FINAL PROJECT REPORT

Project Title: Mapping M. sieversii: A Valuable Genetic Resource for Apple Breeding

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Other funding sources: None

~~**Agency Name:**~~
~~**Amount awarded:**~~
~~**Notes:**~~

Total Project Funding: $54,000

Budget History:

Item	~~Year 1:~~2008	~~Year 2:~~ 2009	Year 3:
Salaries	10,000	10,000	
Benefits	3,500	3,500	
Wages			
Benefits			
Equipment			
Supplies	12,000	15,000	
Travel			

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Miscellaneous			
Total	25,500	28,500	

Objectives

The objectives of the present proposal addressed the following high priority research area defined by the Washington Tree Fruit Research Commission: Continued I.D. of genes and improvement of gene marker tools for continued increase in the efficiency of apple breeding program as well as improved disease and pathogen resistance.

The objectives were:

1) Produce genetic marker data for a family (**GMAL 4593)** of 190 seedlings derived from a 'Royal Gala' x *M. sieversii* cross using a collection of 159 SSR markers. Use the resulting marker data to produce a basic "framework" map for *M. sieversii*.

2) Identify additional markers (SSR and SNP) to fill any gaps in the evolving map and enrich regions of interest with a high density of markers.

3) Anchor the generated map to other apple maps by using identical SSR markers. In particular, exchange markers with New Zealand Institute for Plant and Food Research who will be mapping another family (**GMAL 4591**) from a different 'Royal Gala x *M. sieversii* cross.

4) 200 SNP markers will be identified by IASMA and placed on the genetic map for the GMAL4593 population.

5) Collaborate with others to define QTLs for important traits as the population is characterized. Specifically, **GMAL 4593** will be assessed for fire blight resistance in 2008 and 2009.

Note: **The finished maps and marker data will be deposited in the Genomic Database for the Rosaceae (GDR) in order for it to be available to the entire apple breeding and research community.**

Significant Findings

Objective 1 – One hundred of the 159 SSR markers available for the study have been screened on the entire population of 190 seedlings. Of the SSR primer combinations screened, 64 SSRs were polymorphic within the family. Fifty segregated in *M. sieversii* (MS) and sixty-four segregated in 'Royal Gala' (RG). The markers were assigned to linkage groups using Joinmap® 3.0. Statistics for both the SSRs and SNPs are presented in **Table 1**.

Objective 2 – A linkage map for both *M. sieversii* and 'Royal Gala' have been constructed using Joinmap® 3.0 and presented in **Figure 1**. Some linkage groups are represented as several sub-groups. It is expected that as the mapping effort continues all subsets of a linkage group will be able to be combined into a single linkage group. To accomplish this several hundred additional SNPs specific to the missing linkage group are currently being screened at FEM-IASMA, additional SSRs are being screened, and efforts to identify *sieversii*-specific SNPs are in progress. These extended efforts are part of new proposal submitted to the WTFRC for 2010.

Objective 3 –Additional SNP primer sets were obtained from the New Zealand Institute for Plant and Food Research covering all 17 linkage groups. These SNPS were screened in our population using HRM analysis on the Roche LightCycler and added to the genetic framework map (**Figure 1**).

Objective 4 – While the genetic framework maps for *M. sieversii* and 'Royal Gala' are incomplete, a sufficient number of loci have been established to allow comparisons with existing maps. These comparative maps are presented in **Figs. 2 and 3.** Mapping of the GMAL 4591 population by the New Zealand Institute of Plant and Food Research was not conducted due to a lack of funding so this comparison could not be made.

Ojective 5 – In collaboration with FEM-IASMA, 287 SNP markers were screened in a 96-member subset of the GMAL4593 population by FEM-IASMA. One hundred and seventy-nine were informative and placed on the genetic framework map (**Figure 1)**. As mentioned, this effort is continuing and several hundred additional SNPS are being screened at FEM-IASMA focusing on the missing and incomplete linkage groups.

Objective 6 - All the individual members of the GMAL4593 population have been scored for fire blight resistance. A replicated copy of the mapping population GMAL4593 was planted in Kearneysville in the fall of 2008. Further fire blight resistance evaluation and scoring will be conducted on these individuals in the spring of 2010 in order to obtain a more comprehensive data set to use for QTL analysis. This will also provide time to enrich the *M. sieversii* genetic map so that a meaningful QTL analysis of fire blight resistance can be conducted. The framework map will also be available to those conducting other phenotypic analyses of the GMAL4593 population.

