AN EXPANDED GENETIC MAP OF *VITIS RUPESTRIS* X *MUSCADINIA ROTUNDIFOLIA* FOR FINE SCALE MAPPING AND CHARACTERIZATION OF PIERCE'S DISEASE RESISTANCE

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Reporting Period: The results reported here are from work conducted from June 1, 2002 to November 1, 2002.

INTRODUCTION

This project is funded by the UC Pierce's Disease Grant Program. It expands a genetic mapping effort originally funded by the California Grape Rootstock Improvement Commission, the Fruit tree, Nut tree and Grapevine Improvement Advisory Board, the California Table Grape Commission and the AVF. That study examined the genetics of resistance to the dagger nematode and found that this resistance segregated within several *V. rupestris* x *M. rotundifolia* F1 populations. A resistant and a susceptible sibling were selected from one of these populations, 8909, and they were crossed to produce the 9621 "F2" population (8909-15 x 8909-17). The 8909 and 9621 populations also segregate for resistance to *Xylella fastidiosa* (*Xf*). A genetic map of 116 individuals from the 9621 population was created primarily with AFLP markers. The work proposed here will increase the mapping population to 188 individuals and add at least 100 SSR markers. The addition of SSR markers will help to link the *V. rupestris* x *M. rotundifolia* map to other mapping efforts around the world through their universally comparable nature. This is a new project with funding finalized in September 2002.

OBJECTIVES

1. Expand an existing genetic map created within *V. rupestris* x *M. rotundifolia* focused on resistance to *Xf* by adding 100 more individuals and 100 SSR and 20 EST markers.

Completion of this objective will allow further identification of DNA markers that are tightly linked to *Xf* resistance so that marker-assisted selection strategies can be employed in the breeding program. It will also more fully support efforts to locate and identify the gene(s) responsible for *Xf* resistance.

RESULTS AND CONCLUSIONS

A genetic map of the 9621 population was completed and submitted for publication (M. Doucleff, Y. Jin, F. Gao and M.A. Walker. A Genetic Linkage Map of *Vitis rupestris* x *Muscadinia rotundifolia*. Submitted to Theoretical and Applied Genetics). This map was initiated several years ago, was based on the pseudo-testcross strategy, and used primarily AFLP markers. Over the past two years we have used 15 new AFLP primers, 7 new ISSR primers, and 9 new SSR primers to score over 200 additional molecular markers for 116 F2 individuals in the 9621 population. Ambiguous genotypes were rerun or left as unknown. After scoring and rechecking each marker, approximately 10% of the markers were discarded because they were not consistently scored. Chi-square tests found that about 20% of the markers had significantly distorted ($P < 0.05$) genotype ratios. The remaining markers with $P > 0.05$ (100 AFLP, 32 ISSR, and 18 SSR) combined with the existing 275 AFLP and 25 RAPD markers were used to create a framework map for each F1 parent using MapMaker UNIX 3.0 and PGRI.

A total of 474 polymorphic markers were scored with 298 segregating 1:1 and 176 segregating 3:1. Approximately 7.5% of the bands displayed skewed segregation ratios (Table 1). Of the 298 1:1 markers, 158 were heterozygous in the female (8909-15) and 140 were heterozygous in the male (8909-17). Overall linkages were robust with $p \leq 0.3$ and $X2 \geq 0.001$ (equivalent to LOD score ≥ 3). At $X2 = 0.00001$ and $p \leq 0.25$, 16 linkage groups were formed for 8909-15 and 20 linkage groups for 8909-17 (Table 2). A framework map for each parent was constructed based on a 90% confidence level for correct order using a PGRI bootstrapping algorithm. Markers not ordered with a confidence level $\geq 90\%$ were added to the framework maps as accessory markers. Together the two framework maps covered 1630 cM. This map was based on 116 individuals with 375 AFLP, 32 ISSR, 25 RAPD and 18 SSR markers. Two measures of *Xf* resistance (ELISA values indicating limited *Xf* movement beyond the point of inoculation; and the uneven lignification) both map to the same general area on the same Linkage Group.

Table 1. Data on mapping markers within the 8909-15 x 8909-17 mapping population.

Marker Information	1:1 8909-15 Female Markers	1:1 8909-17 Male Markers	3:1 Markers
Total markers scored	158	140	176
Percent distorted (□2, df=1, p=0.05)	10.8	3.6	7.4
AFLP markers	125	111	160
ISSR, RAPD markers	25	21	14
Microsatellite markers	8	8	2
Framework markers	90	101	NA
Accessory markers	51	30	NA
Missing data %	3.8	5.4	4.0

Table 2. Data on linkage groups on the genetic maps

Framework Map Linkage Group Information	8909-15 Female Parent	8909-17 Male Parent
Total number of groups	16	20
Total size (cM)	730	900
Avg. group size (cM)	45.6	45.6
Avg. distance between markers (cM)	11.0	8.8
Avg. PCO	91.6 ± 4.7	95.5 ± 4.0

Before efficient efforts to locate *Xf* resistance genes can be undertaken, more individuals and markers are needed on the map. We are now in the process of adding this data. Thus far, DNA has been extracted from 188 individuals and we have produced the some marker data (Table 3).

Table 3. Data on number of markers tested and useful for the 8909-15 x 8909-17 mapping population.

Markers	Tested	Amplified	Useful for Map
SSR	111	92	65
EST (D. Adams)	20		14
Total	131		79

We are continuing to add SSR markers and are preparing to retest the entire population for AFLP markers.

FUNDING AGENCIES
Funding for this project was provided by the CDFA Pierce's Disease and Glassy-winged Sharpshooter Board, and the University of California Pierce's Disease Grant Program.