Development of a Set of Organelle Markers for Image Analysis of S_2-SLF1 of *Petunia inflata* Involved in Pollen Specificity of Self-Incompatibility

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Background Information

Self/non-self recognition is regulated by the polymorphic *S*-locus; matching of the pollen *S*-haplotype with one of the two pistil *S*-haplotypes results in inhibition of pollen tube growth. The *S*-locus houses *S-RNase* for pistil specificity, and, for both S_2- and S_3-haplotypes, 17 *S-locus F-box* (*SLF*) genes for pollen specificity. All SLFs are assembled into similar SCF complexes, also containing Rbx1, pollen-specific Cullin1, and pollen-specific Skp1-like protein. According to the collaborative non-self recognition model, for a given *S*-haplotype, each SCF complex interacts with a subset of non-self S-RNases to mediate their ubiquitination and degradation by the 26S proteasome in the cytosol of the pollen tube. Previous studies suggested that SLF protein is localized in the cytosol. However, the subcellular localization of SLF protein has not been examined.

Methods

Six transgene constructs, each for expressing a specific organelle marker, were generated by replacing the *35S* promoter with the pollen-specific *LAT52* promoter (Fig. 1). At least three transgenic plants expressing each of the six organelle markers were generated using *Agrobacterium*-mediated transformation. The organelle dynamics and morphologies in pollen tubes germinated from pollen of the transgenic plants were analyzed using a Laser Scanning Confocal Microscope (Fig. 2). Transgenic plants expressing each of the six organelle markers were crossed with transgenic S_2S_3 plants expressing S_2-*SLF1-sfGFP* driven by the S_2-*SLF1* promoter (Fig. 3). Co-localization analysis was performed using a Laser Scanning Confocal Microscope (Fig. 4). A spinning disk confocal microscope was used for examining the expression of S_2-*SLF1-sfGFP* driven by the S_2-*SLF1* promoter in microspores/pollen from different developmental stages of anthers: developing microspores in stage 1 anthers to bicellular mature pollen in stage 5 anthers, using DAPI staining (Fig. 5).

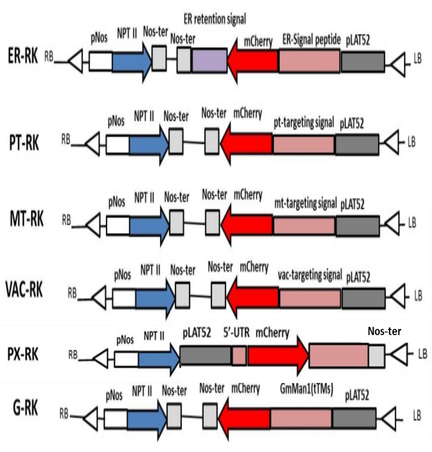


Figure 1. Schematic representation of transgene constructs for expressing six organelle markers in pollen: ER, PT (Plastid), MT (Mitochondrion), VAC (Vacuole), PX (Peroxisome), G (Golgi).

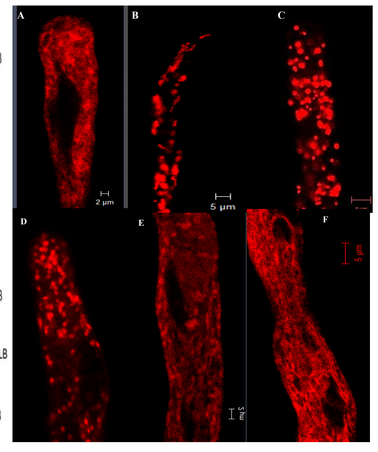


Figure 2. Organelle markers expressed in *Petunia* pollen tubes. A. Vacuole marker; B. Plastid marker; C. Peroxisome marker; D. Golgi marker; E. Mitochondrion marker; F. ER marker.