Results and Discussion

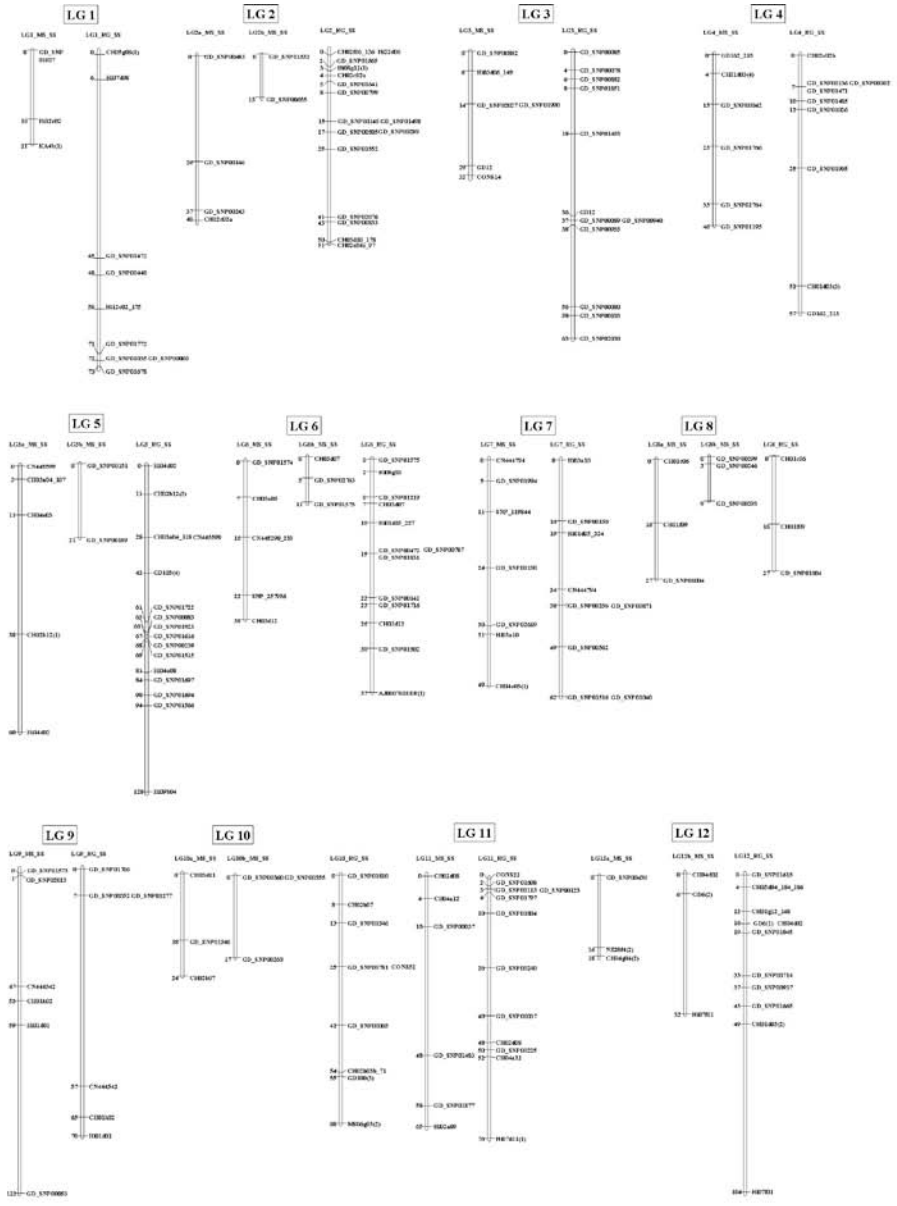
The USDA-ARS Plant Genetic Resources Unit has established a collection of *Malus* from around the world in order to preserve and develop genetic resources important to the apple industry. Among this collection is *Malus sieversii*, the main progenitor of the domestic apple, collected from Central Asia (Khazakhstan). To enable QTL analysis for important traits in *M. sieversii*, we have undertaken the construction of a genetic framework map for the family (F1) GMAL4593 ('Royal Gala' X *M. sieversii* PI 631981 [GMAL 4448]). One-hundred-ninety progeny were analyzed using Joinmap® 3.0 (Van ooijen and Voorips 2001) to establish a framework map for each individual parent of the F1 population GMAL4593 ('Royal Gala' x *Malus sieversii*). Linkage groups were established at a recombination fraction of 0.40 a LOD score ≥ 4.0. The Kosambi mapping function was used for the calculation of map distances.

