

## *Improving Grape Rootstock and Scion Pest and Disease Resistance*

### **ARS LOCATION:**

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### **PROJECT OBJECTIVES:**

1. Genetically improve grape scions through the molecular, genetic, and genomic characterization of disease resistance.
2. Breed, evaluate, and introduce improved grape rootstocks with pest and disease resistance and tolerance to abiotic stress.

### **MAJOR ACCOMPLISHMENTS (2007–2010):**

#### Disease resistance evaluation:

The diverse species and breeding lines maintained in the Geneva germplasm repository store a wealth of resistance mechanisms for incorporation into new varieties. Having submitted for publication the results of screening all 1,400 accessions in the Geneva repository for both powdery mildew and downy mildew resistance, in 2008, we began receiving leaves of breeding lines from grape breeders across the United States for resistance evaluation. Thus far, more than 900 advanced breeding lines, cultivars, and segregating progeny (over 10,000 replicate samples) were screened. Qualitative and quantitative resistance was observed. By providing expertise and support in disease resistance evaluation, this work allows grape breeders to focus on additional traits of interest and more effectively select and develop improved varieties with durable disease resistance.

#### Documenting race-specific resistance to powdery mildew and applying molecular markers to pyramid resistance genes:

Durability of resistance is critical due to the cost of vineyard establishment and the necessary longevity of grapevines. We documented that some resistances in breeding programs could be overcome quickly by common pathogen isolates and communicated a need to pyramid multiple resistance genes in new cultivars. We are applying molecular markers to track these resistance genes in order to further the development of varieties with multiple resistance gene durability. Further, we identified a source of powdery mildew resistance that prevents the pathogen penetration and may be durable by itself or with a single fungicide spray. We are using molecular markers to track the introgression of four resistance genes into high quality raisin, table, and wine grapes. Planting of resistance cultivars would save the average U.S. grape grower up to \$50,000 each year.

Powdery mildew genomics and transcriptomics:

To identify genes required by the pathogen for infection, sporulation, and fungicide resistance, we are actively characterizing powdery mildew genomes (DNA) and transcriptomes (RNA). We led a team of 10 Cornell and ARS scientists in contributing to the international effort to sequence and annotate a reference genome (*Blumeria graminis* f. sp. *hordei*) for powdery mildews. This reference supports our extensive transcriptome sequencing of grape powdery mildew, which we have applied to study the population genetics of the fungus, its mechanisms of overcoming fungicide resistance, the genetic regulation of sporulation, and the effector proteins that are secreted into grape epidermal cells upon infection. These efforts could result in the development of new chemicals or durable disease resistance genes for powdery mildew management.

Development of transgenic hairy root system for grapevine functional gene assay:

One approach to improving grapevine rootstocks for resistance to root-knot nematodes is to express in host plants a gene which can disrupt some critical parasitic functions of nematodes, a concept that was well demonstrated in model species and is actively being pursued in several crops. As a first step of developing such a biotech solution we developed a transgenic hairy root system for evaluation of genes which are resistant to root-knot nematodes in grapes. We evaluated the influences of three *Agrobacterium rhizogenes* strains (15384, A4, and K599), four different tissue types (stem, internode, and petiole on *in vitro* plant, and young shoot excised from *in vitro* plant), and 14 *Vitis* species (*Vitis cinerea*, *V. x champinii*, *V. doaniana*, *V. ficifolia*, *V. flexuosa*, *V. girdiana*, *V. Jacquemontii*, *V. labrusca*, *V. nesbittiana*, *V. x novae-angliae*, *V. palmata*, *V. piasezkii*, *V. treleasii*, and *V. vinifera*) on the induction of transgenic hairy roots. Our results revealed that both 15384 and A4, but not K599, were effective in inducing hairy roots in *Vitis* species and that different *Vitis* species and tissue types responded differently to hairy root induction. Among the 14 species evaluated, *V. cinerea*, *V. labrusca*, *V. treleasii*, and *V. vinifera* produced hairy roots within a 2-week observation period when the induction was carried out on *in vitro* plants. Compared with stem and petiole tissues, the internode tissue showed a higher efficiency for hairy root induction. When young shoots were excised from *in vitro* plants and used as inoculation material, *V. cinerea* and *V. palmata* appeared more responsive to hairy root induction than four other species evaluated, *V. ficifolia*, *V. girdiana*, *V. Jacquemontii*, and *V. vinifera*. On the basis of this work, an optimized hairy-root induction system has been now established and will be used as a functional genomics tool for evaluation of genes that may confer resistance to root-knot nematodes in grapes.

