

The US approach to accelerated grape cultivar development

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Abstract

Grape breeding efforts have resulted in numerous improved wine, table, raisin and rootstock varieties, but are complicated by long generation times, large plant size, and the requirement for a perennial infrastructure. To enable early selection of elite seedlings combining disease resistance, stress tolerance, and fruit quality, U.S. grape breeders and geneticists developed a coordinated strategy for DNA marker discovery and application in a project involving 25 principal investigators. The approach uses centralized trait analysis and high-resolution genetic map development via a Next Generation Sequencing technology known as "genotyping-by-sequencing" (GBS). In this new project (known as "VitisGen"), over 7,000 breeding lines were genotyped in the first year to track alleles introgressed from eleven Vitis species. Our current approach to the analysis of GBS marker data results in the mapping of up to 20,000 genetic markers per population, including alleles not present in the V. vinifera reference genome. These dense genetic maps combined with centralized trait analysis will lead to the development of at least 30 marker sets for alleles controlling traits of current and future interest for grapevine improvement. In addition to the cutting-edge GBS markers, traditional SSR marker analyses contributed to marker-assisted breeding (MAB) efforts in the first year, as the project works to develop direct MAB decisions from GBS data. Together, the 25 principal investigators are seeking to maximize the impact of what is to become an unprecedented level of genetic mapping data relevant to grapevine improvement. Additional information can be found at <http://www.vitisgen.org>.

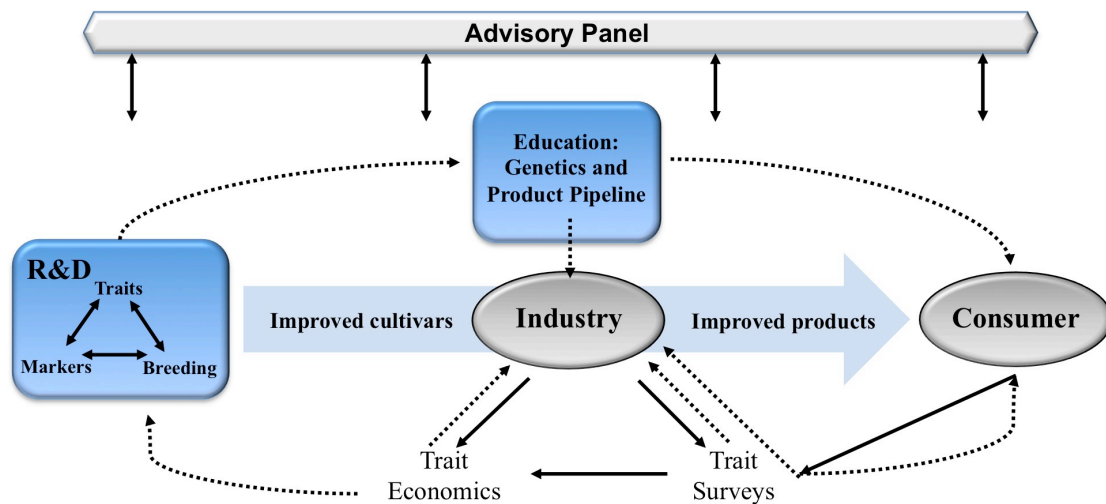
Introduction

Grapevine improvement programs in the United States have until now acted as separate programs, each with their own regional and crop specific goals and objectives. North American breeders meet every other year to share research progress and results, and have often tested each others' selections and shared pollen in the past. However, every program has been run independently and with separate funding streams. This situation has changed substantially with the initiation of the *VitisGen* Project. This project was launched in September 2011 with five years of grant funding from the USDA-National Institute of Food and Agriculture - Specialty Crops Research Initiative. *VitisGen* is a collaborative effort among 11 research institutions (Table 1) and is supported by an Industry Advisory Panel with 25 grape growers, winemakers, processor representatives and others from private industry (Figure 1).