Of 107 SSR primer combinations screened, 81 SSRs were polymorphic within the family. Fifty-three segregated in both *M. sieversii* (MS) and 'Royal Gala' (RG), 15 segregated in MS only and 21 in RG only. Additionally, 287 SNPs were screened in a subset (96) of the population. Ninety-nine SNPs were not informative for mapping in this population and 179 were mapped using Joinmap. 24 of the SNPs segregated in both MS and RG, 158 with RG only and 52 with MS only. The current 'Royal Gala' map consists of 188 molecular markers (124 SNPs and 64 SSRs) assigned to 17 linkage groups and the MS map consists of 107 molecular markers (57 SNPs and 50 SSRs) assigned to 26 linkage groups representative of all 17 linkage groups.

The construction of a framework map for *Malus sieversii* is the first step in enabling the identification of regions of the genome that control important traits such as apple scab and fire blight resistance. This map will be used to place QTLs for fire blight resistance and apple scab resistance. Identifying molecular markers in these regions will help breeders streamline the breeding process by allowing the breeders to make early selections of resistant material. The framework map presented in **Figure 1** will not only serve to identify genomic locations of important traits in *Malus sieversii*, but by using molecular markers common in other apple maps, allow the comparison of these locations in

the *Malus sieversii* genome to other apple genomes. Figure 2 illustrates the cross comparison of *Malus sieversii* linkage groups with the corresponding linkage groups from the apple consensus map recently constructed by N'Diaye et al. (2008) and the 'Fiesta' x 'Discovery' map (www.rosaceae.org and Silfverfberg-Dilworth et al. 2006).

Figure 1 – Genetic framework maps for the parents, *M.sieversii* (MS) and 'Royal Gala' (RG) of mapping population GMAL4593. LG = Linkage group. All SNP-based markers are designated as GD SNP where GD= Golden Delicious. SNPs analysis was conducted by IASMA based on the identification of SNPS from the genomic sequence of 'Golden Delicious.'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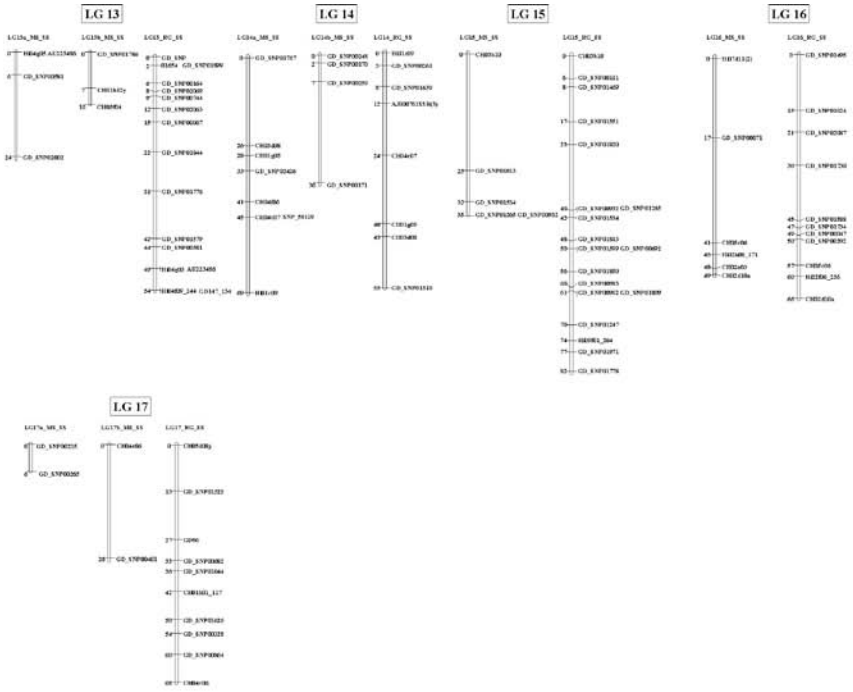
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LG 17

Figure 2 – Anchoring of *M. sieversii* map to the apple consensus map. LG1 is not included because none of the markers from *LG1-M. sieversii* were on the apple consensus map.

