Report to the American Vineyard Foundation

Map-based cloning of a powdery mildew resistance locus in *Vitis*

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Introduction

Molecular maps were developed in two *Vitis* populations in our lab, leading to the identification of an important locus (or gene) affecting powdery mildew resistance. This locus resides in a vine derived from a cross between *V. rupestris* and *V. cinerea*. Map-based cloning of disease resistance genes in plants is a feasible objective, and the small genome size of grapevines facilitates the technical aspects of cloning. We sought to saturate the genetic map in a specific region that includes an important locus for powdery mildew resistance. Our eventual goal was to use these markers to aid in map-based cloning from DNA of the resistant parent.

Objectives for 2001/02, as stated in the proposal:

1. Identification of large numbers of molecular markers (known as AFLPs) on Linkage Group X of Illinois 547-1 (*Vitis rupestris* x *V. cinerea*) where a powdery mildew (PM) resistance gene is situated.

2. Once identified, candidate markers will be used to create a high-density map of the region of Linkage Group X where the resistance gene is located.

At this point in time, the first objective has been completed. This work is described below. Since February 2001, laboratory efforts have focused on Objective 2.

Methods

Plant materials

The original map of Ill. 547-1 was based on data collected from a population of seedlings derived from a cross between 'Horizon' and Illinois 547-1 (*V. rupestris* x *V. cinerea*). There were only 58 individuals used to create the initial genetic map (Dalbó et al., 2000, 2001). In the present work, the population has been expanded to 272 individuals. Among these vines, 70 were planted in 1989 and the rest were planted in 1997 at the Experiment Station research vineyards. Additional seedlings (275) were germinated in 2001.

DNA extraction

Total genomic DNA was isolated from young leaves using a modified CTAB procedure, similar to that described by Lodhi et al. (1995)

Powdery mildew resistance

Data were collected from single vines of seedlings and three vines of parents under natural disease pressure in a fungicide- and insecticide-free vineyard. Data were usually collected during late August or early September, when differences among genotypes were most easily observed. Data have been collected for 8 years from the 70 vines planted in 1989 and for 3 years from the 202 vines planted in 1997.

Powdery mildew resistance was rated according to visual estimates of % leaf area covered by mycelium and scored from 1 to 5 using the following scale:

1 - up to 3% leaf area, resistant (typical rating for Ill. 547-1)

2 - 3 to 12%, partially resistant

3 - 12 to 25%, tolerant

4 - 25 to 50%, susceptible (typical rating for 'Horizon')

5 - >50%, highly susceptible.

Bulked segregant analysis (BSA) using AFLP markers

Preparation of two bulked DNA samples

Two bulked DNA samples were selected from among the seedlings of the 'Horizon' x Ill. 547-1 population. Each bulk contained 18 individuals; one bulk consisted of resistant seedlings and the other of susceptible seedlings. Individuals of the resistant bulk were rated 1.0 to 2.2 and individuals of the susceptible bulk were rated 4.4 to 5.0 according to the powdery mildew rating scale above.

The two bulks should therefore be genetically dissimilar for DNA sequences in the powdery mildew resistant region, but homogenous at other regions (Michelmore et al., 1991).

AFLP (amplified fragment length polymorphism) technique

The AFLP technique produces large numbers of molecular markers in a short period of time. Each of these markers is visualized as a "band" of DNA separated by size on a laboratory gel. AFLP analysis was performed according to Vos et al. (1995) with slight modifications.

DNA was digested 3 hr at 37 C using two restriction enzymes, *Eco R*I and *Mse* I. Two different adapters, designed to avoid the reconstruction of these restriction sites, one for the *Eco R*I sticky ends and one for the *Mse* I sticky ends, were ligated.

Digested-ligated DNA fragments were used as templates for the first amplification reaction, the pre-amplification step. This PCR reaction used primers that were complementary to the adapters with an additional selective 3' nucleotide.

The pre-amplification products were used as templates for the selective amplification. This step was repeated with 64 primer combinations (8 x 8 primer matrix). The PCR reactions were carried out using 35 cycles, each cycle consisted 1 min at 94 C, 1 min for annealing, and 2 min at 72 C. The annealing temperature started at 65 C and decreased 0.7 C per cycle until 56 C. Starting at a high annealing temperature allows for improved primer selectivity. By gradually decreasing the annealing temperature, the efficiency of primer binding increases.