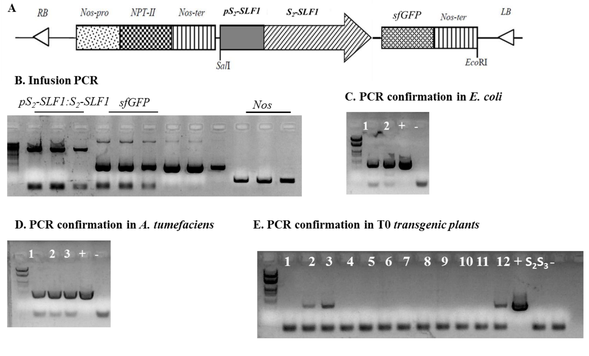
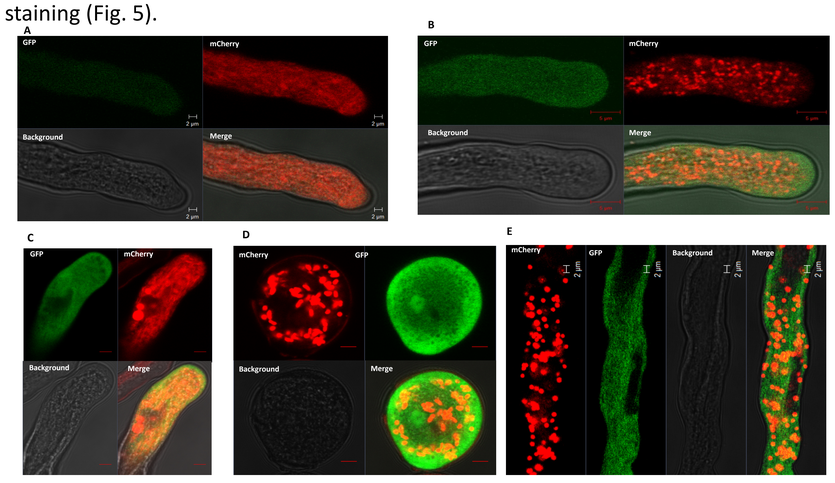


Figure 3. Generation of transgenic plants expressing S_2-SLF1:sfGFP driven by the promoter of S_2-*SLF1*. A. Schematic representation of the construct for pS_2-*SLF1*:S_2-*SLF1:sfGFP*. B. Infusion PCR to obtain DNA fragments. C. PCR analysis to confirm successful transformation of *E. coli*. D. PCR analysis to confirm successful transformation of *A. tumefaciens*. E. PCR analysis to confirm successful generation of transgenic *Petunia* plants.



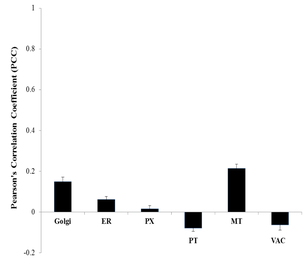


Figure 4. Mean Pearson's correlation coefficients of S_2-SLF1:sfGFP with the six organelle markers. The Pearson's correlation coefficient (PCC) of S_2-SLF1:sfGFP with each organelle marker was determined by examining 40 selected regions of interest (ROI) throughout each *in vitro* germinated pollen tube. The mean PCC for each marker is shown, with the standard error of the mean indicated. Abbreviations: ER, endoplasmic reticulum; PX, peroxisome; PT, plastid; MT, mitochondrion; VAC, vacuole.