Evaluating genes for potential to improve grapevine architecture:

Grapevine plant architecture can have significant impact on both grape production and viticulture management in vineyards. It is a trait which, to a large extent, dictates a grape grower's profitability. On the other hand, whole plant functional testing of grapevine genes is challenging due to large plant size and the time required from transformation to fruiting. Therefore development of a small and rapid cycling grapevine variety for functional genomics research is desired. To meet the needs of these two plant-architecture related research issues, we introduced several plant-growth related genes into *Arabidopsis* and grapevines to determine how these genes might affect plant architecture and growth cycle. One of the genes we evaluated was *GAI* (GIBBERELLIN ACID INSENSITIVE), the "green revolution gene" originally discovered in wheat. *GAI* plays a central role in plant gibberellin signaling. Mutations in a *GAI* gene can result in changes of plant architecture (e.g. reduced plant height)

and other traits, as well demonstrated in wheat, barley, rice and other crops. A mutant with a point mutation in the grapevine *GAI* gene (*Vvgai*) was recently discovered by Australian scientists. The same mutant was recovered by ARS scientist Peter Cousins, in collaboration with David Tricoli of University of California-Davis, through tissue culture. This mutant has dwarf and early flowering properties and was released as a germplasm line, “Pixie”, for research uses. To further explore *GAI* variants for development of desirable grapevines for functional genomics research as well as for the improvement of grapevine architecture, we evaluated several known mutation variants of the *GAI* gene, discovered from other crops, in both transgenic *Arabidopsis* and grapevine plants. We found that different mutant forms of *GAI* can have very different impacts on the reduction of plant internodes length, therefore plant height, and changes of other traits. This work provided direct evidence of the significance of allelic variation and reinforced the needs of exploring new forms of “old” known genes in crop improvement.

Developing root-knot nematode resistant rootstocks:

Root-knot nematodes are a serious vineyard pest. Nematode management using methyl bromide and other nematocidal chemicals is not sustainable and virulent nematodes are emerging that can damage important rootstocks. To improve grape rootstocks for resistance to virulent root-knot nematode populations, crosses were made using grape rootstock germplasm and the resistance of over 4,000 unique seedlings was determined by inoculating the plants with infectious virulent root-knot nematodes, then determining nematode reproduction levels following a suitable incubation period. To evaluate the horticultural characteristics of grape rootstock selections and increase plant material for further evaluation and grower trials, 20 nematode resistant selections were planted in grafted rootstock trials at the University of California Kearney Research and Extension Center, Parlier, California. Three rootstock selections that have completed the first round of rootstock trials were identified for release to grape growers and nursery operators. These selections are easily rooted and grafted and are superior to Freedom in yield:pruning weight.

Improving quality in Vignoles through clonal selection:

Vignoles is a valuable component of the wine grape cultivar portfolio in the eastern United States. Consumers appreciate its distinctive apricot, peach, and citrus notes in wines. It is relatively winter hardy and is grown in such diverse locations as New York, Missouri, Indiana, Ohio, Pennsylvania, Illinois, Nebraska, and Michigan. However, Vignoles has small, tight clusters prone to bunch rot later in the season, and losses up to one third of the crop are possible in rainy years. Despite consumer demand for Vignoles, the production of these wines is limited by bunch rot susceptibility associated with compact cluster architecture. Development of chemical and cultural practices to counter bunch rots in Vignoles remains challenging. Our goal is to select an improved, loose-clustered clone of Vignoles that will contribute to an integrated approach to disease control. Following gamma radiation, irradiated buds and non-irradiated controls were bench grafted onto 3309C rootstock. A total of 2,336 vines, including 30 non-mutagenized Vignoles control vines, were planted in 2007 and 2008 at the Cornell Lake Erie Research and Extension Laboratory (Portland, New York) in the Lake Erie shore grape and wine production region. Screening in the population of clones has identified several clones with looser clusters than control, non-mutagenized Vignoles vines.

