Observation of Pollen Tube Overgrowth in *feronia* and *ralf* Mutants

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Background

Plants are very important organisms. We all rely on crops and agriculture to sustain ourselves. Therefore, the study of plants and how they reproduce is crucial. Flowering plants such as the model plant *Arabidopsis thaliana* have non-motile sperm so they must rely on male-female interactions to ensure successful fertilizations. It starts from pollen hydration on the stigma, then sperm travels down the pistil in pollen tubes, locates to ovules and ends with sperm delivery to the female gametophyte for fertilization (Figure 1). Research has shown 3 main components in the fertilization journey: a receptor kinase, glycophosphatidylinositol (GPI)-anchored protein functioning as a co-receptor, and its ligand rapid alkalinization factors (RALFs) (Li et al 2016; Blackburn et al 2020; Xiao et al 2019).

FERONIA (FER) is a receptor kinase that is on the cell membrane. FER plays important roles in plant development, reproduction and stress related responses (Li et al. 2016). FER works with a GPI-anchored protein co-receptor, LLG1, to receive signals. Once it receives a signal FER directly interacts with ROPGEFs, guanine exchange factors that activate RAC/ROPs who recruit NADPH oxidase to produce reactive oxygen species (ROS). ROS supports root, root hair growth, and in reproduction mediates pollen tube rupture and sperm release to enable fertilization (Figure 2)(Duan et al 2010; Duan et al 2014). RALF6, 7, 16, 36 and 37 found in pollen are also important in pollen tube reception. RALFs bind to the extracellular domain of FER and are known to trigger Ca2+ influx that allows fertilization (Cheung et. al 2022). Under normal circumstances a growing pollen tube terminates at the filiform apparatus and bursts and ejects its sperm. In pollination with *ralf36, 37*, and *ralf6, 7, 16, 36, 37* mutants induces ovules displaying pollen tube overgrowth. Similarly, about 80% of *fer-4* mutants show dramatic pollen tube pile-up due to overgrowth (Figure 3)(Cheung et al. 2022). Over this summer I worked with pollen and pistils and observed pollen tubes and overgrowth phenotypes.

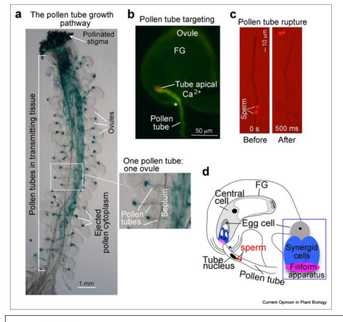


Figure 1. Pollen pistil interactions. For fertilization to occur sperm from pollen must travel from the top of the stigma and be released by pollen tube rupture at the ovule (Cheung et. al 2022)

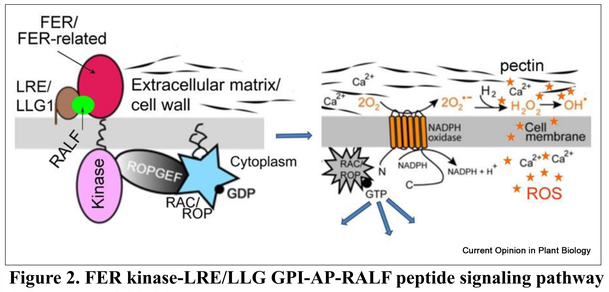


Figure 2. FER kinase-LRE/LLG GPI-AP-RALF peptide signaling pathway (Cheung et. al 2022)

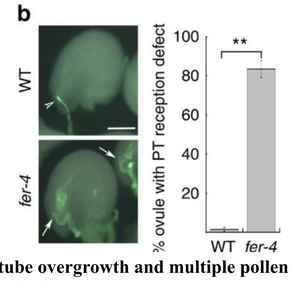
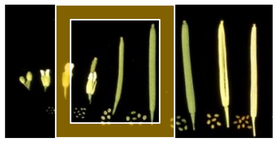


Figure 3. Pollen tube overgrowth and multiple pollen tubes seen in *fer-4* mutant (Duan et. al 2014)

Materials and Methods

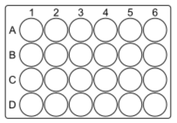
Aniline blue staining (Stains callose in pollen tubes)

(Process relates to figure 4)



