

## Viticulture Consortium – East

### Progress Report for 2006

#### A DIRECTED, NON-TRANSGENIC APPROACH TO RECESSIVE DISEASE RESISTANCE IN *VITIS* TOWARD DEVELOPMENT OF DURABLY RESISTANT CULTIVARS

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*Due to reduced funding, the proposal was revised and resubmitted as follows to fit the budget: Eliminate identification of multiple resistance genes (focus only on Mlo); Hire a 6-month technician rather than 9-month post-doc; Cut grower re-imbursement in half; Do not present or publish results in a formal scientific forum.*

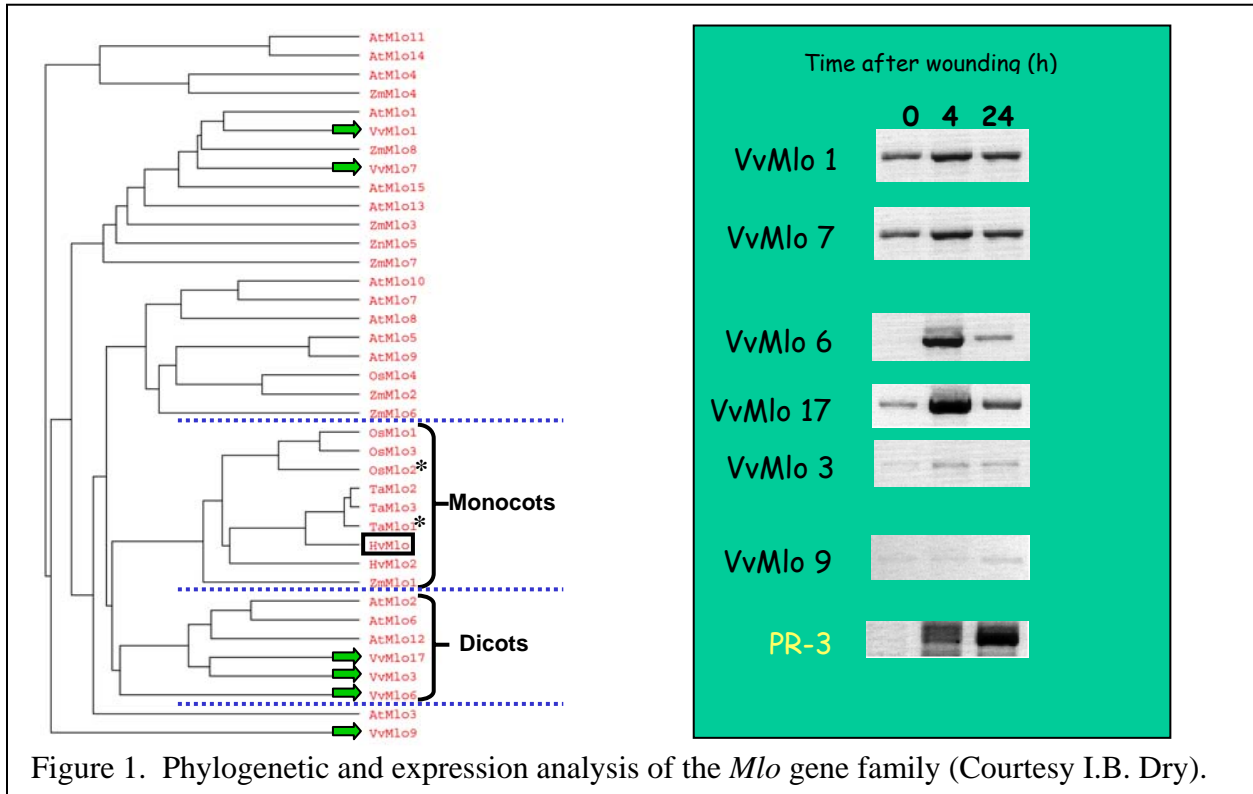
##### Objectives:

- 1) To induce 10,000 independent mutational events in a Chardonnay genetic background.
- 2) To conduct a forward genetic screen of 5,000 selfed mutant seedlings (in the segregating M2 state) for loss of susceptibility to powdery mildew.
- 3) To conduct a reverse genetic screen of M2 seedlings for mutated alleles of the *Mlo* powdery mildew resistance gene.

**Introduction:** Although naturally-occurring grapevine mutations have played a prominent role in crop improvement, with “sports” widely propagated due to improvements in cluster architecture, cold hardiness, berry color, and berry size, for example, induced mutagenesis has been underutilized as a breeding technique in grapevine.

Recessive resistance genes that arise from knocking out genes required for disease provide a durable approach to disease control against biotrophic plant pathogens including viruses (1,9), nematodes (15,18,19), bacteria (2,7), and fungi – most notably, powdery mildews (4,6,16). Recessive resistance against host-specific powdery mildews has been identified in apple, barley, cucumber, melon, pea, tomato, *Arabidopsis*, and both wild and domesticated wheat (3,5,8,11-13,17). One example is barley *mlo* (4,6). A monogenic, race non-specific, durable resistance gene, *mlo* has broad appeal to barley breeders, leading to its incorporation in over 130 barley cultivars. Currently, over 50% of certified barley seed sold in Europe contains mlo resistance, and it remains durable.

