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RESEARCH FOCUS

VitisGen: Mapping the Way to the Next Generation of Grapes

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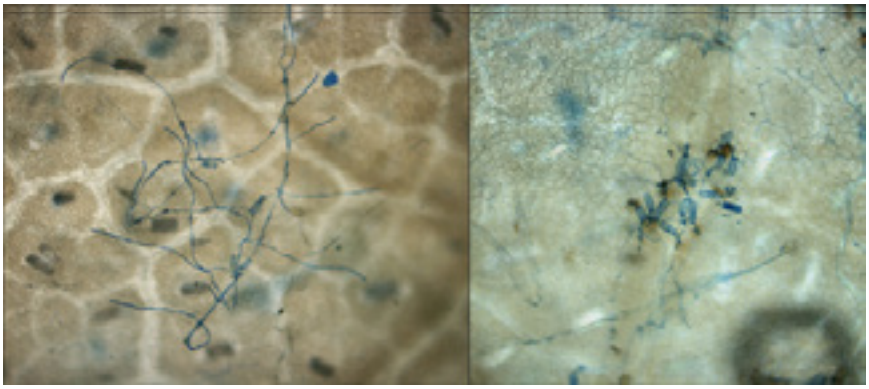


Powdery mildew spores on leaf tissue from vines that are susceptible (left) and resistant (right) to infection. The presence of hyphae, the thin strands extending from the spore on the left, indicates that the fungus extensively infected the leaf. Very short hyphae developed from spores on the resistant leaf on the right. Evaluating powdery mildew resistance and developing DNA markers for resistance genes is a major goal of the USDA-supported VitisGen project.

Photo by Jim Monahan, Finger Lakes Grape Program, Cornell University

The process of evaluating potential new grape cultivars can take a long time – in some cases, over 30 years can pass between the time the initial cross is made and when a variety is released for commercial use. *VitisGen* is a multi-disciplinary collaborative project focused on decreasing the effort and cost involved in developing the next generation of grapes. It will allow researchers to focus on selecting elite seedlings already harboring important traits from the start. Led by Bruce Reisch, professor of horticulture at Cornell University's New York State Agricultural Experiment Station in Geneva, NY, *VitisGen* incorporates cutting edge genomics technology, precision measurement of traits, and economic research into the traditional grape breeding and evaluation process. This research aims to accelerate the ability to identify genes related to high value traits like disease resistance and fruit quality. Identifying genes related to these traits and others will help grape breeding programs develop new varieties more efficiently that will appeal to a wide range of consumers, while also addressing the needs of growers and processors.

KEY CONCEPTS

- The *VitisGen* project is a national, collaborative effort by grape breeders and geneticists to identify and implement genetic markers for marker-assisted selection of wine and table grapes.
- *VitisGen* researchers aim to identify markers and incorporate genes for disease resistance, cold-hardiness, and fruit quality into new varieties of grapes.
- Grapevine breeding proceeds slowly because of the time, space and costs of producing mature vines that can be evaluated in the field and winery.
- The number of progeny a breeder can evaluate is limited. Many progeny fail to have the combination of traits desired by the breeder, and thus are discarded.
- Modern genetic tools such as DNA markers allow grape breeders to test grapevines at the seedling stage, before going to the expense of growing them in the field.
- This process, called marker-assisted selection, allows breeders to know, for the first time, which specific genes are present in each seedling.
- *Marker-assisted selection* will accelerate development of improved grape varieties by allowing breeders to discard more seedlings and concentrate further evaluation on only those vines with appropriate traits.
- *Marker-assisted selection* allows breeders for the first time to identify seedlings with multiple powdery mildew resistance genes, to improve a vine's efficacy, longevity of resistance, as well as reduce the need for pesticides.

Introduction. Launched in 2011 with a grant from the National Institute of Food and Agriculture Specialty Crop Research Initiative (NIFA-SCRI), *VitisGen* marks an important advance in conventional grape breeding programs. This five-year project is accelerating the discovery of new grape varieties with desirable qualities and traits preferred by consumers of raisins, wine, juice and table grapes. In consultation with both industry and the grape genetics and breeding communities, *VitisGen* identified three priority traits: resistance to powdery mildew, improved low temperature responses (e.g., better winter hardiness, delayed budbreak to avoid spring frosts) and key fruit quality characteristics. This project brings together 24 scientists from 11 different institutions across the United States and Canada, developing scientific resources that will allow both scientists and breeders to address topics of regional significance.

