FINAL PROJECT REPORT

Project Title: Identifying fire blight resistance in M. sieversii for scion breeding

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Other funding sources: None

Total Project Funding: $54,000

Budget History:

Item	Year 1: 2010	Year 2: 2011	
Salaries	$4,854[1]	$4,854[1]	
Benefits	$156	$156	
Wages	0	0	
Benefits	0	0	
Equipment	0	0	
Supplies	$10,000[2]	$10,000[2]	
Travel	0	0	
Plot Fees	0	$10,000[3]	
Miscellaneous	$14,000[4]	0	
Total	$29,000	$25,000	

Footnotes: [1]Salary is for summer undergraduate student to assist Norelli in determining fire blight resistance of GMAL4593 population (Obj. 1). [2]Supplies are to identify additional molecular markers (SNP) in *M. sieversii* (Obj. 2). [3]Plot fees were for establishment of *M. sieversii* at USDA-ARS, Kearneysville, WV ($4,000) and at WSU-TFREC, Wenatchee, WA ($6,000) (Obj. 3). [4]Miscellaneous year 1 was to propagate 10 replicates of 200 *M. sieversii* accessions at Van Well Nursery, East Wenatchee, WA (Obj. 3).

Original Objectives:

1. Identify genetic markers for fire blight resistance in the *M. sieversii* GMAL4593 mapping population for use in scion breeding programs.

2. Identify *M. sieversii*-specific molecular markers to add additional markers to the genetic map of *M. sieversii.*

3. Establish plantings of *M. sieversii* in WA and WV for the identification of additional sources of fire blight resistance in the future.

Significant Findings:

- Fire blight resistance in GMAL4593 mapping population was associated with molecular markers on chromosomes (linkage groups) 10 and 8 of *M. sieversii.*

- Both environment and the strain of the pathogen used for inoculation had an effect on the association of specific genetic markers to the fire blight resistance trait. However, three markers located between 20-30 cMs on linkage group 10 were consistently associated with fire blight resistance in the field in both 2010 and 2011.

- The total number of molecular markers in the *M. sieversii* GMAL4593 map increased from 107 (Wisniewski 2009 final report) to 163 markers.

- This project succeeded due to collaboration with RosBREED project to re-sequence *M. sieversii* parent of GMAL4593 and identify *M. sieversii*-specific molecular markers to fill gaps within the molecular map.

- All available field and greenhouse data on fire blight resistance of the *M. sieversii* collected by the USDA in Kazakhstan was analyzed and 191 accessions were selected for the identification of additional sources of fire blight resistance. Replicate trees of 202 selected accessions and control cultivars were propagated at Van Well Nursery, East Wenatchee, WA. Field plantings will be established this spring at WSU-TFREC, Wenatchee, WA and USDA-ARS, Kearneysville, WV.

Results & Discussion:

Objective 1: Identify genetic markers for fire blight resistance in the *M. sieversii* GMAL4593 mapping population for use in scion breeding programs.

The GMAL4593 mapping population consists of seedling progeny derived from a cross between 'Royal Gala' and *M. sieversii* PI613981. To successfully map a genetic locus for fire blight resistance within this population, the population needs to segregate for resistance. In other words, when challenged with the fire blight pathogen, some individuals within the population need to be consistently resistant to the disease, and others need to be consistently susceptible to the disease. Previous (2008) greenhouse evaluation of GMAL4593 for fire blight resistance by Herb Aldwinckle (Cornell University) and Phillip Forsline (USDA-ARS) in Geneva, NY found the population to be segregating for fire blight resistance.