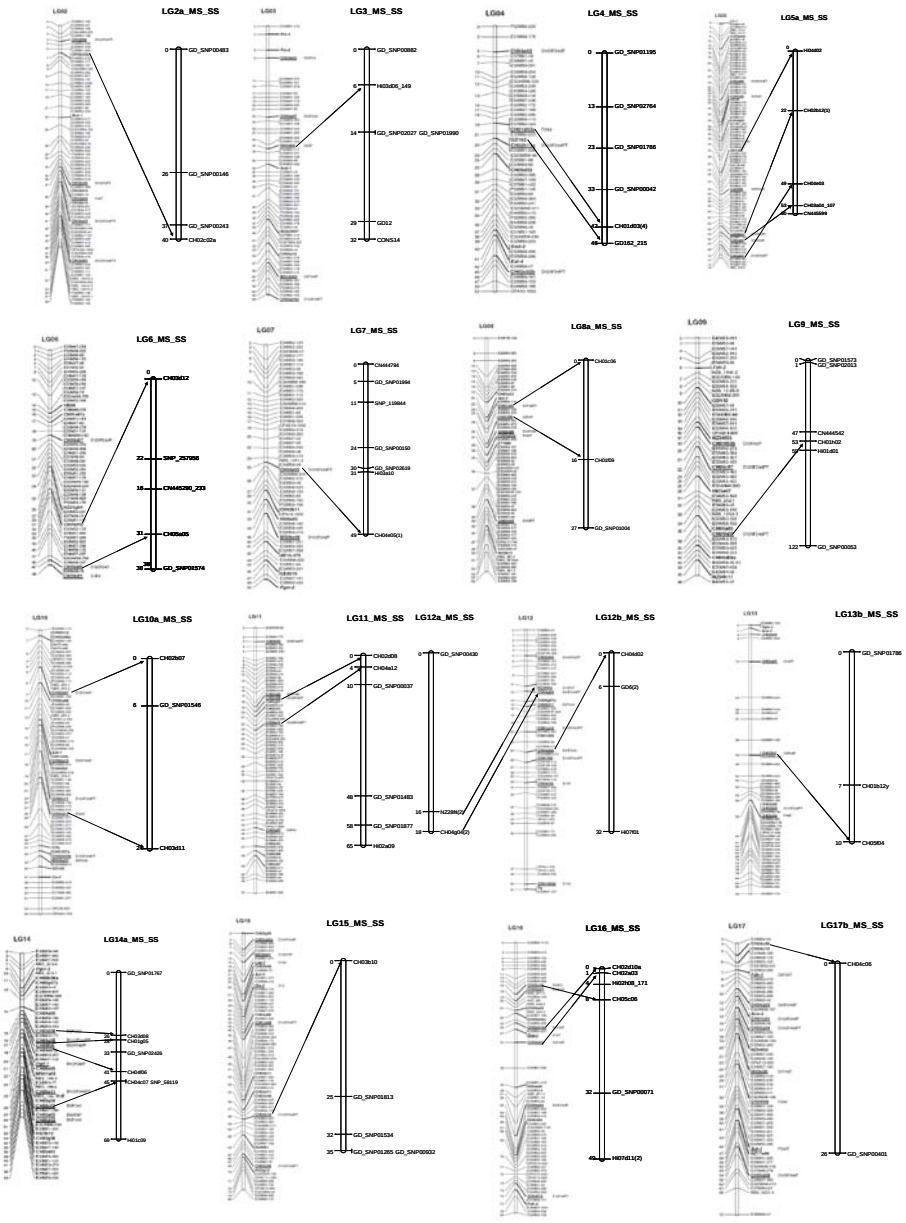

Table 1 – Mapping statistics for each parent in GMAL4593 ('Royal Gala' x *M. sieversii*).
LG = Linkage Group Number, **#loci** = total number of loci identified on the linkage group, **#SSR** = number of SSR markers mapping to the linkage group, **#SNP**s = number of SNPs mapping to the linkage group, **CM distance** = centimorgans of distance spanning the linkage group by the collective SNP and SSR markers.

Malus sieversii

LG	#loci	#SSR	#SNPs	CM distance
1	3	2	1	21.182
2a	4	1	3	39.827
2b	2	0	2	13.242
3	6	2	4	31.911
4	6	2	4	46.343
5a	5	5	0	59.873
5b	2	0	2	21.637
6a	5	3	2	37.565
6b	3	1	2	11.41
7	7	3	4	48.937
8a	3	2	1	26.922
8b	3	0	3	9.065
9	6	3	3	121.687
10a	3	2	1	24.302
10b	3	0	3	17.329
11	6	3	3	64.69
12a	3	2	1	17.692
12b	3	3	0	31.599
13a	4	2	2	23.832
13b	3	2	1	10.11
14a	8	5	3	68
14b	4	0	4	29.634
15	5	1	4	35.253
16	6	5	1	49.341
17a	2	0	2	5.773
17b	2	1	1	26.275
totals	100	47	53	832.422