1. Collect old pollinated flowers and young siliques

2. Transfer siliques to wells

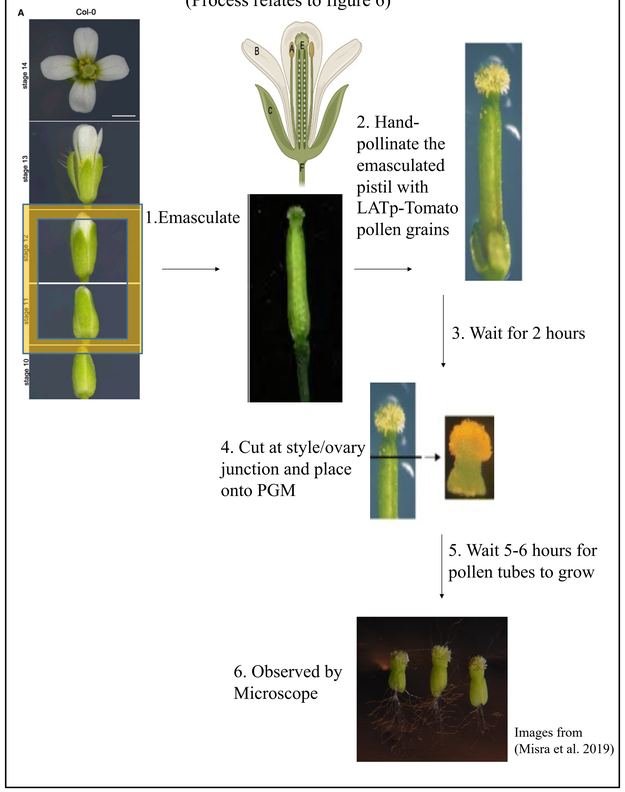


3. Fixation in acetic acid/EtOH (1:3) solution
4. Rehydration 70%, 50% and 30% EtOH
5. Alkaline Treatment with 2M NaOH
6. Washing distilled water
7. Staining with aniline blue solution

8. Observed with microscope under UV irradiation condition.

Emasculation and semi–*in vivo* pollination

(Process relates to figure 6)



Results

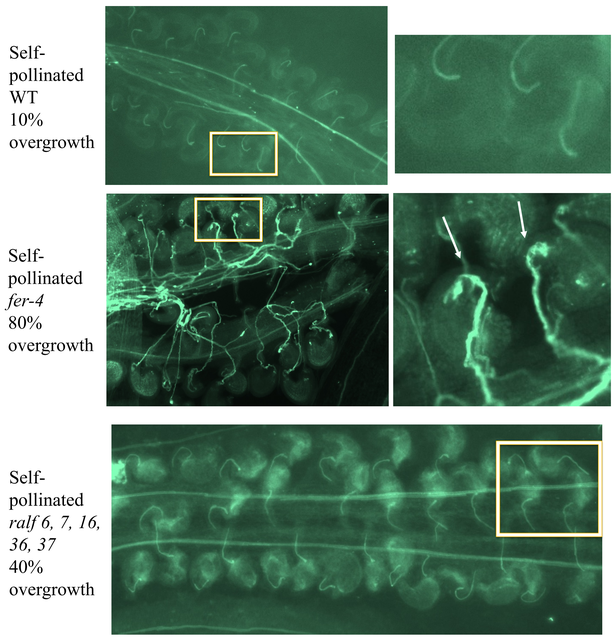


Figure 4. Pollen tube pictures of WT, *fer-4*, and *ralf6, 7, 16, 36, 37* after aniline staining process. White arrows indicate pollen tube overgrowth.

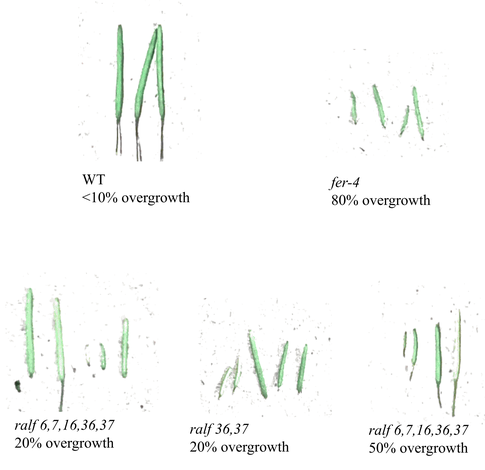


Figure 5. Silique sizes of self-pollinated WT, *fer-4, ralf6,7,16,36,3, ralf36,37* mutants and associated overgrowth.

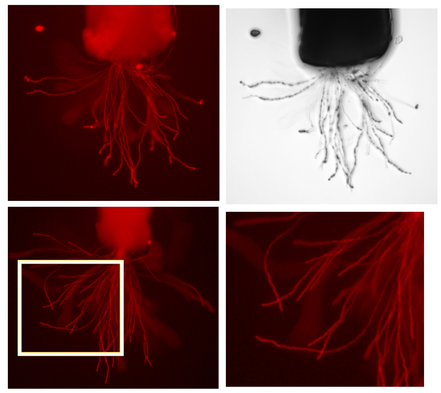


Figure 6. Semi-*in vivo* Pollen tubes from WT pistils pollinated with LAT promoter-Tomato pollen.

Future Directions

Over the summer I learned and practiced these techniques and plan to use them in research this coming semester. I plan to work with understanding more about how FER works with another receptor kinase, ANJEA, on stigma papilla cells. A separate direction includes understanding more about how calcium signaling is involved in regulating the processes in fertilization.

Acknowledgements

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