In 2010, approximately 2,700 vigorously growing shoots on 3-4 replicate field grown trees of the same 190 GMAL4593 individuals used in the 2008 greenhouse trial were challenged with fire blight. Similar to the 2008 trial, disease varied among individuals in the population with the percent of the current season's shoot length that became diseased ranging from 5 to 100%. However, the disease rating obtained in the 2008 greenhouse and 2010 field tests were not correlated. In other words, the individuals found resistant in the greenhouse test were not the same as those identified as resistant in the field trial. Furthermore, when fire blight resistance was mapped within the GMAL4593, fire blight resistance determined in the greenhouse was associated with markers on linkage group (LG) 8, whereas resistance determined in the field was associated with markers on LG10.

There were several possible causes for this discrepancy. The 2008 greenhouse test was conducted with a native NY fire blight strain (Ea273) whereas 2010 field test was conducted with a strain from the AFRS collection and it was possible that the genetic loci controlling fire blight resistance in GMAL4593 are specific to different strains of the fire blight bacteria. To address this possibility, the GMAL4593 population was challenged with three strains of the pathogen in 2011 from NY (Ea273 used in 2008 trial), WV (AFRS used in 2010 trial) and WA. Other possible causes for the discrepancy included: 1) differences between the greenhouse and field environments, 2) both genetic loci on LG10 and LG8 were contributing to resistance, and 3) the fire blight resistance observed within the GMAL4593 was due more to environment than genetics and reliable markers for resistance could not be determined.

In 2011, approximately 2,900 vigorously growing shoots on 3 replicate field grown trees were challenged with 3 strains of the fire blight pathogen. The percent of the current season's shoot length that became blighted (%SLB) amongst the individuals of the GMAL4593 population ranged from 3 to 100%. The AFRS strain caused the most disease with an average of 88 %SLB, followed by Ea273 with 41 %SLB and the WA strain with 33 %SLB. The results obtained with strain AFRS in 2011 were very similar to those obtained in 2010 and resistance was associated with markers on LG10 (Table 1, next page) rather than LG8 (Table 2, subsequent page). The results obtained with strain Ea273 and the WA strain were very similar to each other. Although there was some variation in the evaluation of fire blight resistance in the GMAL4593 population obtained with the Ea273 and WA strains and that obtain with AFRS, all the strains showed highly significant association with 3 markers on LG10: GDsnp00307 (21.1 cM), U50187SSR (29.7 cM) and USDA-ARS_DIP1 (30.1 cM) (Table 1). These markers were also highly associated with resistance in the 2010 trial using the AFRS strain (Table 1). Although the markers also had some association with resistance in the 2008 greenhouse trial, it was not highly significant (Table 1). In conclusion, these three markers on LG10 were the markers most consistently associated with fire blight resistance in the GMAL4593 population and will be validated in future studies.

The resistance observed when the population was challenged with strain Ea273 in the field (2011) also showed significant association with markers on LG08 (Table 2). However, only one of the LG8 markers (GDsnp1643539, 41.8 cM) that was highly associated (P=0.005) with resistance in the 2008 greenhouse trial using strain Ea273 was also associated with resistance in the 2011 trial with strain Ea273. Markers CH05a02, NH005b and GDsnp00584 which highly associated in the 2008 trial, showed no association in the 2011 trial.

The analysis of fire blight resistance at both of these loci (LG10 and LG8) is continuing, however additional funds for this aspect of the research are not being requested from WTFRC. Currently, we plan to incorporate the fire blight resistance found in GMAL4593 into genetic backgrounds with superior fruit quality using Fastrack breeding, or accelerating breeding that takes advantage of shortened juvenility of apple by transgenic expression of transcription factors controlling flowering. To validate the markers identified in this study, progeny resulting from these crosses will be screened

for resistance using both markers on LG10 and LG8 and by controlled inoculation with the fire blight pathogen.

Table 1: Association between fire blight resistance and genetic markers on **LG 10** determined by rank sum test of Krusgal-Wallis. Significance levels: -:not significant, *:0.1, **:0.05, ***:0.01, ****:0.005 and *****:0.001.