Royal Gala

LG	#loci	#SSR	#SNPs	CM distance
1	9	3	6	72.594
2	16	6	10	50.712
3	12	1	11	63.342
4	9	3	6	57.299
5	16	7	9	127.644
6	13	5	8	37.495
7	9	3	6	62.442
8	3	2	1	27.139
9	6	3	3	70.111
10	9	4	5	67.596
11	12	3	9	73.066
12	11	6	5	103.555
13	15	4	11	53.873
14	8	5	3	54.535
15	19	2	17	81.892
16	11	3	8	66.413
17	10	4	6	68.245
totals	188	64	124	1137.953

Executive Summary

Malus sieversii, the main progenitor of the domestic apple, collected from Central Asia (Khazakhstan), is an important genetic resource for apple breeders developing new cultivars for the industry. In addition, to being a source of drought, pest and disease resistance, it offers the potential of many new fruit quality traits. As opposed to many more exotic apple genetic resources, elite selections of *M. sieversii* have large, palatable fruit. Breeding apple varieties for novelty and superior quality, meeting grower demands and satisfying consumer requests is a challenging task. Marker-assisted selection (MAS) is a very promising genomic approach to make the breeding process more efficient and precise. This method uses molecular markers linked to the genes of desirable traits to monitor how the desired traits of each parent have combined in individual seedlings. Although marker assisted selection does not replace the need to carefully evaluate mature trees for complex traits like flavor, texture and aroma, it provides the breeder with a tool to identify plants with unfavorable combinations of genes when they are young seedlings. By eliminating the need to grow unfavorable seedlings to maturity before identifying them as "losers", the breeder can maximize their limited resources. Marker assisted selection also allows the breeder to "pyramid" different resistance genes to enhance the durability of resistance in new varieties, a process which is extremely difficult by current apple breeding methods.

Essential to the process of marker-assisted selection is the availability of detailed genetic maps. Small, repetitive DNA sequences in the genome or DNA of organisms called 'simple sequence repeats' (SSRs, aka microsatellites) can be used as markers to create genetic maps. Genes controlling desirable traits are then mapped to a specific location between two markers on the chromosome map. The regions of a chromosome that are associated with a greater or lesser portion of a multi-gene trait are referred to as quantitative trait loci (QTLs) that can likewise be mapped or defined by specific markers (Fig. 2). **To enable QTL analysis for important traits in *M. sieversii*, we have undertaken the construction of a genetic framework map for the family (F1) GMAL4593 ('Royal Gala' X *M. sieversii* PI 631981). One-hundred-ninety progeny were analyzed using Joinmap® 3.0 (Van ooijen and Voorips 2001) to establish a framework map for each individual parent of the F1 population GMAL4593 ('Royal Gala' x *Malus sieversii*).**

Over 100 SSRs and 300 SNP markers have been screened in our mapping population and those that were informative (i.e. had segregating alleles) were mapped to either or both parents. Using this data, we have created a basic genetic framework map for both the *M. sieversii* and 'Royal Gala' parents of the GMAL4593 mapping population. Additionally, the entire population has been evaluated and scored for fire blight resistance. The GMAL4593 population has also been propagated and established as a replicated planting at the USDA-ARS, Appalachian Fruit Research Station, Kearneysville, WV for additional fire blight resistance screening. Using the genetic framework map, the collective data on fire blight resistance can be used to determine the genetic location (QTL) of the fire blight resistance in *M. sieversii*.

The genetic framework maps for *M. sieversii* and 'Royal Gala' are incomplete at this stage. Some linkage groups are represented by only a few markers and some linkage groups are represented as subsets. The placement of additional markers on the map in order to enrich the map and join all the subsets of a linkage group into a single linkage group is being pursued. Specifically, several hundred more SNPS are being screened by FEM-IASMA and once the results have been obtained, the additional markers will be added to the parental framework maps. **Identifying markers that segregate in the *M. sieversii* parent has been difficult and many more markers are mapping to the 'Royal Gala' parent. This was not anticipated and the identification of *M. sieversii*-specific SNPS by re-sequencing in order to enrich the *M. sieversii* genetic map and the identification of QTLs for fire blight resistance is the subject of a new proposal submitted to the WTFRC, entitled, "Identifying fire blight resistance in *M. sieversii* for scion breeding."**