Table 1. The 11 research institutions involved in the USDA-ARS Specialty Crops Research Initiative project known as “VitisGen”

Cornell University
 United States Department of Agriculture - Agricultural Research Service
 University of Minnesota
 South Dakota State University
 Florida A&M University
 Missouri State University
 University of California, Davis
 Oklahoma State University
 Oklahoma City University
 Mississippi State University
 Dalhousie University

Figure 1. VitisGen Project Workflow.



Overview

The vision for the *VitisGen* Project has four primary elements:

- To identify high priority vine performance and fruit quality traits with documented economic value to the grape industry and to the consumer;
- To discover, identify, and improve high priority traits using both traditional and modern biological approaches;
- To implement this strategy through development of molecular trait markers and improved grape varieties; and
- To enhance communication regarding the value of improved knowledge of grape genomics, new varieties, new technologies, and evolving needs of the grape industry and consumers.

So what does all of that really mean? In a nutshell, we are trying to develop new genetic (DNA-based) markers that indicate the presence or absence of genes controlling traits

such as powdery mildew resistance, low temperature responses, and various fruit quality characteristics. These genetic markers can be used to identify or select plants as seedlings that will retain high priority traits. This will help to speed up the breeding and evaluation process, so these traits can be incorporated into new grape varieties more quickly, benefiting both consumers and the grape industry. The US grape industry, particularly in areas where *V. vinifera* grapes are difficult to grow, is not at all resistant to trying entirely new varieties. Consumers are willing to try them, and wine-producing industries in some regions rely heavily upon grapes developed for areas with extremely cold winters, or areas with unusually high disease pressure. So whatever can be done to accelerate the identification and development of new varieties has the potential for great economic impact.

Much of the work of the VitisGen project is focused upon 19 different “mapping populations”. These populations are groups of full-sibling seedlings resulting from matings made by various breeders in the project. Each mating was done with certain strategic goals in mind, such as to study the genetics of disease resistance, nematode resistance, or low temperature response. By creating genetic maps from each population while at the same time characterizing traits of interest for each seedling, the project intends to develop a solid base of information on the association of genetic markers with genes affecting traits of interest to breeders.

A five-member Executive Committee, each of whom leads one of five project teams, heads the VitisGen Project:

Dr. Bruce Reisch leads the *Breeding Team*, which includes nine breeders from six states (California, South Dakota, Missouri, Minnesota, Florida, and New York). The major roles of this team are to maintain the plants that make up the *VitisGen* mapping populations and to provide this plant material to the Genetics Team, which is responsible for the genetic analysis of the plants, and to the Trait Evaluation Team, which evaluates plants for traits of interest. Altogether, 12 *Vitis* species are represented among the populations maintained by the breeding team. In the project's first year, the Breeding Team submitted a combined total of samples from more than 7,000 vines to the Genetics and Trait Evaluation Teams. Locally, breeders are also evaluating traits such as flower type; flowering time; resistance to various fungal diseases, foliar phylloxera, and nematodes; and several fruit attributes (e.g., berry size, berry shape, skin color, and seedlessness).

Dr. Anne Fennell of South Dakota State University leads the *Trait Evaluation Team*. The team has established three “centers,” or sets of scientists in different locations, to evaluate the mapping populations for low temperature responses, powdery mildew resistance, and fruit quality. The group focused on low temperature responses is based in South Dakota, and measures freezing tolerance, chilling fulfillment, and the rate of budbreak.

The powdery mildew center is based at Cornell University and USDA-ARS. This group maintains a genetically diverse collection of grape powdery mildew strains and looks at how effective plants with different genetic profiles are at preventing fungal infection (Figure 2). Leaf tissues are infected with a single strain of powdery mildew, and the response of each leaf to the fungus is characterized at the microscopic level.



Figure 2. Powdery mildew infected leaf.

