation cross than was done with the chimeric plants. Since a wild type plant is being pollinated, there is no chance that the transgene could interfere with that plant's SI. However, the pollen carries the transgene and should exhibit the same interaction pattern that the self-cross produced. As of now, only *S_2-SLF2/S_2S_7* plants have been crossed into wild type S_2S_7 plants and it was found that there is no fruit produced in this cross, which is as expected. Cross data for each plant tested in this experiment is shown in **Table 9** and a summary of interactions of these proteins with S_7-RNase is shown in **Table 10**.

Table 9. Individual plant data for S_2-SLF2, 12, 14, 16 interactions.

Transgene	Genotype	Plant Number	PCR GFP Check	Pollen GFP Check	Self Cross	Confirmation Cross
S_2-SLF2	S_2S_7	4	Yes	Yes	1x SC	2x SC
	S_2S_7	5	Yes	Yes	1x SC	2x SC
	S_2S_7	9	Yes	Yes	1x SC	3x SC
	S_2S_7	13	Yes	Yes	1x SC	N/A
	S_2S_7	14	Yes	Yes	1x SC	1x SC
S_2-SLF12	S_2S_7	1	Yes	Yes	3x SI	N/A
	S_2S_7	4	Yes	Yes	3x SI	N/A
	S_2S_7	12	Yes	Yes	3x SI	N/A
	S_2S_7	15	Yes	Yes	3x SI	N/A
	S_3S_7	17	Yes	Yes	3x SI	N/A
S_2-SLF14	S_2S_7	3	Yes	Yes	3x SI	N/A
	S_2S_7	4	Yes	Yes	3x SI	N/A
	S_2S_7	5	Yes	Yes	3x SI	N/A
	S_2S_7	22	Yes	Yes	3x SI	N/A
S_2-SLF16	S_2S_7	17	Yes	Yes	2x SI	N/A
	S_2S_7	20	Yes	Yes	3x SI	N/A
	S_2S_7	27	Yes	Yes	3x SI	N/A

These plants have a different confirmation cross than what was done with the transgenic plants with the chimeric SLF proteins.

Table 10. Summary table for S_2-SLF2, 12, 14, and 16 interactions with S_7-RNase.

	SLF			
	S_2-SLF2	S_2-SLF12	S_2-SLF14	S_2-SLF16
S_7-RNase	+	–	–	–

"+" indicates an interaction, "–" indicates no interaction.

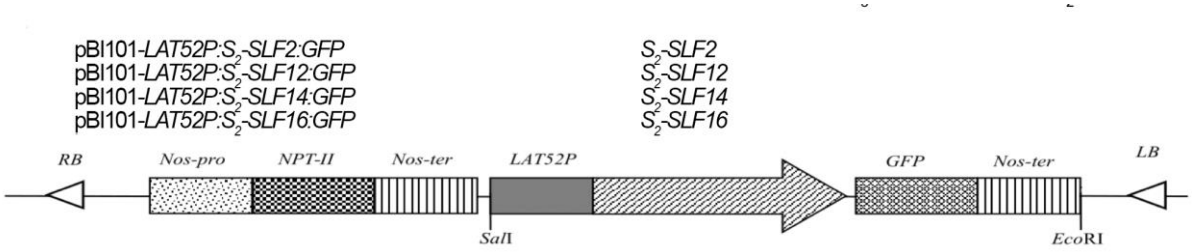

Figure 9. Schematic of S_2-SLFx constructs.

This figure was adapted from Wu et al. (2017).

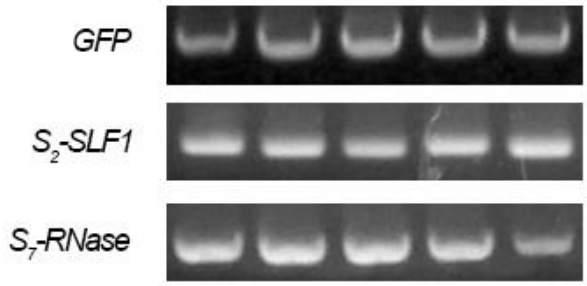

Figure 10. PCR gene verification of *S_2-SLFx/S2S7* plants

Each plant was genotyped and checked for the presence of the transgene through PCR. The presence of *GFP* indicates that the plants contain the transgene. The presence of *S_2-SLF1* and *S_7-RNase* indicates the plant is of *S_2-* and *S_7-*haplotype.

