THE GENETICS OF RESISTANCE TO PIERCE'S DISEASE

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INTRODUCTION

This project was part of the American Vineyard Foundation Long Term Project on Pierce's Disease. Our component of this project focuses on understanding the genetics of resistance to *Xylella fastidiosa* (*Xf*), the causal agent of Pierce's disease (PD). The studies include understanding the inheritance of resistance to *Xf* in *Vitis rupestris* x *Muscadinia rotundifolia* and developing genetic markers for *Xf* resistance. It integrates into two other projects – a fine-scale mapping project for *Xf* resistance, and the breeding of PD resistant table and raisin grapes.

These *Xf* resistance studies are being carried out on potted and replicated plants under greenhouse conditions using needle inoculation with the 'Stag's Leap' strain of *Xf*. The plants are grown for 12 to 16 weeks and then evaluated for the presence of *Xf* and irregular shoot lignification symptoms. ELISA is used to test stem samples from 10 cm above and 10 below and at the point of inoculation. This system has proved to be highly reliable, efficient and quantifiable.

OBJECTIVES

1. Complete analysis of a series of crosses (Design II mating scheme) allowing the quantitative inheritance of *Xf* resistance to be evaluated.
2. Complete a genetic map of a *Vitis rupestris* x *Muscadinia rotundifolia* seedling population using AFLP (amplified fragment length polymorphism) markers to allow the identification of DNA markers to *Xf* resistance and eventual identification of *Xf* resistance genes and their genetic engineering into *vinifera* cultivars.
3. Develop and utilize genetic markers to assist and accelerate the introgression of *Xf* resistance into table, raisin and wine grapes.

RESULTS AND CONCLUSIONS

Inheritance studies of Xf resistance in a M. rotundifolia background:

Last year, Alan Krivanek (PhD student) completed a broad series of crosses within a Design II mating scheme among siblings from the 8909 (*V. rupestris* x *M. rotundifolia*) population. From among those families he is currently screening a total of 2,100 plants for *Xf* resistance under a randomized complete block design using our greenhouse evaluation system. The crosses include 9 Resistant x Resistant families, 3 Susceptible x R families, 3 R x S families and 1 S x S family. From these crosses 3-4 cuttings from 20-38 seedlings from each of the 16 families have been propagated potted and inoculated twice with the 'Stags Leap' *Xf* isolate. Parents of the 4x4 Design II were chosen as follows: Females – 8909-02 R, 8909-07 S, 8909-15 R, 8909-16 R?; Males – 8909-01 R, 8909-08 R, 8909-19 S, 8909-26 R.

Genetic mapping of a V. rupestris x M. rotundifolia population (9621 – 8909-15 x 8909-17):

A genetic map of *V. rupestris* x *M. rotundifolia* has been 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 is now being expanded and fine scale mapping undertaken to better localize *Xf* resistance genes and markers linked to them (Please see the report on this project by Walker and Riaz within these Proceedings).

Development of genetic markers for Xf resistance:

This study is being carried out in the 9621 mapping population (8909-15 x 8909-17, both *Xf* resistant). To date, about 70 primer combinations have been evaluated out of a goal of 150 to 200 primer combinations. It is expected that 2-4 markers flanking the *Xf* resistance gene within a 2-cM window will be identified. Several candidate markers liked to resistance in the male parent and the female parent have been identified. Candidate markers will be confirmed by separately evaluating marker patterns on each individual within the bulk. SCAR primers will be developed from the tightly flanking markers and run on the 145 genotypes previously screened for resistance. A total of 145 genotypes with marker data should yield a

mapping resolution of approximately 2cM. This resolution will be used to confirm the order and distance of the SCAR markers around the resistance locus on its linkage group.

Susceptible bulk: 9621-025, 9621-034, 9621-039, 9621-045, 9621-062, 9621-094, 9621-116, 9621-118, 9621-167, 9621-219, and 9621-277.

Bulked segregant analysis (BSA) was also carried out on a population composed of four different populations. The male in all four was 8909-08 (*V. rupestris* x *M. rotundifolia*). The females were advanced seedless *V. vinifera* table grape selections from D. Ramming: B90-116 (population 501), C63-83 (502), C33-30 (503), and P79-101 (504). A total of 120 plants were tested for *Xf* resistance under our greenhouse screen and evaluated with ELISA. From these, 14 were used in a resistant bulk and 16 were used in a susceptible bulk. These bulks were screened with 114 different AFLP primer combinations. Out of these 114, 11 were primer combinations that had already been mapped (see above) in a related cross (8909-15 x 8909-17). These primer combinations were from Group 15 of this map, as is *Xf* resistance. None of these 11 markers were close enough to show up in the BSA analysis.

Results from eight of the 114 markers tested suggest that they are candidate markers. These markers linked with *Xf* resistance in the bulk analysis, and need to be further tested on the individual genotypes. Most of these candidate markers were faint bands, indicating that they may not be present in all of the resistant genotypes, and therefore recombination events prevented tighter linkages.

Genetic markers were also sought in the 0023 population (8909-15 R x B90-116 S (advanced seedless selection from D. Ramming)). One hundred and eight seedlings were inoculated under our greenhouse system in a randomized block design. Plants were inoculated twice. After 16 weeks the plants were evaluated for *Xf* resistance based on cane lignification ELISA. Fifty-four of the 108 seedlings had mean bacteria numbers of less than 500,000 cfu/ml which in other populations is the cut off point for resistance. The genotypes: 0023-19, 0023-54, 0023-63, 0023-98 had stem bacteria numbers of less than 60,000 cfu/ml and were crossed to advanced table grape selections in order to establish a large breeding population. Approximately 1,800 seeds have been collected. Markers linked to resistance in the mapping and BSA portions of this project will be used on this population. Correlation of the markers with *Xf* resistance will be confirmed and calculated if different from the original mapping population.

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