Research Plan – Kyle Cheung –Multifaceted approach to eradicate the lethal plant pathogen, Botrytis cinerea, by examining the role of proteins ADF4, ILR3, XanDH, and FLS2 - Plant Sciences Category

Rational:

Botrytis cinerea (gray mold) is a widespread and destructive necrotrophic fungal disease rotting more than 500 plant species[1], targeting both ornamental plants, fruits, and vegetables, that results in economic losses ranging from $10 billion to $100 billion worldwide[2]. To combat the fungus, many methods including chemical control, biological control, and resistance inducers have been established. However, chemical and biological methods are often ineffective since *B. cinerea*'s genome is adaptable and prone to developing drug resistance genes[1]. Resistance inducers can be utilized integrating the use of resistance genes in the host plant as a defense against pathogens[3]. Although, many plant defense mechanisms in relation to *Botrytis cinerea* is largely unknown. Several proteins including ADF4, ILR3, XanDH, and FLS2 have been selected to study because these proteins interact in plant immunity processes and/or imputing in fungal/bacterial defense. To examine their role in *B. cinerea*, these proteins were removed through agrobacterium genetic insertions in Arabidopsis thaliana and infected with the fungus. The level of susceptibility can indicate the role of the knockout gene in the plant immunity process against *Botrytis cinerea.* By learning more about the impact of these immunity genes, further studies regarding gene overexpression or nullification can be initiated and could lead to proper protection against *B. cinerea*.

Research Questions:

The goal of this project was to examine the roles of protein ADF4, ILR3, XanDH, and FLS2 in the process of plant immunity against fungal pathogen *Botrytis cinerea* through lesion measurement and reading of fungal biomass.

Procedure:

***Botrytis cinerea* Infection:**

In order to maintain a culture of *B. cinerea*, it was inoculated into a V8 agar media - since the V8 stimulated common fruits and vegetables. The BO5.10 strain of Botrytis was employed to the culture and began infecting the solution. After *B. cinerea* was grown for approximately 7-14 days, the solidified plates were inoculated by transferring a triangular piece of the infected agar to the center of new V8 media plate to ensure the Botrytis continues growth.

With the infected V8 media with *B. cinerea*, the spore solution for infection can be prepared. A 1% maltose solution was made using Difco Sabouraud maltose broth (BD) in conjunction with 1/6th of infected V8 media agar. After the solution of media and maltose solution were filtered through a cheesecloth filter, the spore count were then calculated and spore concentration measured with a hemocytometer.

Arabidopsis Planting/Growth Conditions:

The Arabidopsis mutants were obtained through SALK institute. These genes included ADF4, bHLH 105 (SALK 043690), bHLH 105 (SALK 004997), ABA 2-1, and FLS2 compared to COL-0. COL-0 acted as the wild type and was grown in the same conditions as others. 10 pots were planted and placed in the 21°C growth chamber (12 hr light/dark cycle with 60a light intensity of 100 μE). After the plants grown for 6 weeks, pots were infected with *Botrytis cinerea*.

Risk and safety

Botrytis cinerea is a very infectious fungus and was dealt with many precautions to keep contained. *Botrytis cinerea* can cause an allergic reaction and form of pneumonitis known as "winegrower's lung".

This fungus was always handled with gloves. Autoclave was used and safety was taken with training courses involving the machine.

Data analysis

Infecting Arabidopsis with Botrytis:

After Arabidopsis was grown for 6 weeks, 3 leaves from each gene mutation were inoculated with a drop (5 µl each) of the *B. cinerea* spore solution directly on the plant leaf. Lesion diameters on the leaves were measured 3 days post inoculation to quantify the spread of the pathogen.

Quantifying fungal biomass by WAC chitin assay

Plant leaf tissue was collected from the inoculated leaves. They were then placed into tubes with volume of 1 M KOH containing 0.1% (vol/vol) Silwet L-77. The tissues were autoclaved on a standard sterilization cycle and were then washed 50 mM Tris, pH 7.0 and resuspended in it acting as a buffer solution. The plant tissue/solution was then macerated through hand grinding with a micropipette generating a uniform suspension of tissue. Solution of WGA-FITC dissolved in water was added into a centrifuge tube and the tissue suspension was added. Three samples were prepared for each mutant tissue and centrifuge tubes were utilized. Following staining, the samples were centrifuged. The pellet was resuspended in 50 mM Tris, pH 7.0. The solution was then transferred to trays for fluorometry and chitin fluorometric measurements were tested with the SpectraMax M2e

NO CHANGES EXIST

Bibliography

1. Hua, et al. “Pathogenic Mechanisms and Control Strategies of Botrytis Cinerea Causing Post-Harvest Decay in Fruits and Vegetables.” OUP Academic, Oxford University Press, 24 July 2018, academic.oup.com/fqs/article/2/3/111/5057759.

2. “Pathogens of Autotrophs.” *ScienceDirect*, Academic Press, 11 Dec. 2015, www.sciencedirect.com/science/article/pii/B9780123820341000086.

3. Marolleau, et al. “When a Plant Resistance Inducer Leaves the Lab for the Field: Integrating ASM into Routine Apple Protection Practices.” *Frontiers*, Frontiers, 27 Oct. 2017, www.frontiersin.org/articles/10.3389/fpls.2017.01938/full.