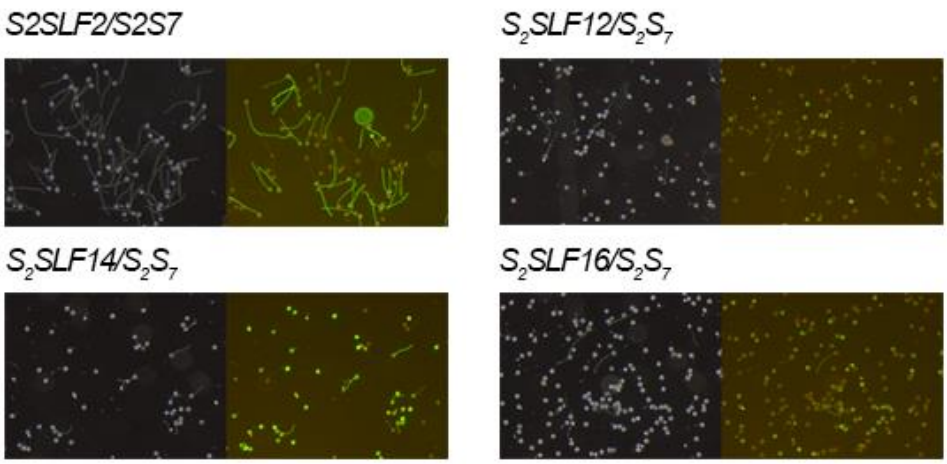

Figure 11. Expression of S_2-SLFx proteins in pollen tubes.

Pollen was induced to grow and further observed under a fluorescent microscope. Fluorescence of these pollen grains and tubes indicates expression of our transgene. Both pollen under visible light and fluorescent light are shown.

Discussion

Throughout the years of examining SI in *Petunia inflata*, many advances have been made. The discovery of the interactions of the S-RNase protein and multiple SLF proteins has revealed to us the deep mystery of SI. Among the SLF proteins that have been examined, S_2-SLF1 and S_3-SLF1 have 88.7% of the amino acid sequence in common, yet they interact differently with three S-RNases. With these proteins, it is possible to identify which amino acids determine the interaction specificity, and therefore, a better idea of which amino acids are present at the interaction surface of S-RNases and SLF proteins.

F322, a chimeric protein made with FD 1 of S_3-SLF1 and both FD 2 and FD 3 of S_2-SLF1, should have its own characteristic pattern of interaction with all the S-RNases tested in this experiment. This protein was created to determine whether or not FD 1, which has 15 amino acid differences between S_2-SLF1 and S_3-SLF1, contains amino acids contributing to the interaction specificity of these SLF proteins (Wu et al., 2017). Should this protein follow the interaction pattern of S_2-SLF1, we will know that FD 1 does not contribute to the interaction specificity of S_2-SLF1 since the interaction pattern did not change from S_2-SLF1. If this protein were to follow the interaction pattern of S_3-SLF1, then we would expect that FD 1 contributes to the interaction specificity of S_2-SLF1 since there was a difference in the interaction pattern due to the change of FD 1. For all the S-RNases tested, F322 followed the interaction pattern of S_2-SLF1. As seen in **Table 8**, the major difference in interaction pattern between S_2-SLF1 and S_3-SLF1 lies in the interaction with S_3-RNase, S_7-RNase, and S_{13}-RNase; S_2-SLF1 is able to interact

with all of these S-RNases while S_3-SLF1 cannot. From this set of experiments, we know that F322 does interact with S_7-RNase and according to Wu et al., 2017, F322 does interact with S_3-RNase. Therefore, we can say that FD 1 does not contribute to the interaction specificity of S_2-SLF1 with these two S-RNases.

The idea that FD 1 does not contribute to the interaction specificity of S_2-SLF1 or S_3-SLF1 is not entirely surprising. With sequence analysis, we have been able to determine that FD 1 corresponds to the F-box domain of these SLF proteins. Within an SCF complex, we know that the F-box domain does not interact with the substrate of the E3 ubiquitin ligase, but rather connect the SLF protein to the rest of the SCF complex (Cardozo and Pagano, 2004). While necessary to the proper functioning of SLF proteins in SI (Meng et al., 2011) this domain of the protein should not be interacting with the substrate, which in this instance is the non-self S-RNase. Ideally, in order to double check these results, the construction of a new chimeric protein, F233, is needed. F233 would contain FD 1 from S_2-SLF1 and FD 2 and FD 3 from S_3-SLF1 and would expect to follow the interaction pattern of S_3-SLF1. Indeed, in Wu et al. (2017), F233 was introduced to *Petunia inflata* and was tested in its interaction with S_3-RNase. F233 did not interact with this S-RNase, just like S_3-SLF1. However, no tests were done beyond this and it is unknown whether or not F233 continues to act like S_3-SLF1 with other S-RNases. Results from other chimeric protein tests have pointed to FD 2 and FD 3 housing the amino acids responsible for interaction specificity within S_3, S_7, and S_{13} pollen (Wu et al., 2017).