Group	Position (cM)	environment:	green-house	field	field	field
		year:	2008	2010	2011	2011
		pathogen strain:	Ea273	AFRS	AFRS	Ea273
		Locus	KW significance			
LG10	0.0	GD_SNP00260b	-	-	-	**
LG10	2.0	GD_SNP01710	***	-	**	**
LG10	2.3	CH01f12	**	*	*	****
LG10	4.5	CH03d11	-	-	-	-
LG10	8.9	GD_SNP00869	-	*	*	*****
LG10	14.2	DIP47HRM	-	-	-	**
LG10	20.1	GD_SNP00307HRM	**	****	****	****
LG10	29.7	U50187SSR	**	****	*****	****
LG10	30.1	DIP1HRMa	**	*****	****	***
LG10	34.0	GD_SNP00360b	-	***	*	-
LG10	34.1	GD_SNP00355b	-	**	-	-
LG10	38.6	GD100	-	*	-	-
LG10	38.8	Hi22a07x_202	-	-	-	-
LG10	39.7	Hi05b02	-	*	-	-
LG10	42.3	DIP5HRM	-	-	-	-

Markers consistently associated with resistance in field

Table 2: Association between fire blight resistance and genetic markers on **LG 08** determined by rank sum test of Krusgal-Wallis. Significance levels: -:not significant, *:0.1, **:0.05, ***:0.01, ****:0.005 and *****:0.001.

Group	Position (cM)	environment:	green-house	field	field	field
		year:	2008	2010	2011	2011
		pathogen strain:	Ea273	AFRS	AFRS	Ea273
		Locus	KW significance			
LG08	0.0	GD_SNP00246b	-	-	-	-
LG08	0.2	GD_SNP00299b	-	-	-	*
LG08	7.2	DIP21HRMb	**	-	-	****
LG08	8.4	GD_SNP00293a	-	-	-	*
LG08	17.2	GD_SNP1699552	-	-	-	-
LG08	28.8	CH05a02_126	****	-	-	-
LG08	29.3	NH005b	****	-	-	-
LG08	29.4	CH05a02_114	****	-	-	-
LG08	30.5	CH01f09	-	-	*	*
LG08	32.9	GD_SNP01004	-	-	-	**
LG08	41.8	GdSNP1643539	****	**	-	***
LG08	42.1	GD_SNP00584HRM	*	-	-	-
LG08	44.2	GD_SNP00584HRM	****	-	-	-
LG08	46.4	CH01e12	*	-	-	-
LG08	47.0	CH01c06	*	-	-	-
LG08	47.0	CH01c06b	**	-	-	**
LG08	47.2	CH01c06_155	**	-	-	**
LG08	50.9	DIP17HRM	-	-	-	-
LG08	61.1	GD_SNP00175HRM	**	-	-	-
LG08	70.5	DIP18HRM	-	*	-	**

The resistance observed when the GMAL4593 population was challenged with the AFRS strain did not show highly significant association with markers ('Locus' in table) on LG08 (2 middle columns under 'KW significance'). Although results with strain Ea273 did show significant association with markers on LG08, the results obtained for specific markers were generally inconsistent in that the markers most associated with resistance in 2008 greenhouse trial were not associated with resistance in 2011 field trials (except for GDsnp1699552).

Objective 2: Identify *M. sieversii*-specific molecular markers to add additional markers to the genetic map of *M. sieversii*.

A total of 921 molecular markers were screened in the GMAL4593 mapping population. These included simple sequence repeats (SSRs), single nucleotide polymorphisms (SNPs) and deletion-insertion polymorphisms (DIPs). In order for the markers to be informative in the *M. sieversii* map they must segregate either in *M. sieversii* or in both parents. Among the SSR markers, ca. 40% of the markers were informative for *M. sieversii* PI613981, whereas ca. 65% were informative for 'Royal Gala'. Among the SNP markers, only ca. 20% of markers were informative for *M. sieversii*, compared with 50% for 'Royal Gala'. The lower occurrence of SNP markers in *M. sieversii* is probably due to the broader "genetic base" or greater diversity of genes in *M. sieversii* compared to domesticated apple, making it less likely that any specific SNP markers will be present in a specific individual.

