MPB Report 2019

**Deciphering insect resistance in potato dihaploids with Phureja chromosome fragments**

PI: Ek Han Tan, SBE, University of Maine
Co-PI: Andrei Alyokhin, SBE University of Maine

**1. Generation of diploid potato population with Phureja chromosome fragments**

The Phureja haploid inducer (HI) parent used in all crosses was IVP48, and is known to be an efficient haploid inducer. When crossed with tetraploid cultivars (such as Atlantic, Caribou Russet or NY121), some of the resulting progeny will be diploid, as determined by chloroplast counting and sequencing.

2018 Crosses

| Female parent | Male parent | No. of flowers pollinated | No. of fruits | Berry setting rate (%) | Potential Dihaploids |
|---|---|---|---|---|---|
| Atlantic | IVP48 | 550 | 92 | 17 | 152 |

**Table 1. Crosses made in 2018, along with the potential dihaploids.**

Our collection of in vitro diploid potato dihaploids has been initiated and will be maintained in aseptic tissue culture. We used a fluorescent chloroplast staining protocol to determine the potential diploid potato dihaploids from the crosses. Seen below in Figure 1 is an example of this for the cross.

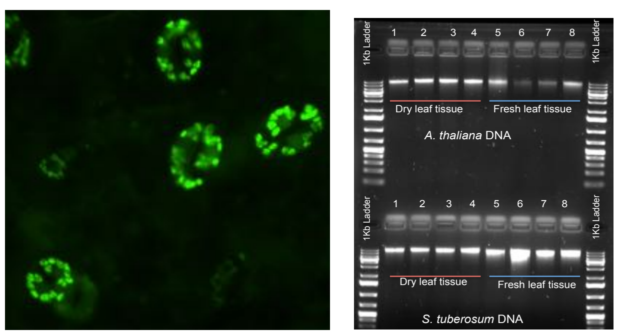


**Figure 1. Chloroplast staining of a potential Atlantic dihaploids, showing 6-8 chloroplast per guard cell (L panel). DNA isolation protocol using dried versus fresh tissue to be used for sequencing (R panel).**

DNA from all 152 samples has been isolated using a non-toxic DNA isolation protocol that we have developed recently (Figure 1). Once we have the number of DNA samples required, these will be sent for whole genome sequencing on the Illumina platform. We anticipate including this set with the 2019 crosses. This allows us to maximize the cost of sequencing. Aneuploid dihaploids with Phureja chromosome introgressions will be determined using sequencing methods.

2019 Crosses

| Female parent | Male parent | No. of flowers pollinated | No. of fruits | Berry setting rate (%) |
|---|---|---|---|---|
| Atlantic | IVP48 | 450 | 273 | 60.7 |
| NY121 | IVP48 | 292 | 66 | 22.6 |
| Caribou.Russet | IVP48 | 368 | 201 | 54.6 |

**Table 2. Ongoing crosses in 2019 to include more elite cultivars of importance to Maine.**

We are able to obtain much higher berry setting rates in 2019 based on our experiences from 2018, and will continue to make crosses this winter.

**2. Testing aphid resistance of Desiree, Atlantic and IVP48 (Phureja Haploid Inducer)**

To test the difference in genetic background to the green peach aphid (*Myzus persicae*), we designed experiments using excised potato leaflets from IVP48, Atlantic (AV2) and Desiree.

Experimental setup

Each potato leaflet were incubated with 5 adult aphids in a standard petri dish with wet kimwipes. For each experimental setup, each cultivar were present in triplicates. We performed two sets of experiments for a total of 2 biological replicates, and a total of 6 technical replicates per cultivar tested. We performed the inspections at the same time each day for each 24 hour period beginning Day 1, which is 24 hours after inoculation. We counted the number of dead aphids, the number of adult aphids and the number of nymphs. The experiment is terminated after Day 5.

Analysis

Data were determined not to follow normal distribution according to Wilk-Shapiro test ($P<0.0001$; PROC UNIVARIATE, SAS Institute, 2018) and transferred using rank transformations (PROC RANK, SAS Institute, 2018). Differences in the number of adult aphids and nymphs were then compared among potato accessions using repeated measures ANOVA (PROC MIXED, SAS Institute, 2018).