F232, a chimeric protein made from FD 1 of S_2-SLF1, FD 2 from S_3-SLF1, and FD 3 from S_2-SLF1, was created to examine how changing FD 2 would change the interaction pattern of S_2-SLF1. FD 2 contains 13 of the total 44 differing amino acids between S_2-SLF1 and S_3-SLF1 (Wu et al., 2017). Should F232 act like S_2-SLF1 in its interaction pattern with the 8 chosen

S-RNases, then we would know that the amino acids in FD 2 does not contribute to the interaction specificity of S_2-SLF1 since FD 2 of S_2-SLF1 was swapped out with FD 2 of S_3-SLF1. If F232 has a similar interaction pattern to S_3-SLF1, then the amino acids within FD 2 will contribute to the interaction specificity of S_2-SLF1 with the selected S-RNases. For the tests performed, it seems that F232 is similar to S_2-SLF1 in its interaction with S_3-RNase while also being similar to S_3-SLF1 in its lack of interaction with S_7-RNase and S_{13}-RNase. Therefore, it seems that amino acids from different Functional Domains of the protein contribute to interaction specificity with different S-RNases.

In the instance of the interaction of S_2-SLF1 and S_3-SLF1 with S_3-RNase, another chimeric protein had been tested, F332. This protein, which essentially is S_3-SLF1 but with FD 3 of S_2-SLF1, interacted with S_3-RNase just like S_2-SLF1. Therefore, it is thought that FD 3 contains the amino acids requisite for recognition specificity of S_3-RNase (Wu et al., 2017). Yet, F232 was found to behave like S_3-SLF1 in its non-interaction with S_7-RNase and S_{13}-RNase even though the molecule is essentially S_2-SLF1 with FD 2 of S_3-SLF1. Therefore, FD 2 and FD 3 contain the amino acids necessary for specifically recognizing S_7-RNase and S_{13}-RNase. As the test with F332 was done prior to this experiment, these results were initially shocking. However, it is not unreasonable to believe that an SLF protein has slightly different amino acid contacts within the interacting domain depending on which S-RNase is being recognized. With this mechanism, one SLF protein can recognize multiple non-self S-RNases and promote outcrossing of progeny.

In order to fully corroborate these results, more chimeric proteins would need to be created. For example, chimeric protein F323 would be a check for the results of F232. If the amino acids within FD 2 contribute to the interaction specificity of S_2-SLF1 with S_7-RNase and

S_{13}-RNase, then F323 should exhibit the interaction pattern of S_2-SLF1 for the same reasons that F232 exhibits the interaction pattern of S_3-SLF1. Yet, this does not completely answer the question of what amino acids contribute to the interaction specificity of S_2-SLF1 with S_7-RNase and S_{13}-RNase, because FD 2 contains 13 differing amino acids. It is not known which specific amino acids engage in recognizing the S-RNase. One method that has been used to narrow down which amino acids in FD 3 contribute to the interaction of S_2-SLF1 with S_3-RNase is to divide up the FD into smaller regions. FD 2 could be divided into smaller and smaller subdomains and tested using the same approach as this chimeric scheme to determine which amino acids contribute to the interaction specificity of the SLF protein. Another method to determine this is to co-crystallize S_2-SLF1 with S_7- or S_{13}-RNase and identify the part of the protein that is at the interaction surface.

One goal of the SI researchers is to determine the various interactions between SLF and S-RNase. As seen, the wide variety of SLF proteins and S-RNases make this goal difficult to reach. Due to the wide variety of SLF proteins, the collaborative non-self recognition model has been proposed as the mechanism by which SI is accomplished within the pistil of *Petunia inflata* (Kubo et al., 2010). With the thought that there are multiple SLF proteins involved in SI, we hypothesize that each non-self S-RNase is recognized by at least two SLF proteins. It is thought that having multiple SLF proteins is evolutionarily advantageous for *P. inflata* because if something were to happen to one of the SLF proteins, like a mutation in the coding sequence, then there would still be another one available to recognize a designated non-self S-RNase. This idea has recently been explored through the use of CRISPR/Cas9. *S_2-SLF1* had been knocked out in *S_2S_3* transgenic plants and then these transgenic plants were used to pollinate *S_2S_7* plants. At the time, only *S_2-SLF1* had been found to interact with S_7-RNase. Yet, the cross produced fruit,

meaning there was at least one more SLF protein that can recognize S_7-RNase and mediate its subsequent degradation via the 26S proteasome. The results of this preliminary experiment led to the observation of the relationship between S_2-SLF2, S_2-SLF12, S_2-SLF14, and S_2-SLF16 with S_7-RNase.