Results

All the organelles labeled with this set of six markers showed an actin-myosin-dependent movement in the cytoplasm and displayed characteristic morphologies (Fig. 2) consistent with previous observations by a Laser Scanning Confocal Microscope (Nelson et al. 2007). The transgenic plants expressing each organelle marker were crossed with the transgenic plants expressing S_2-*SLF1-GFP* to obtain double-transgenic plants for subcellular localization analysis of S_2-SLF1 by confocal microscopy (Fig. 3). Co-localization coefficient analysis of mCherry-tagged markers with sfGFP (super-folder GFP)-tagged S_2-SLF1 showed that S_2-SLF1 had poor co-localization with plastids, peroxisomes, and vacuoles, but fair co-localization with Golgi and ER, suggesting that some S_2-SLF1 molecules are localized in the Golgi and ER but the majority are localized in the cytosol of the pollen tube (Fig. 4). A spinning-disk confocal microscope was used for a time series analysis of S_2-SLF1-sfGFP expression, driven by the S_2-*SLF1* promoter, from developing microspores in stage 1 anthers to bicellular mature pollen in stage 5 anthers. After DAPI staining of microspores/pollen, co-localization analysis revealed that S_2-SLF1-sfGFP fluorescence became detectable in bicellular microspores in late stage 3 anthers (Fig. 5A,B,C), consistent with the real-time PCR results (Fig. 5D) showing that S_2-*SLF1* transcripts started to accumulate in stage 3 anthers, were most abundant in stage 4 anthers, and decreased in stage 5 anthers.

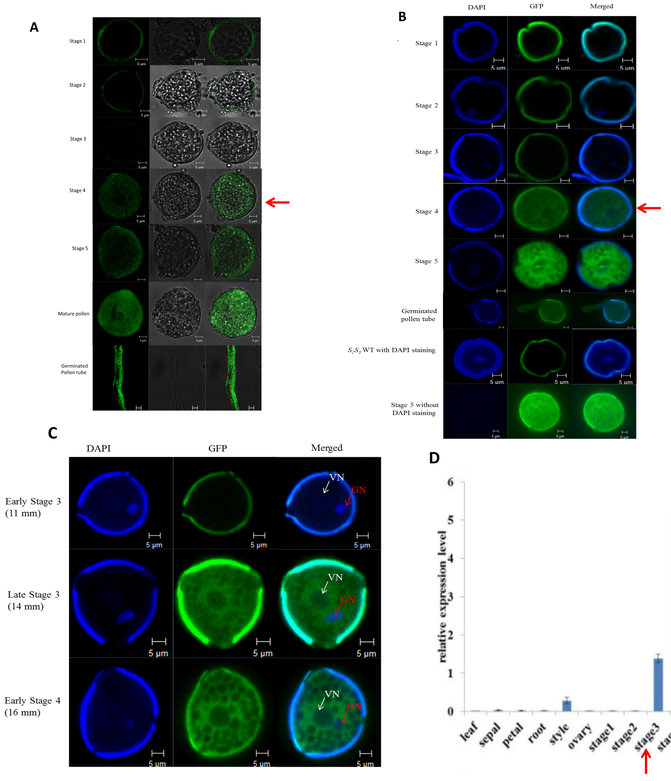


Figure 5. Expression of S_2-SLF1:sfGFP in microspores/pollen of anthers at different developmental stages. A. Laser scanning microscopic images. The red arrow indicates the stage when sfGFP fluorescence begins to be detectable. B. Spinning-disk confocal microscopic images. The red arrow indicates the stage when sfGFP fluorescence begins to be detectable. C. sfGFP fluorescence at early stage 3 (bud size = 11 mm), late stage 3 (bud size = 14 mm), and early stage 4 (bud size = 16 mm). White arrows indicate vegetative nucleus, and red arrows indicate generative nucleus. SfGFP fluorescence signals surround both nuclei. D. Expression of a typical S_2-*SLF* gene in different tissues. The red arrow indicates anther stage 3 when S_2-*SLF* transcripts begin to accumulate.

Conclusion

This study showed that S_2-SLF1 is produced and localized in the cytosol of pollen before pollen grains land on the pistil, and that S_2-SLF1 is specific to the generative cell (after completion of microspore mitosis in stage 3 anthers), consistent with our previous finding that an artificial microRNA expressed by the S_2-*SLF1* promoter, but not by the vegetative-nucleus-specific promoter, *LAT52*, suppressed expression of S_2-*SLF1* in S_2 pollen (Sun and Kao 2013).

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References

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