The first nymphs appeared on excised potato leaflets starting on the second day after inoculation, and then steadily increased throughout the experiment (Fig. 1; $df=5,75$, $F=66.32$, $P<0.0001$). Overall, there was no statistically significant difference in aphid fecundity among the tested potato accessions ($df=2,15$, $F=1.23$, $P=0.3200$). However, the interaction between potato accession and day since inoculation was significant ($df=10,75$, $F=2.12$, $P=0.0326$), with a quicker population growth on Desiree (Fig. 1).

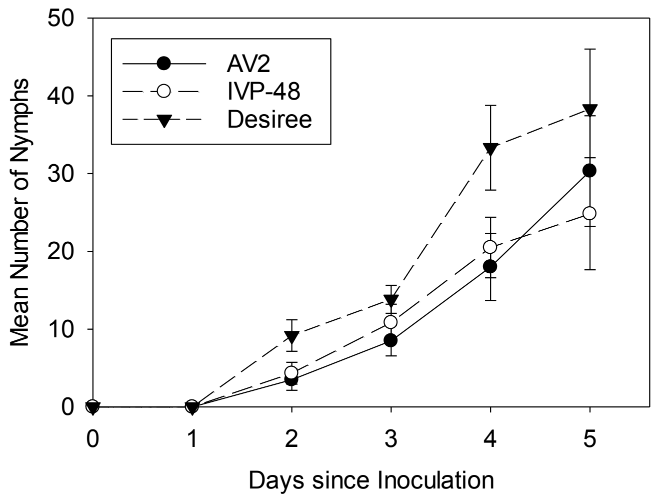


**Figure 2. Number of green peach aphid nymphs on excised potato leaflets in Petri dishes. Error bars denote standard errors.**

Numbers of adult aphids also increased significantly as nymphs matured into adults (Fig. 2; df=5,75, F=12.91, P<0.0001). Although adult populations appeared to grow at a quickest pace on Desiree (Fig. 2), neither effect of the accession (df=2,15, F=0.85, P=0.4476) nor its interaction with the day since inoculation (df=10,75, F=1.40, P=0.1953) were statistically significant.

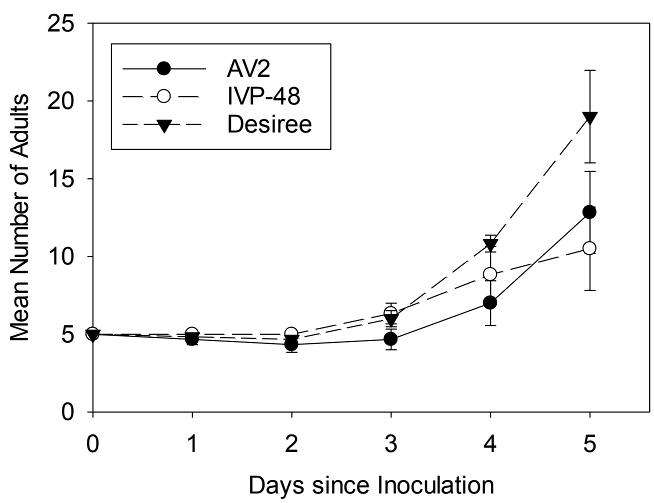


**Figure 3. Number of adult green peach aphids on excised potato leaflets in Petri dishes. Error bars denote standard errors.**

Based on our results, AV2 and IVP-48 are less suitable aphid hosts compared to Desiree. However, the observed differences among accessions were not very strong.

**Future work**

We aim to continue our dihaploids extraction efforts and characterizing the diploid individuals as part of the collaborative effort of all the public potato breeding institutions in the US to revolutionize potato breeding at the diploid level. Even though the effect is not large, we were able to detect advantages of IVP48 over Desiree in terms of the colonization of green peach aphid nymphs but not over Atlantic. It would be possible to test the setup with Caribou Russet and NY121 in the future and ascertain if any advantage can be conferred via IVP48 to future diploid progeny from these cultivars.