Grape varieties that can resist disease and tolerate low temperature stress often have undesirable aromas and flavors. The scientists at the fruit quality center, also based at Cornell University, are working on how characteristics like negative aroma and flavor compounds, organic acids, and other undesirable characteristics in fruit are influenced by genetics.

Dr. Lance Cadle-Davidson of the USDA-ARS Grape Genetics Research Unit leads the *Genetics Team*, which is centered at Cornell University. This group is able to take advantage of advanced laboratory and computational facilities such as the Cornell University Biotechnology Resource Center, the Institute for Genomic Diversity, and the Bioinformatics Facility. The Genetics Team includes molecular biologists, plant geneticists, and computational biologists, and is using a new technology called



Figure 3. Bruce Reisch (right) and Steve Luce harvest leaf samples from seedlings to submit to the genotyping center

genotyping-by-sequencing (GBS) to discover new genetic markers that are closely associated with genes controlling certain traits. In just the first year of the project, the Genetics Team processed 7,200 GBS samples, generating more than one billion data points. In addition to generating this large amount of data for new markers, the genetics team also generated data for genetic markers already known to be associated with traits (e.g. genes for powdery mildew resistance, downy mildew resistance, flower sex and seedlessness) that breeders were using (Figure 3). This dataset was returned to breeders and is already being used to improve speed and efficiency in grape breeding programs.

Dr. Julian Alston of the University of California, Davis, is the lead for the *Trait Economics Team*, with a primary focus to identify top priority traits and document their value. To accomplish this, the trait economics team is conducting research on the value of disease resistance in viticulture and is developing surveys for grape breeders, growers, industry, and consumers. Thus far, the research has concentrated on Pierce's disease and powdery mildew disease resistance. Determining the value of powdery mildew resistance in viticulture is twofold. First, the prevalence of powdery mildew must be identified across different segments of industry and the country. Second, the consequences in terms of yield losses, investment in preventative efforts, or other costs imposed are determined. The first *VitisGen* survey was aimed toward identifying grape breeder's perceived priorities in grape genetics research. Future surveys are being developed for grape growers, industry members, and consumers.

The Extension and Outreach Team is led by Hans Walter-Peterson from Cornell Cooperative Extension. This team is responsible for educating consumers and the industry about the project and the benefits that this work will have for both the industry and consumers. So far, the group has developed publicity materials for the project, including a logo and project brochure, and is working on a new website for the project as well. In the near future, the group will be developing more materials highlighting certain aspects and accomplishments of the project, including online videos, webinars, newsletters, glossaries (Table 2) and more.

Table 2. A glossary of terms used to describe the VitisGen project

Genetic markers: pieces of DNA with a known location on a chromosome

Molecular-trait markers: genetic markers linked to traits

Genomics: the study of genomes or an organism's complete hereditary information

Genotype: the genetic make-up of an organism

Genotyping-by-sequencing (GBS): a next-generation DNA de-coding technology used to analyze an organism's DNA

Phenotype: the observable characteristics of an individual organism

Progeny: offspring

Mapping population: a group of related organisms used to construct a genetic map

Conclusion

How do all five of the teams work together? The breeding, trait evaluation, and genetics teams function together as a research and development unit. The trait evaluation and genetic data are integrated to generate new trait-associated markers. Breeders use these markers to screen progeny and discard those that do not have the desired trait(s). This reduces the overall costs related to vineyard establishment and vine evaluation. The trait economics team identifies top priority traits through breeder, grower, industry, and consumer surveys, which help to steer the focus of the research and development unit. The extension and outreach team communicates with industry and consumers to provide education about new technologies and genomic resources. Overseeing the entire project is an Industry Advisory Panel, which provides guidance and non-federal matching funds to support the *VitisGen* Project. This type of collaborative effort will result in the development of new tools and techniques that will lead the way in developing the next generation of grape cultivars.

For more details on the VitisGen Project, visit the project's website at <http://www.vitisgen.org>.