Currently, there are a total of 375 informative markers for *M. sieversii* PI613981. Of these, 252 could be assigned to specific chromosomes or "linkage groups" and 163 were mapped. All of the 17 chromosomes of apple are covered by the current map. Coverage of the individual linkage groups ranges from 50% to 95% of the 'Golden Delicious' genome reference map with an overall average of 73% coverage. Based upon the genome reference map there is on average one marker per 8.3 cM of genetic distance. Within linkage groups 8 and 10 that were associated with fire blight resistance, there is on average one marker per 3.7 and 6.3 cM, respectively.

The current map provides an excellent foundation for additional research on this population and *M. sieversii*. The map will be deposited and publicly available at the Genome Database for the Rosaceae (GDR). We are currently resolving segregation distortion amongst some of the SSR markers by reanalyzing the data using alternative software and expect to have a map completed for deposit at GRD within 3 months. In addition to this work on fire blight, resistance to apple scab (*Venturia inaequalis*), resistance to blue mold (*Penicillium expansum*) and water use efficiency is being evaluated within the GMAL4593 population at USDA-ARS Kearneysville, WV.

Objective 3: Establish plantings of *M. sieversii* in WA and WV for the identification of additional sources of fire blight resistance in the future.

Although many different *M. sieversii* accessions in the USDA-ARS collection have shown high levels of fire blight resistance, we do not know if these other potential sources of resistance contain the same fire blight resistance genes present in GMAL4593 or if they represent distinct, and perhaps more useful, sources of resistance. The purpose of the new *M. sieversii* plantings being established here are to obtain reliable fire blight data to identify the best sources of resistance for use in scion breeding.

Amongst the several thousand seed of *M. sieversii* collected in Kazakhstan, a CORE collection of 110 accessions has been selected which represent a broad range of the genetic and character diversity found throughout Kazakhstan. To select accessions to be included in this trial, we first selected 37 individuals from the CORE collection that appeared resistant to fire blight and had superior fruit quality. Superior fruit quality was based upon available GRIN data for flavor (rejecting bitter, astringent or sour flavors), fruit weight, harvest date (favoring late), soluble solids and juice quality. Because a ratio of 1 susceptible: 1 resistant plant will be advantageous for later association mapping studies, we then selected accessions derived from sister seed (seeds collected from same mother plant in Kazakhstan) of the selected CORE accessions to result in a predicted ratio of 1 susceptible:1 resistant accession. Observations have previously been made on the natural occurrence of fire blight in approximately 1,000 field grown accessions of *M. sieversii*. Accessions damaged by natural fire blight infection in the field can be considered susceptible. However, in cases where no fire blight damage occurred it is not known if these accessions are resistant to the disease or if they escaped exposure to the pathogen during favorable conditions for infection. To estimate the rate of escape, a subset of plants without field infection were propagated and challenged with fire blight bacteria in greenhouse. Approximately 60% of plants without natural fire blight infection were found to be resistant when challenged in the greenhouse. This information was then used to predict a 1 susceptible: 1 resistant plant ratio among selected accessions.

In addition to selecting accessions for a balanced fire blight resistant : susceptible ratio, additional accessions were selected based upon resistance to both fire blight and apple scab, and superior fruit quality. The remainder of the CORE collection was also included to insure full genetic and character representation of *M. sieversii* in the plantings.

Standard cultivars were included in trials so that results obtained in different locations and years can be directly compared. Resistant control cultivars ('Delicious', 'Splendour' and 'Goldrush') were included to establish the lower limit for a "resistant" rating. Two highly susceptible cultivars ('Jonathan' and 'Gala') were included to establish the high end of the disease scale when comparing tests and to ensure that a minimum disease pressure threshold is achieved in every test. Moderately resistant ('Empire' and 'Golden Delicious') and highly resistant (Geneva.41 and Robusta 5) cultivars were also included to establish the mid and low end of the disease scale when comparing tests.