PAGE (polyacrylamide gel electrophoresis) and Silver Staining

AFLP markers were visualized on large polyacrylamide sequencing gel units (45 x 43

cm) to separate AFLP products that differed in length by as little as 1 base. We used silver staining methods to observe bands on polyacrylamide gels.

Results and Discussion

Powdery Mildew Resistance Analysis

We collected powdery mildew resistance ratings from 70 vines for 8 years. The most resistant and susceptible categories totaled 10% of these 70 vines (Figure 1A). Susceptible vines (rated 3.6 to 4.5) comprised 44% of the population.

Powdery mildew resistance was also rated between 1999 and 2001 on 202 vines that were planted in 1997. In this new population, resistance was normally distributed. The total population comprising plants from both 1989 and 1997 plantings also showed a normal distribution for powdery mildew resistance.

The choice of the right population is one key factor in the detection of important genes and the creation of a high-density map near these genes. Our results indicate that a normal distribution can be achieved by increasing the population size. By combining genetic data from both the old and the new population, accuracy in the creation of a high-density map will be improved.

Marker Analysis and Screening

We used AFLP markers for the analysis of the powdery mildew resistance locus on Linkage Group X of Illinois 547-1 (*V. rupestris* x *V. cinerea*). In our bulked segregant analysis survey, we examined approximately 5,500 bands (DNA segments) resulting from 64 primer combinations. We searched for the expected markers near a powdery mildew resistant gene(s) by comparing the two parents and the bands produced from the 2 bulked DNA samples described earlier. With 37 of the 64 primer combinations, we identified 68 markers (1 to 4 markers per primer pair) that were potentially linked to the locus for powdery mildew resistance. We will refer to these markers as "candidate markers". There were three primer combinations giving rise to four candidate markers each. Of the 68 candidate markers, 39 were found in the Ill.547-1 parent and resistant bulk but not in Horizon and the susceptible bulk. On the other hand, 29 markers were found in Horizon DNA and in the susceptible bulk, but not in the Ill. 547-1 and the resistant bulk. Among 68 total markers, 29 showed high intensity and were easily scored.

We then focused our efforts primarily on the 29 markers that were of high intensity and easily scored, plus other markers that were amplified in the same reactions and not as easy to score. From 26 primer combinations, 57 of the 68 candidate markers were examined. Of these 57, 29 markers produced useful data (others did not produce clear and distinct bands), and seven have so far been tentatively placed on the map in the region between two markers identified earlier (Dalbó et al. 2001) that flanked the powdery mildew resistance locus. The precise mapping distances are still to be defined. Of these seven, four were on the Illinois 547-1-derived linkage group and three were on the Horizon linkage group.

In addition, the complete population of 272 vines was analyzed for segregation of the two original markers identified by Dalbó et al. (2001). On the Horizon map, the distance between the two markers changed from 9.5 centimorgans (cM) based on the original 58 seedlings to 5.6 cM. On the map of the Illinois 547-1 linkage group, the distance expanded from 1.8 cM to 12.0 cM. It is not surprising that the distances should change as the population size grows, but the distances calculated based on the very large population of 272 vines is expected to change

little.

Summary

We analyzed powdery mildew resistance in 272 vines from the cross between Horizon (susceptible) and Ill. 547-1 (resistant). Segregation for resistance was normally distributed. Our work shows that the actual distance between two DNA markers for powdery mildew resistance identified earlier is actually 12 cM, rather than the 1.8 cM calculated earlier from a small population.

As a result of bulked segregant analysis using AFLP markers, we found 68 candidate markers for mapping to the powdery mildew resistance locus. Efforts have begun to saturate the genetic map in the region of this resistance gene locus. To date, 57 of the 68 markers were examined, yet of these just seven were tentatively placed on the map in the same region as the powdery mildew resistance locus.

New technologies are being developed in other laboratories that may allow a more thorough and detailed analysis of the many genes involved in a plant's defense again disease. Our project to date has been focused on just a single gene (or a chromosomal region with several genes). Given the relative difficulty to clone even a single gene based on map position, I have decided to pause this project pending examination of the best techniques currently available to reach our goal of cloning grapevine genes for disease resistance and understanding more about the grapevine disease resistance responses. Undoubtedly, the material we now possess is unique, and our present knowledge of the map location of this gene for powdery mildew resistance will be of value as new approaches are examined to reach our original goals.

Literature cited

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