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# Contributions of the *Vitis*Gen2 project to grapevine breeding and genetics

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## Summary

The *Vitis*Gen projects (2011-2022) have improved the tools available for breeding new grapevine cultivars with regional adaptation, high quality, and disease resistance. *Vitis*Gen2 (the second project in the series) was a multi-state collaboration (USDA-Geneva, New York; University of California, Davis; USDA-Parlier, California; Cornell University; Missouri State University; University of Minnesota; South Dakota State University; Washington State University; North Dakota State University; and E&J Gallo, California) to develop improved genetic mapping technology; to identify useful DNA marker-trait associations; and to incorporate marker-assisted selection (MAS) into breeding programs. A novel genetic mapping platform (rhAmpSeq) now provides 2000 + markers that are transferable across the *Vitis* genus. rhAmpSeq has been used in California, New York, Missouri, and South Dakota to identify new QTL for powdery and downy mildew resistance. In addition, fruit/flower traits that would normally take years to phenotype have been associated with predictive markers accessible from seedling DNA (e.g. malate metabolism, anthocyanin acylation, bloom phenology and flower sex). Since 2011, the project has used MAS to screen thousands of grape seedlings from public breeding programs in the United States and has produced "RenStack" public domain lines to enable simultaneous access to 4 or 6 powdery mildew resistance loci from single source genotypes. High-throughput phenotyping for powdery and downy mildew resistance has been revolutionized with the Blackbird automated-imaging system powered by artificial intelligence for image analysis. Affordable DNA sequencing along with phenotyping innovations are transforming grapevine breeding.

## Keywords

***Vitis*, breeding, marker-assisted selection, QTL, disease resistance, insect resistance, phenotyping, molecular markers**

## Introduction

The *Vitis*Gen projects started in 2011 with funding from the US Department of Agriculture, National Institute of Food and Agriculture, Specialty Crop Research Initiative (SCRI). Matching funds from industry (E&J Gallo and the National Grape Research Alliance) and university participants were required to match $4.5 million in SCRI funding through 2016. The five-year *Vitis*Gen2 project started in September 2017 and was funded with $6.55 million in support of its breeding, genetics, economics, and extension objectives. A team of 25 investigators across ten institutions was advised by an Industry/Scientific Advisory Panel, providing valuable feedback over the course of a complex project. The *Vitis*Gen2 project focused on the "Application of next-generation technology to accelerate grapevine improvement" (see https://vitisgen.org). There were six teams, each with a Team Leader: Genetics (J. Londo); Breeding and Local Phenotyping (B. Reisch); Powdery Mildew (L. Cadle-Davidson); Fruit Quality (G. Sacks); Trait Economics (J. Alston); and Extension (T. Martinson). A Postdoctoral Associate level Project Manager (U. Ikeogu) facilitated research coordination, group meetings, reports, and publication activities, while also contributing to the overall research effort. This paper will focus on some of the most important accomplishments of the Genetics, Breeding, Powdery Mildew (PM) and Fruit Quality Teams, especially those most relevant to breeding and genetics of *Vitis* spp.

### *Molecular markers and mapping technology*

To associate important traits with chromosomal locations that affect those traits, genetic mapping technology with molecular markers is an invaluable tool. In the early stages of *Vitis*Gen2, the Genetics team developed rhAmpSeq haplotype mapping procedures (Zou *et al.*, 2020) that were implemented in grape breeding and germplasm collection programs over the course of the project. The team started with genome sequences derived from 10 genomes representing *V. vinifera*, interspecific hybrids, and North American species. A core genome was developed based on regions that were found among all genomes examined. These were used to de-



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velop 2,000 unique markers physically distributed across all 19 chromosomes and spanning 99% of the grape genome. Across four test families representing a broad range of *Vitis* breeding parents, 92% of the markers worked.

Each marker resulted from a two-amplification process followed by Illumina sequencing yielding 2x150 bp reads. Each allele was defined as a specific series of Single Nucleotide Polymorphisms (SNPs) within the sequence read. So, each allele consisted of a haplotype of multiple SNP markers.

## *Genetics of flower sex and broad implementation of marker-assisted selection*

Collaborative efforts across the Genetics and Breeding Teams led to research further illuminating the genetics of grapevine flower sex. Sequence data from the *Vitis*Gen2 project were evaluated, along with sequence data already published, to closely examine the sequence of the flower sex locus among cultivars, as well as species from North America, Europe, and Asia (Zou *et al.*, 2021). Emergence of self-fertile vines with perfect flowers played a central role in the domestication of *V. vinifera*. Wild grapevines have either pistillate or staminate flowers, but having perfect flowers eliminates the need for a nearby pollinator and produces well-filled clusters on productive vines. We identified two distinct H (hermaphrodite) alleles, *H1* and *H2*, suggesting that there were at least two distinct domestication events. From a grape breeder's perspective, the work reported by Zou *et al.* (2021) also resulted in a robust set of genetic markers to predict flower sex (both genotype and phenotype) from young seedling DNA across a broad range of *Vitis* germplasm. This shaves years off the time it otherwise takes to identify flower types in breeding programs.

Through the course of the *Vitis*Gen2 project, all participating breeding programs (New York, Minnesota, California, North Dakota, and Missouri) carried out marker-assisted selection. Markers identified by the *Vitis*Gen2 project along with others published elsewhere were converted to rhAmpSeq marker sets, which were applied to new breeding populations following validation.

## *High-throughput phenotyping of disease resistance*

A major effort of the Powdery Mildew Team resulted in a robotic, nondestructive technique to evaluate PM resistance (Bierman *et al.*, 2019). Each robotic imaging system has the capacity to image 3,000 leaf disks per day at 1.2 µm resolution and can document growth of PM hyphae over the course of a 10-day period. Reactions of leaf tissue to separate PM strains can also be documented with accuracy. Artificial intelligence methods have been employed to quantify disease severity (Qiu *et al.*, 2022).