Novel root-knot nematode resistance allele identified in grape rootstock germplasm:

An accession of *Vitis nesbittiana* resistant to *N*-virulent nematode strains was identified and progeny testing determined that the accession is heterozygous for a single dominant resistance allele with specificity different from that of *N*. We hypothesized that the new allele is found at a distinct resistance locus and is not an allele of the *N* gene. The relationship of the novel allele, tentatively *R*, to *N* was determined using progeny testing. The resistant accession (genotype *nnRr*) was crossed with the rootstocks Freedom and Harmony, homozygous for the *N* allele (*NNrr*) and susceptible to *N*-virulent nematodes. Progeny from the Freedom x *V. nesbittiana* cross were screened with *N*-virulent nematodes and resistant seedlings (genotype *NnRr*) were selected. Pollen was collected from flowering resistant seedlings and used to make test crosses with a nematode-susceptible female, the rootstock 161-49C (*nnrr*). Test cross progeny (*NnRr* x *nnrr*) were screened with *N*-avirulent nematodes and segregated 3:1 for resistance, indicating that *N* and *R* are non-allelic and represent two distinct resistance loci. This knowledge will facilitate the use of the resistant *V. nesbittiana* accession in breeding to produce new rootstock cultivars resistant to *N*-virulent nematodes.

**TECHNOLOGY TRANSFER/OUTREACH:**

- Segregation of resistance to root-knot nematodes in a grape rootstock germplasm hybrid population. Oral presentation, American Society for Enology and Viticulture-Eastern Section annual meeting, Geneva, NY, July 2010.
- A root-knot nematode resistance allele from *Vitis cordifolia* is not allelic to the *N* nematode resistance allele. Oral presentation, American Society for Enology and Viticulture national conference, Seattle, WA, June 2010.
- Vignoles Clonal Selection. Lake Erie Regional Grape Program Summer Growers Conference, Cornell Lake Erie Research & Extension Laboratory, Portland, NY, July 2010.
- Breeding and evaluating improved root-knot nematode resistant rootstocks. University of California Kearney Grape Day, Kearney Agricultural Center, Parlier, CA, August 2009.
- Evaluation of modern rootstocks for Concord grapevines in the Lake Erie production region. Poster presentation, GiESCO Symposium, Davis, CA, July 2009.
- Quantitative genetics of root-knot nematode resistance in a *Vitis* rootstock hybrid population. Oral presentation, American Society for Enology and Viticulture annual meeting, Napa, CA, June 2009.
- Rootstock Use and Evaluation in Eastern North America. Invited oral presentation, Rootstock Symposium of the American Society for Enology and Viticulture 2009 annual meeting, Napa, CA, June 2009.
- Using Rootstocks to Enhance Grape and Wine Quality and Vineyard Sustainable Practices. Keynote Viticulture Presentation, Texas Viticulture and Enology Research Symposium, Granbury, TX, June, 2009.
- Rootstocks for Nematode Management. Washington Association of Wine Grape Growers, Kennewick, WA, February 2009.
- Grapevine rootstocks: What they are, what they contribute, and how we are improving them. A Day with Grape Germplasm Resources at Geneva, New York State Agricultural Experiment Station, Cornell Cooperative Extension, Geneva, NY, August 2008.
- USDA ARS Research on Grapevine Rootstock Influence on Pierce's Disease, Rio Grande Valley Grape Growing Field Day, Rio Farms, Monte Alto, Texas, June 2008.