The VitisGen Project Team. *Led by Cornell professor Bruce Reisch, VitisGen involves 25 scientists from 10 institutions in the US and Canada. Participants based at Cornell and USDA ARS units at Geneva and Ithaca include: Bruce Reisch and Beth Takacs (Horticulture); Hans Walter-Peterson (Extension), David Gadoury, Bob Seem, Wayne Wilcox (Plant Pathology), Gavin Sacks and Anna Katharine Mansfield (Food Science); and USDA grape germplasm research scientists (Geneva) Gan Yuan Zhong, Lance Cadle-Davidson, Jason Londo, Chris Owens, and Ed Buckler.*

The strategy for improving traditional plant breeding program efficiency is based on an intensive search for molecular markers. These markers will assist grape breeders with successful identification of grapevines harboring priority traits to include in their breeding programs. *VitisGen* is using Genotyping-by-Sequencing, a cutting-edge DNA sequencing technique that allows cost-effective, detailed mapping of the entire genome of each grapevine in a breeding program. This genomic data will be used to precisely develop new grapevines, while also providing grape breeders with significant information about the grapevines in their programs. By identifying DNA markers to use in a technique called marker-assisted selection, this process will remove much of the guesswork that has impeded traditional breeding programs, and also increase favorable outcomes.

This project is a collaboration among five complementary teams – Breeding, Genetics, Trait Evaluation, Trait Economics and Extension and Outreach. This integrated approach will enable *VitisGen* to meet the needs of breeders, growers, fruit processors and consumers by producing novel grape varieties beneficial to all in the grape industry.

Powdery mildew resistance: An example. Powdery mildew (PM), caused by Erysiphe necator, is the most common disease affecting both native and hybrid cultivated grapes worldwide. PM infects leaves, young berries, and other green tissues, leaving the crop unusable for quality grape and wine production. PM control is often considered the salient management practice in commercial grape production.

The Five *VitisGen* Teams

VitisGen teams are collaborating to create novel grape varieties with powdery mildew resistance, low temperature tolerance and fruit quality.

Breeding. This team produces new crosses and maintains existing breeding lines. By selecting existing grape lines with priority traits, discovered DNA markers guide identification of new germplasm for novel crosses. This process speeds up selection by allowing breeders to select only grape seedlings with desired traits. *Leader and Project Director: Bruce Reisch, Horticulture, Cornell.*

Genetics. The genotyping team develops genetic maps of promising grape breeding families and uses them to identify DNA markers with priority traits. To date, they have screened individuals from 62 families as well as individuals representing diverse grape traits. *Leader: Lance Cadle-Davidson, Grape Genetics Research Unit, USDA-ARS, Geneva, NY.*

Trait Evaluation. This team screens grapevines from the Breeding Team for priority traits. Each year, the team evaluates samples for PM resistance, low temperature response and fruit quality. Goals include developing standard, objective trait evaluation methods to use in conjunction with genetic maps to identify new DNA markers. *Leader: Anne Fennell, South Dakota State University.*

Trait Economics. This team's goal is to better understand economic implications by determining stakeholder interest and quantifying the economic benefits of utilizing priority traits. For example, the team evaluated returns from a PM resistant variety by analyzing how deploying resistant cultivars would change current production costs. *Leader: Julian Alston, UC-Davis.*

Extension and Outreach. This team communicates and shares project results with key stakeholders including producers, grape breeders and geneticists and consumers. They produced a website, an ongoing seasonal newsletter, and informational videos (For example "VitisGen - Breeding Crosses" and "*VitisGen* - Tracking Resistance"). *Leader: Hans Walter-Peterson, Cornell Cooperative Extension, Penn Yan, NY.*

In the U.S. alone, grape growers apply 31 million pounds of sulfur annually, as well as modern fungicides, to manage PM. Since the 1980s, a succession of modern fungicides have been used successfully in grape production, but their use is often limited to a handful of growing seasons, due to the rapid development of fungicide resistance by the PM fungus. Today's growers must be aware of fungicide resistance, avoid overuse of particular products, and rotate among PM fungicides with different modes of action.

High costs of PM management are compounded by the genetics of popular grape varieties. Most of today's V. vinifera grape varieties are derived from a small pool of PM-susceptible ancestors bred hundreds of years ago. Classic wine varieties such as Riesling, Chardonnay, and Cabernet Sauvignon were selected for attributes such as flavor or color, not for PM resistance. This is because the pathogen—native to North America--had not yet appeared in Europe. All of these varieties are highly susceptible to PM, however, many of the 30+ Vitis species present in North America evolved to co-exist with powdery mildew. Therefore, many sources of PM resistance are present within these wild species, but resistance needs to be combined with favorable flavor and aroma attributes. As modern cultivars with PM resistance are developed and released, their fruit quality must meet or exceed that of the classic varieties to gain marketplace acceptance.

