Replacing the Manchurian crabapple pollinizer for apple orchards

Carmen Swannack

WSU Tree Fruit Research and Extension Center

Wenatchee, WA

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Overview

Washington state is the leader in U.S. apple production, producing more than 50% of the U.S. apples and exporting apples to more than 60 countries.[5] The apple industry is an important asset to the state's economy; a study conducted in 2014 found that the apple industry bolstered the state's economy by over $7.5 billion in 2012-2013 through product output, employment, and employee compensation.[1] The goal of this project is to directly address a major issue facing the Washington State apple industry: the use of the Manchurian crabapple (*Malus mandshurica*) as a pollenizer in commercial orchards. Manchurian is susceptible to pathogens such as *Sphaeropsis pyriputrescens* (Sphaeropsis rot) and *Phacidiopycnis washingtonensis* (speck rot), which are considered quarantine diseases in some international markets. With widespread use of susceptible varieties, inoculum of these pathogens can accumulate in orchards and contribute to post-harvest diseases. This results in complications with exportation, especially into Asian markets where these are quarantine diseases. The overall aim of this project, led by Prof. Musacchi at the WSU Tree Fruit Research and Extension Center in Wenatchee, WA, is to find suitable alternatives to Manchurian as pollenizers in apple orchards. Having disease resistances that limit the issues presented by Manchurian is the most important trait for potential pollenizers in this project, however, pollen performance and genetic compatibility are also important characteristics. Screening the candidate pollenizers for pollen viability and self-incompatibility genotyping were the focus of this internship.

S-genotyping

Introduction:

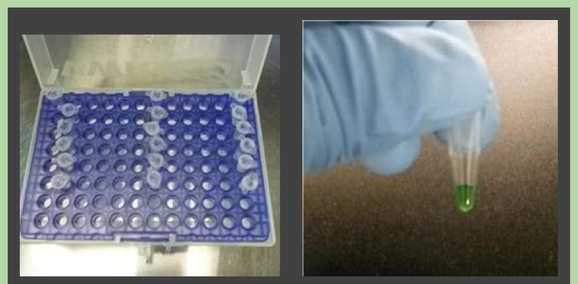
- **Most apple varieties exhibit a gametophytic self-incompatibility system (GSI)[3] that promotes outcrossing**
- **The GSI mechanism is controlled by a multiallelic *S*-locus**
- **The *S*-locus encodes an RNase in the pistil and a corresponding pollen S-determinant**
- **Full incompatibility: same genotype at the *S*-locus; pollination cannot occur**
- **Full compatibility: each have two different alleles at the *S*-locus; pollination can occur**
- **Partial compatibility: one of the alleles is the same while the other is different; pollination can occur with pollen containing the allele that is not the same**

The goal of the *S*-genotyping portion of this project:

- **determine the *S*-allele composition of many common apple cultivars as well as potential pollenizer varieties**
- **Use the S-genotypes to help select future pollenizer varieties with relatively rare *S*-alleles that are compatible with a wider variety of commercial apple cultivars**

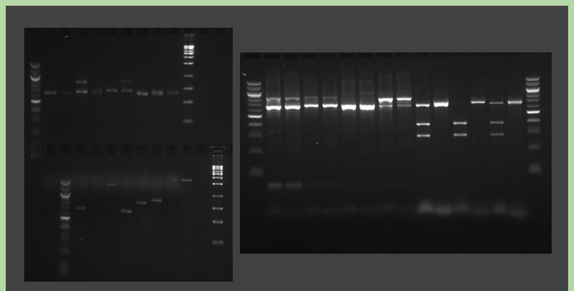
Methods:

- **Extract genomic DNA from young leaves using the DNeasy Plant Mini Kit (Qiagen)**
- **Use consensus primers to amplify genomic *S*-fragments with PCR[2,4]**
- **Use restriction enzyme digestion or allele-specific PCR to screen for the presence of *S*-haplotypes by restriction fragment length polymorphism (RFLP) of by presence of the expected fragment size visualized on an agarose gel**
- ***S*-fragments are ligated into the pGEM-T Easy Vector (Promega) and sequenced to confirm the results of the allele-specific PCR and RFLP methods**



Set up PCR in 0.2 mL tubes

Prepare, load, and run an agarose gel in elechtrophoresis chamber



Visualize gel results using a UV transilluminator

Pollen Germination

Introduction:

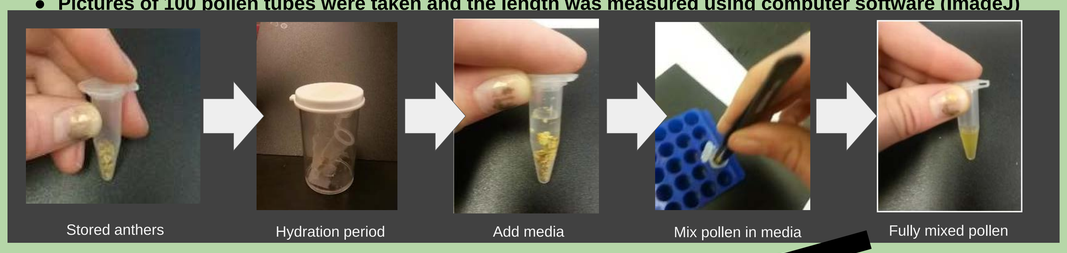
- **It is important to analyze the pollen viability of candidate pollinizer varieties to replace Manchurian**
- **As a pollenizer, it is important that a variety produces pollen that germinates well**
- **A high pollen germination rate will allow maximum pollination to occur throughout an orchard**
- **Without a viable pollen source, the flowers will not be fertilized and fruit can not develop**