- Rootstocks: What are Their Capabilities and Limitations? Lake Erie Regional Grape Growers Conference, Fredonia, NY, March 2008.
- New rootstocks for San Joaquin Valley vineyards. San Joaquin Valley Grape Symposium, Easton, CA, January 2008.
- USDA ARS Rootstock Breeding Program: Vineyard Trials Update. Kearney Grape Day, University of California Kearney Agricultural Center, Parlier, CA, August 2007.
- Co-hosted one-day GGRU and Grape Industry Workshop with National Grape Cooperative Association/Welch's in Dunkirk, NY, in December, 2007.
- Hosted GGRU Focus Group meetings May 2007 and October 2009 in Geneva, NY.
- Partnered with Cornell University in hosting various field tours, grape growers' meetings, and other outreach activities.
- Molecular markers developed by ARS Geneva for powdery mildew resistance were applied along with markers for seedlessness when appropriate across 2,000 breeding lines in New York and California grape breeding programs.

**EXTERNAL SUPPORT:**

- American Vineyard Foundation and California Table Grape Commission: Durable powdery mildew disease resistance in *Vitis vinifera*: proof-of-function and discovery of genetic variation. \$70,000
- Viticulture Consortium East and NY Wine and Grape Foundation: Silencing recessive disease resistance genes in *Vitis vinifera* for the development of durably resistant cultivars. \$52,912
- Viticulture Consortium East: Targeting genomic elements of grape powdery mildew to manage disease: \$95,300
- California Table Grape Commission: Next generation markers to accelerate grape cultivar improvement. \$10,000
- Viticulture Consortium East (2010): Improve Concord Grape Variety Through Mutation Discovery. \$16,660
- Viticulture Consortium East, New York Wine and Grape Foundation: Quality Improvement in Vignoles through Clonal Selection (2007-2010): \$10,000
- Breeding Rootstocks Resistant to Aggressive Nematodes: Funded cooperatively by: American Vineyard Foundation, California Table Grape Commission, and California Raisin Marketing Board since 2010; funded by California Grape Rootstock Improvement Commission (2010) and California Grape Rootstock Research Foundation (2008) \$88,000 for 2010.

**COLLABORATORS:**

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**RECENT PUBLICATIONS:**

- Garris, A., Cousins, P., Ramming, D. and Baldo, A., 2009. Parentage analysis of Freedom rootstock. *American Journal of Enology and Viticulture* 60:357-361.
- Moyer, M.M., Gadoury, D.M., Cadle-Davidson, L., Dry, I.B., Wilcox, W.F., and Seem, R.C. 2010. Effects of acute low temperature events on development of *Erysiphe necator* and susceptibility of *Vitis vinifera*. *Phytopathology*, *in press*.
- Cadle-Davidson, L., Wakefield, L., Seem, R.C., and Gadoury, D.M. 2010. Specific isolation of RNA from the grape powdery mildew pathogen *Erysiphe necator*, an epiphytic, obligate parasite. *Journal of Phytopathology* 158:69-71.
- Cadle-Davidson, L. 2008. Variation within and between *Vitis* species for foliar resistance to the downy mildew pathogen *Plasmopara viticola*. *Plant Disease* 92:1577-1584.
- Cadle-Davidson, L. 2008. Monitoring the quiescence and activation of *Botrytis cinerea* in developing grape berries. *American Journal of Enology and Viticulture* 59: 387-395.
- Gee, C.T., Gadoury, D.M, and Cadle-Davidson, L. 2008. Ontogenic resistance to *Uncinula necator* varies by genotype and tissue-type in a diverse collection of grapevine (*Vitis spp.*). *Plant Disease* 92: 1067-1073.
- Zheng P, Allen W, Roesler K, Williams M, Zhang S, Li J, Glassman K, Ranch J, Nubel D, Solawetz W, Bhatramakki D, Llaca V, Deschamps S, Zhong GY, Tarczynski M, Shen B. (2008) A phenylalanine in DGAT is a key determinant of oil content and composition in maize. *Nature Genetics* 40:367-372.
- Myles S, Chia J-M, Hurwitz B, Simon C, Zhong GY, Buckler E, Ware D (2010) Rapid Genomic Characterization of the Genus *Vitis*. *PLoS ONE* 5: e8219