Budwood of 202 selected accessions and control cultivars was collected at the USDA-ARS-Plant Genetic Resources Unit in Geneva, NY in late July 2010 and sent to Van Well Nursery, East Wenatchee, WA for propagation. The material was propagated on M.7 rootstock. The trees were dug and stored in fall 2011 and will be planted at sites both WSU-TFREC, Wenatchee, WA (3 reps per accession) and USDA-ARS, Kearneysville, WV (4 reps per accession). Each planting in WA and WV will include the same 202 *M. sieversii* accessions and controls.

Executive Summary

Project Title: **Identifying fire blight resistance in M. sieversii for scion breeding**

This project had two main goals: 1) to identify molecular markers for fire blight resistance in the *M. sieversii* GMAL4593 mapping population and 2) establish plantings of *M. sieversii* at WSU-TFREC, Wenatchee, and USDA-ARS, Kearneysville, WV to be able to identify stronger sources of fire blight resistance for scion breeding in the future.

Approximately 60 additional molecular markers were added to the *M. sieversii* GMAL4593 in order to identify markers for fire blight. All of the 17 chromosomes (linkage groups) of apple are covered by the current map. Coverage of the individual linkage groups ranges from 50% to 95% of the 'Golden Delicious' genome reference map with an overall average of 73% coverage. The current map provides an excellent foundation for additional research on this population and *M. sieversii*. The map will be deposited and publicly available at the Genome Database for the Rosaceae (GDR). In addition to this work on fire blight, resistance to apple scab (*Venturia inaequalis*), resistance to blue mold (*Penicillium expansum*) and water use efficiency is being evaluated within the GMAL4593 population at USDA-ARS Kearneysville, WV using the genetic map generated in this project.

Fire blight resistance in GMAL4593 mapping population was associated with molecular markers on chromosomes (linkage groups) 10 and 8 of *M. sieversii*. The population was evaluated for fire blight resistance in the greenhouse and in the field over multiple year using multiple strains of the pathogen to insure that the effects of both environment and genetics were taken into consideration in the genetic analysis. Although both environment and the strain of the pathogen used for inoculation had effects on fire blight resistance, three markers located between 20-30 cMs on linkage group 10 were consistently associated with fire blight resistance in the field in both 2010 and 2011. The resistance locus identified on LG8 was more greatly affected by both environment and strain of the pathogen. The analysis of fire blight resistance at both of these loci (LG10 and LG8) is continuing, however additional funds for this aspect of the research are not being requested from WTFRC. Currently, we plan to incorporate the fire blight resistance found in GMAL4593 into genetic backgrounds with superior fruit quality using Fastrack breeding, or accelerating breeding that takes advantage of shortened juvenility of apple by transgenic expression of transcription factors controlling flowering. To validate the markers identified in this study, progeny resulting from these crosses will be screened for resistance using both the markers identified on LG10 and LG8 and by controlled inoculation with the fire blight pathogen.

The GMAL4593 population was created based upon the appearance of the *M. sieversii* growing in Kazakhstan before the several thousand seed of *M. sieversii* collected in Kazakhstan were characterized and genetically analyzed. To identify better sources of fire blight resistance for future breeding, all available field and greenhouse data on fire blight resistance and fruit quality of the *M. sieversii* collected by the USDA in Kazakhstan was analyzed and 191 accessions were selected for evaluation. Replicate trees of 202 selected accessions and control cultivars were propagated at Van Well Nursery, East Wenatchee, WA. Field plantings will be established this spring at WSU-TFREC, Wenatchee, WA and USDA-ARS, Kearneysville, WV. Additional funds are being requested from WTFRC to challenge these planting with the fire blight pathogen and determine the best *M. sieversii* accessions to be used as sources of fire blight resistance in the WSU apple breeding program.