The Role of Membrane Proteins in Plant-Microbe Interactions

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Citations

Introduction

Plants display a wide variety of interactions with microbes in the rhizosphere (the root system). Some beneficial microbes promote plant health, while other pathogenic bacteria employ a host of detrimental biochemicals, including pore-forming toxins (PFTs) that create harmful pores in the cell membrane (Li et al., 2021). The exact mechanisms that plants use for regulating the microbial environment are still being explored. However, research demonstrates that plants possess an innate mechanism to distinguish between harmful pathogens and beneficial rhizosphere bacteria (Thoms, 2023). Despite this, much is still unknown about the specifics of the underlying immune mechanisms. Our project asks the question: what membrane repair proteins are involved in the differentiation of harmful from beneficial bacteria in the rhizosphere? We hypothesize that membrane-bound proteins are crucial to the plant's innate immune ability to differentiate between harmful and beneficial bacteria. To investigate our hypothesis, We used a model system consisting of the well-established plant system *Arabidopsis thaliana* and two strains of the bacteria *Pseudomonas fluorescens*.

Methods

- ***Arabidopsis thaliana*** (thale cross) model system
- 3D-printed **MYCroplanter** seed trays (Chen, 2025)
- Six genotypes of Arabidopsis thaliana germinated in agar gel:
  - **Controls:**
    - **Col-0** (wild type)
    - **BBC** mutant (BAK1, BKK1, CERK1)
  - **Mutants:**
    - **SYT5** mutant (synaptotagmin)
    - **SYT1** mutant (synaptotagmin)
    - **SOBIR1** mutant (receptor-like kinase)
    - **NDR1** mutant (integrin-like protein)
- After five days, transferred to 96-well plate inoculated with bacteria (***Pseudomonas fluorescens***) in competition.
  - **WSC365** – commensal strain with mNeonGreen fluorescence protein
  - **N2C3** – pathogenic strain with mScarlet fluorescence protein
- Seven days after inoculation, measure plant health and bacterial concentrations with a flatbed scanner and florescence reader.
- Convert florescence readings to optical density using standard curve
- Analyze data using modifies ANOVA test

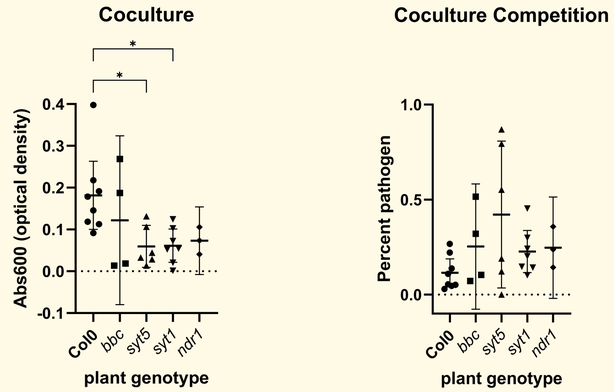

Figure 1 & 2: Overall concentration of bacteria (left) and ratios between N2C3 and WSC365 (right) are compared between mutants (n=1; temporal replicate).

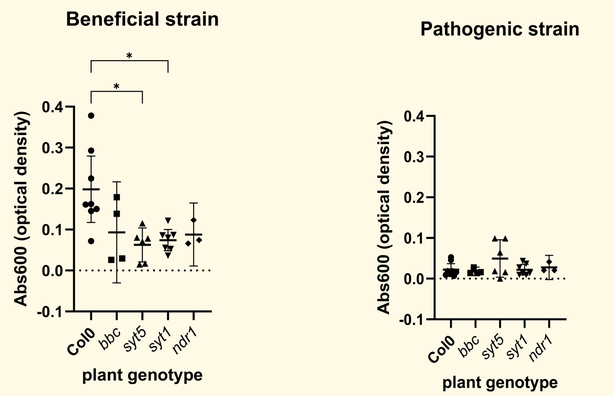

Figure 3 & 4: Concentrations of WSC365 (left) and N2C3 (right) are compared between mutants (n=1; temporal replicate).

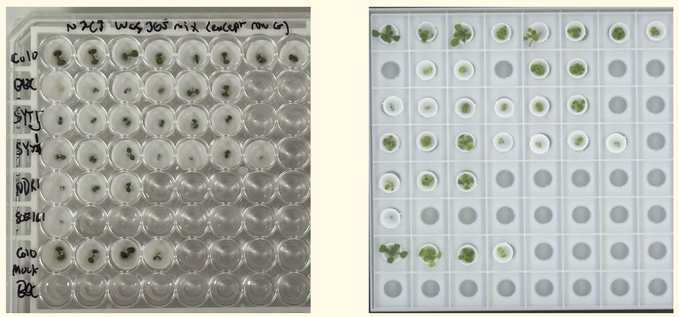

Figure 5 & 6: Arabidopsis Thaliana immediately after inoculation (left) and seven days after inoculation (right)

Results

We found that significant differences existed in the overall concentration of bacteria present in the synaptotagmin (SYT) mutants as compared to the wild-type control (Figure 1). Additionally, the synaptotagmin mutants showed significantly lower concentrations of beneficial WSC365 as compared to the wild-type control (Figure 2). However, the concentration of pathogenic bacteria remained constant across the strains, with the possible exception of SYT5 (Figure 4). The ratio of pathogenic to beneficial bacteria is displayed in Figure 5, and, while no significant differences exist due to a high amount of variance, it is important to note the high ratio of pathogens to commensals for SYT5. These results are from n=1 temporal replicate, and we anticipate trending results will become significant once n=3.

Discussion

- Our preliminary findings, while not conclusive, suggest that SYT5, a synaptotagmin protein integral in vesicle trafficking, plays a role in the innate immune system's differentiation of beneficial and harmful microbes (Lewis & Lazarowitz, 2010).
- The presence of pore-forming toxins (PFTs) in N2C3 contributes to these findings (Ulhuq et al., 2022). Synaptotagmin likely senses pores in the cell membrane by triggering endocytosis in the damaged membrane.
- By knocking out synaptotagmin, and thus the plant's ability to recognize PFTs, the plant can no longer differentiate between the harmful N2C3 and the beneficial WSC365 as effectively, leading to the ratios illuminated in Figures 2, 3, and 4. It is important to note that this discussion is theoretical, and further research is necessary to understand the systems at play.
- Limitations include small sample sizes, mold contamination, and seed viability.
- Future research should focus on repeating the results from the preliminary trials as well as further investigating the mechanism behind SYT5's connection to innate immunity.
- This research has implications across agricultural and pharmaceutical fields because it adds to the body of knowledge covering the immune response of plants, which could deepen researchers' understandings of blight, plant disease patterns, pathogen interactions, and even animal immune responses.

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