Recently, three *mlo* alleles each conferring resistance to powdery mildew on *Arabidopsis* were discovered and found to be closely related to functional barley *mlo* (14). In tomato, the recessive powdery mildew resistance gene *ol-2* co-localizes with a *mlo* homologue and shares all of the hallmarks of *mlo* resistance (R. Panstruga, personal communication). The finding of *mlo*-conferred resistance in dicotyledonous plants suggests that this gene may provide resistance in a broad range of hosts. Recently, three *mlo* homologues with the classic hallmarks of *mlo* resistance have been cloned and characterized in grapevine (Fig. 1).



## Results:

*Objective 1: To induce 10,000 independent mutational events in a Chardonnay genetic background.*

In 2005 and 2006, we extracted and tracked seed from individual clusters of treated Chardonnay vines. In 2005, we collected 612 clusters and over 1500 clusters in 2006. Based on our estimations, this represents approximately 100,000 independent mutational events, providing sufficient material for several forward genetic screens and reverse genetic projects over the next decade.

We tested the efficacy of mutagenesis using the M2 seeds collected in 2005 by measuring survival rate. We germinated two batches of seeds harvested in 2005 (n=14,139 and n=13,220) representing three levels of EMS mutagenesis and non-mutagenized control clusters. Normalizing the control to 100%, the three treatments averaged 50.0%, 46.8%, and 41.5% survival relative to the control, suggesting efficient levels of mutagenesis.

Because the product of this project will be breeding lines with recessive resistance incorporated, in addition to our Chardonnay objective, we initiated mutagenesis on two potential parents for cross-hybridization, based on input provided by Dr. Bruce Reisch. We obtained approximately 20,000 independent mutational events per parent. If we can identify the same recessive resistance gene in Chardonnay and one or both of these parents, cross hybridization could directly result in an improved variety with recessive resistance.

*Objective 2: To conduct a forward genetic screen of 5,000 selfed mutant seedlings (in the segregating M2 state) for loss of susceptibility to powdery mildew.*

To date, we have screened 4163 seedlings for both downy mildew and powdery mildew resistance. This process involves inoculating batches of seedlings with  $10^5$  downy mildew sporangia per mL, incubating for 2 weeks and re-inoculating non-symptomatic plants. After the downy mildew screen, plants are cleaned up, revived, and inoculated twice in the same manner with  $10^5$  powdery mildew conidia. Although we have identified and subsequently confirmed almost 100 Chardonnay M2 seedlings with strong levels of downy mildew resistance in the screen and in detached leaf assays (many immune), none of the 43 seedlings that were non-symptomatic throughout the powdery mildew screen were immune when inundated with powdery mildew conidia in a detached leaf assay. Regardless, the 43 powdery mildew resistant seedlings from the resistance screen will be planted in a nursery in 2007 and tested for powdery mildew field resistance, the desired trait. We will continue this forward genetic screen in Spring 2007 to surpass 5,000 seedlings.

*Objective 3: To conduct a reverse genetic screen of M2 seedlings for mutated alleles of the Mlo powdery mildew resistance gene.*

A targeted, reverse genetic approach is being pursued based on PCR-based TILLING technology (10). ARS is funding a TILLING pilot project in collaboration with the Seattle TILLING Project to optimize TILLING in a Chardonnay M2 mutant population of 768 individuals. The purpose of this pilot project is to determine mutation rates and appropriate protocols and population sizes for discovering mutations in a gene-of-interest. *Mlo* is one of the pilot project's targets. We were able to harness data from the Genoscope 5x genome sequencing project to identify 14 members of the *Mlo* multigene family, compared with 15 members identified in *Arabidopsis*. Based on sequence phylogeny and expression patterns, four of these *Mlo* genes were selected as strong candidates for a role in susceptibility to powdery mildew. The 768 M2 mutants in the pilot project are currently being screened for mutations in the four *Mlo* candidates and four additional genes. The identification of mutations in *Mlo* will be followed by testing of those seedlings for a resistance phenotype.

## **Appendix:**

**a. Impact Statement:** We have identified *Vitis vinifera* plants with recessive resistance to downy mildew or powdery mildew, likely to be durable resistance. Given the broad susceptibility of *vinifera* cultivars, these resistant plants are likely to be valuable breeding material. In addition to traditional breeding approaches and our development of perfect molecular markers for marker assisted selection, our approach will facilitate direct and rapid incorporation of resistance into any available cultivar using biotechnology approaches.

b. Presentations of this Viticulture Consortium-East funded project:

Cadle-Davidson, L. 2006. Forward and reverse genetic screens for resistance to powdery mildew and downy mildew of grapevine. *Phytopathology* 95:S18.

Cadle-Davidson, L. 2006. Cellular phenotypes of prehaustorial, hypersensitive, and ontogenic resistance against grapevine powdery mildew on developing leaves. *Phytopathology* 95:S17.

Cadle-Davidson, L., Cadle-Davidson, M. M., Cousins, P., Garris, A. and Owens, C. L. 2006. Novel methods and techniques for genetic improvement of grape (*Vitis*). ASPB: Plant Biology 2006.

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