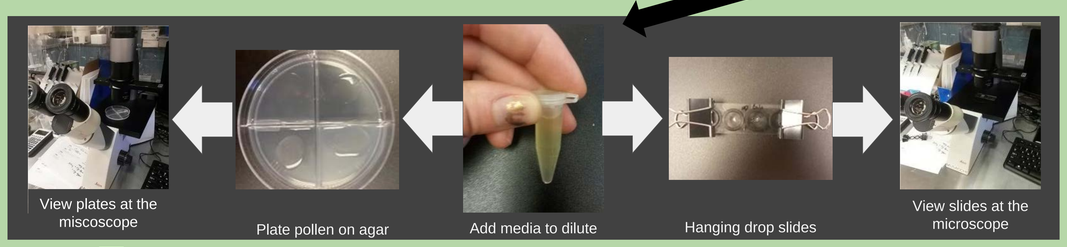
The goal of the *in vitro* pollen germination portion of this project:

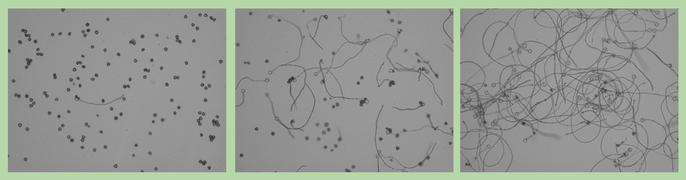
- **Screen the collection of potential replacement pollenizers for their pollen viability**
- **The pollen germination rate will help determine whether a certain cultivar has enough viable pollen to be a successful pollenizer**
- **The threshold established by this project for an acceptable candidate as a pollenizer variety is a 50% or higher pollen germination rate**
- **Long pollen tubes that can more successfully fertilize a flower are also desirable**

Methods:

- **Flowers were harvested in the spring**
- **Petals were removed and anthers were separated and transferred into 2 ml tubes**
- **Anthers were dried under a lamp for 24 h and stored at -20 °C**
- **Samples were hydrated for 2 hours in a humid jar at room temperature**
- **Pollen germination media was added to the sample**
- **Anthers were gently squeezed with a wide tweezers to promote the release of the pollen grains**
- **Pollen suspension was plated on agar plates and slides with 4 replications per sample**
- **Both hanging drops and agar plates were used to determine which method produced better results**
- **Agar plates and slides were incubated for 4 h at room temperature**
- **Pollen was viewed under an inverted microscope and the germination rate was calculated by counting the pollen grains with and without pollen tubes until a total of 400 pollen grains have been counted**
- **Pictures of 100 pollen tubes were taken and the length was measured using computer software (ImageJ)**







Examples of samples with a low (left), medium (middle) and high (right) pollen germination rate

Summary:

My summer internship at the WSU Tree Fruit Research and Extension Center in Wenatchee, Washington has been a rewarding experience. I have had the opportunity to expand my skill set while on the job, gaining practical experience that will greatly benefit me as I continue on to graduate studies and a career in plant sciences. I have gained many valuable skills relating to my career interests, including learning how to visualize PCRs with gel electrophoresis and how to ligate PCR fragments into a plasmid to prepare them for sequencing. I also had the opportunity to work with an inverted microscope and help set up *in vitro* pollen germination experiments. Though I gained a lot of career experience, I also had the opportunity to build general life skills. For example, I was expected to always be active in the learning process and analyze situations, adjusting my procedures accordingly. Throughout the internship experience I have learned many new skills while working on a project that will greatly benefit the Washington apple industry.

References:

[1]**Globalwise, Inc. 2014. The Washington Apple Industry: Updated Evaluation of Contributions to the State Economy and the Important Role of Exports.**

[2]**Kim, H., H. Kakui, N. Kotoda, Y. Hirata, T. Koba, and H. Sassa. 2009. Determination of partial genomic sequences and development of a CAPS system of the *S-Rnase* gene for the identification of 22 *S* haplotypes of apple (*Malus x domestica* Borkh.). Molecular Breeding. 23: 463-472.**

[3]**Sassa, H. 2016. Molecular mechanism of the S-RNase-based gametophytic self-incompatibility in fruit trees of Rosaceae. Breeding Science. 66(1): 116-121.**

[4]**Sanzol, J. 2009. Genomic characterization of self-incompatibility ribonucleases (S-RNases) in European pear cultivars and development of PCR detection for 20 alleles. Tree Genetics and Genomes. 5(3): 393-405.**

[5]**Washington Apple Commission. 2017. http://bestapples.com/resources-teachers-corner/fun-facts/.**