## *RenStack selections with multiple loci for PM resistance*

To enable the grape breeding and genetics community to access multiple genes for disease resistance, two crosses were made by Breeding Team members; one resulted in four sibling vines harboring four loci for PM resistance (REN1, RUN1, REN6 and REN7); the other resulted in four vines harboring six loci for PM resistance (REN1, RUN1, REN3, REN4, REN9 and REN10). These eight genotypes were shared with Foundation Plant Services, Davis, CA (fps.ucdavis.edu). Once vines have been virus-checked and attain sufficient maturity, they will be available without intellectual property protection for domestic distribution of cuttings and pollen (Martinson *et al.*, 2023).

## *Newly discovered loci for resistance and fruit quality*

The *Vitis*Gen2 projects in mapping and phenotyping resulted in the identification of new loci governing biotic stress resistances as well as fruit quality. A multiyear study identified the *REN11* locus from *V. aestivalis* (Karn *et al.*, 2021b). This Chr15 locus confers effective resistance to PM on leaves, rachises, berries, and often on stems. Another major locus for PM resistance, *REN12* from *V. amurensis*, was discovered by the Powdery Mildew and Breeding Teams (Cadle-Davidson, Ledbetter *et al.*, unpublished).

The PM phenotyping robot proved to be highly useful in quantifying downy mildew (DM) growth as well. Efforts to phenotype this trait in segregating biparental populations resulted in the identification of previously unknown resistance loci from *V. amurensis* on chromosomes 10 and 13 (Sapkota *et al.*, unpublished). Loci for DM resistance were also identified from *V. X doaniana* – one (*RPV33*) controlled leaf resistance and the other controlled fruit resistance (Sapkota *et al.*, unpublished). Prior to the use of robotics to assess disease incidence and severity, computer imaging was used to identify five minor loci (*RPV17* to *RPV21*) for downy mildew resistance (Divilov *et al.*, 2017; Divilov *et al.*, 2018).

In studies led by investigators from the Univ. of Minnesota, a QTL for foliar phylloxera resistance (*Rdv3*) was mapped to Chr14 at 4.8 Mbp (Clark *et al.*, 2018). Markers that could be employed for marker-assisted selection were developed, and candidate resistance genes at *Rdv3* were identified (Yin *et al.*, 2022).

When *Vitis*Gen2 resources turned to studies in fruit quality, environmental effects on trait variation likely caused difficulties in delineating the inheritance of secondary metabolites. Yet some advances were made, notably related to acylation of anthocyanin pigments; hexenal quantities and related "grassy"-smelling odorants; and malic acid. Acylated anthocyanins are more stable than non-acylated anthocyanins in juices and wines. In a multi-year study characterizing anthocyanin acylation in *Vitis* spp. populations in New York, a major locus was identified at 15.85 Mbp of Chr3, explaining up to 85% of the phenotypic variance (Karn *et al.*, 2021a). With this information, rhAmpSeq markers were developed and implemented in breeding programs. Another success from the Fruit Quality Team resulted in the identification of a locus on Chr2 influencing levels of hexenals (Alahakoon *et al.*, 2022). These six-carbon compounds are responsible for grassy odors in some hybrid grapes, so the possibility now exists to devel-

op markers that enable the pre-selection of seedlings with low levels of hexenals. This same study also investigated genetic controls of malic acid, the major contributor to acidity and sourness in wild *Vitis spp*. The *Vitis*Gen2 team determined that in contrast to *vinifera*, malic acid in wild *Vitis* is not degraded (Burzynski-Chang *et al.*, 2020). Follow-up work using five years of data from a mapping population (Reshef *et al.*, 2022) identified significant QTL on Chr1, 7, and 17, but determined that these were not transferable to other populations. These results, along with a review of past literature, led to the conclusion that there are diverse physiological mechanisms that regulate the levels of malate, and associated loci are reported on all 19 chromosomes.

### *VitisGen2 teamwork enabled discoveries contributing to grape cultivar development*

The *Vitis*Gen2 project resulted in significant advances in grapevine genetics, supported by next-gen sequencing technology, new chromosome mapping strategies, and advances in high-throughput phenotyping. Contributory factors leading to project success included the following: long-term funding of investigators with complementary skills (pathology, breeding, genetics, genomics, computational biology, chemistry, enology, etc.); dedication to teamwork; creative and thoughtful input from postdoctoral associates, students, and staff; and Advisory Panel input from science as well as industry perspectives. To continue the momentum that began with the first *Vitis*Gen project in 2011, a *Vitis*Gen3 project entitled "Completing the grapevine powdery mildew resistance pipeline: From genes-on-the-shelf to sticks-in-the-ground" (see https://vitisgen3.umn.edu/) was funded and work began in September 2022. This project was awarded $10 million for a four-year period.

A highly important goal of our work was to facilitate the improvement of technology available for the development of new cultivars that will be needed to reduce pesticide inputs, enable sustainable grape production, and support resilience to climate change while still producing fruit of superior quality. To this end, our team members have employed new technologies to produce new selections and potential new cultivars, some of which are described here: https://www.vitisgen2.org/home-2/grape-selections-from-the-vitisgen-and-vitisgen2-projects/. Grapevine cultivar releases still require 12 to 25 years from crossing to release, but the process now used is much more efficient due to *Vitis*Gen2 and other projects around the world. There are many potential cultivars in the pipeline that will hopefully contribute to the future of the wine and grape industries.

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## Conflicts of interest

The authors declare that they do not have any conflicts of interest.

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