From the *in vivo* tests accomplished, we found that S_2-SLF2 interacts with S_7-RNase while S_2-SLF12, S_2-SLF14, and S_2-SLF16 do not interact with S_7-RNase. These results are shown in **Table 10**. This would indicate that *S_2*-haplotype *P. inflata* has indeed evolved multiple SLF proteins, S_2-SLF1 and S_2-SLF2, to interact with S_7-RNase. Since the interaction of *S_2*-haplotype pollen with S_7-RNase has evolved redundancy in recognizing and subsequently detoxifying S_7-RNase, it is possible that other interactions have also evolved redundancy. However, results indicate that pollinating *$S_{13}S_{13}$* plants with the *S_2-SLF1* knockout plants did not result in fertilization by the *S_2* pollen. This indicates that there is no redundancy in this interaction as only S_2-SLF1 can interact with S_{13}-RNase. While having redundancy in all interactions is theoretically beneficial, they may not be in place as of yet and it may take thousands of years before the fail-safe strategy is adopted for all non-self S-RNases in *P. inflata*.

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Wu, L., Williams, J.S., Wang, N., Khatri, W.A., San Román, D., and Kao, T.-h. (2017). Use of Domain-Swapping to Identify Candidate Amino Acids Involved in Differential Interactions between Two Allelic Variants of Type-1 S-Locus F-Box Protein and S_3-RNase in Petunia inflata. Plant Cell Physiol.

Zhang, B., Yang, X., Yang, C., Li, M., and Guo, Y. (2016). Exploiting the CRISPR/Cas9 System for Targeted Genome Mutagenesis in Petunia. Scientific Reports *6*, 20315.

ACADEMIC VITA

PROFESSIONAL EXPERIENCE

Teh-hui Kao Lab **University Park, PA**
Research Assistant *Sep 2014 – Present*
Project 1: To verify the amino acids involved in recognition specificity of the SLF/S-RNase interaction using chimeric SLF proteins
Project 2: To determine the interaction relationship of various S_2-SLFx proteins and S_7-RNase

- Construct transgenic plants and test self-pollination crosses to check for rejection of pollen
- Utilize gDNA isolation techniques and PCR in order to identify genes of interest in *P. inflata*
- Grow pollen for observation of GFP-tagged transgene products with fluorescence microscopy

Claude dePamphilis Lab **University Park, PA**
Research Assistant *Aug 2012, June 2013 – Aug 2013*
Project 1: To make a cDNA library of *L. philippensis*, a non-parasitic member of the Orobanchaceae family, in its seedling stage to compare it to *T. versicolor*, a known plant parasite of the Orobanchaceae family

- Performed RNA isolations on *L. philippensis* in various seedling stages
- Analyzed PCR and gel electrophoresis for presence of genes of interest in *T. versicolor*
- Measured *T. versicolor* growth when parasitizing various host plants, such as tomato or maize

PEER-REVIEWED PUBLICATIONS

- Wu, L., Williams, J.S., Wang, N., **Khatri, W.A.**, San Román, D., and Kao, T.-h. (2017). Use of Domain-Swapping to Identify Candidate Amino Acids Involved in Differential Interactions between Two Allelic Variants of Type-1 S-Locus F-Box Protein and S_3-RNase in *Petunia inflata*. Plant Cell Physiol. Doi.org/10.1093/pcp/pcx176

MANUSCRIPTS IN PREPARATION OR UNDER REVIEW

- Sun, L., Williams, J.S., Li, S., **Khatri, W.A.**, Stone, P.G., Keebaugh. M.D., and Kao, T.-h. (In Preparation). Complex Interaction Relationships between SLF Proteins and S-RNases in Self-Incompatibility of *Petunia*.

EDUCATION

The Pennsylvania State University | Schreyer Honors College **University Park, PA**
Bachelor of Science in Microbiology | Honors in Biochemistry & Molecular Biology *Expected Graduation: Spring 2018*

Université Laval | École de Langues **Quebec City, Quebec, Canada**
French Foreign Language Program *May 2016 – June 2016*

LEADERSHIP EXPERIENCE

PSU Swing Dance Club **University Park, PA**
President *Apr 2016 – Present*

- Organize the large-scale workshops with swing dance professionals to gather 130 attendees
- Cooperate with university officials to obtain upwards of $13,000 of funding for workshops
- Teach biweekly, hour long lessons on various topics and sequences in swing dance to 20 regular attendees

Upward Bound Math & Science: Summer Experience in the Eberly College of Science **University Park, PA**
Research Mentor *Mar 2015 – July 2015*

- Oversaw the completion of a project, by high schoolers, involving the creation of a plasmid