Identifying powdery mildew resistance. Traditionally, searching for PM resistance is a costly and slow process. Breeders first needed to rigorously study the natural variation in PM resistance to select parents to be used to combine PM resistance with other desirable traits. Visually rating PM in the field, for example, can require several years of observation because environmental conditions vary. Breeders make numerous such crosses each year, and return when fruit are ripe to harvest the resulting berries and extract the seeds. These are germinated to produce seedlings in the following year, and seedlings are tested immediately for powdery mildew resistance under no-spray conditions. It takes 3-5 years from the time a cross is made until the new seedlings bear sufficient fruit for evaluation. For wine grapes, sample batches of wine must be evaluated over several years as well. Thus, the breeder may be faced with the expensive and time-consuming maintenance of many plants that may ultimately be undesirable.

For this reason, the *VitisGen* project is developing DNA markers (short sequences of DNA adjacent to genes of interest) that will allow breeders to test and select vines at the seedling stage, and discard those lacking DNA markers that indicate the presence of disease resistance genes (or fruit quality or other traits). This process, called *marker-assisted selection*, allows breeders to discard undesirable seedlings and concentrate on planting only the select group of seedlings with the desirable combination of characteristics.

Pyramiding powdery mildew resistance

Bruce Reisch has been breeding for PM resistance at Cornell for the past 25 years. But until molecular markers were made available, he had no way of knowing which genes were present in his PM resistant progeny. Now, with genetic markers available for numerous PM resistance genes (Table 1), he can go back to his 'library' of previous crosses (and released cultivars) and find out which genes are responsible for the observed resistance. Going forward, he can also evaluate seedlings genetically and combine several PM resistance genes into one seedling. This process, called gene stacking, will lead to selections that are likely to have more durable resistance, because they have several genetic sources of resistance.

Just as PM evolves quickly to overcome modern fungicides, it also evolves to overcome single resistance genes. For example, *Run1* is the most widely used PM resistance gene around the world – and one of the most effective. But within three years of planting in the Cornell breeding program, PM was already found growing and reproducing on *Run1* vines. Just as growers rotate different fungicides to maintain chemical efficacy, breeders combine, or stack, different resistance genes in a single vine to maintain genetic durability. There is no way to stack genes by simply testing vines for PM resistance - vines with one gene or five genes for resistance may all look nearly alike when tested for resistance. But DNA markers can tell us which vines have one, two, three, or more resistance genes. This is a major advantage for a breeding program – allowing us to do something not possible by traditional phenotyping techniques.

Table 1. Major powdery mildew resistance loci tracked in *VitisGen.*

Locus	Chromosome	Source of Resistance	Origin of resistant species
Ren1	13	*Vitis vinifera* cv. 'Kishmish vatkana'	Central Asia
Ren2	14	*Vitis cinerea*	North America
Ren3	15	'Regent' [1]	North America
Ren4	18	*Vitis romaneti*	Eastern Asia
Run1	12	*Vitis rotundifolia*	North America
Run2	18	*Vitis rotundifolia*	North America

[+1] Interspecific hybrid derived from 'Chambourcin', which descends from numerous North American Vitis species.

Phenotyping. While a vine's genes are the basis for its resistance or susceptibility, the expression of those genes affecting a plant's characteristics, known as the phenotype, is variable. The powdery mildew phenotyping process (see sidebar on page 5) starts with a few simple tools: a hole punch, a baking dish, and scores of spores. Leaf samples are harvested with the hole punch and then transferred to a controlled environment (otherwise known as a 9x13 glass baking dish), where they are each exposed to equal amounts of PM. PM growth is observed under a microscope to determine which individuals are resistant and which are susceptible. This process is labor-intensive and time-consuming, but it provides much more reliable observations than those from the field.

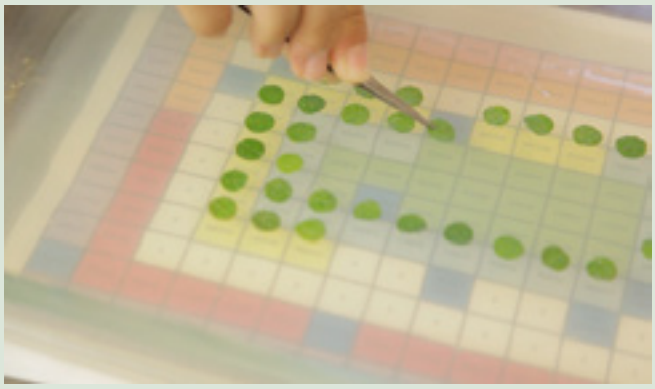
The Extension and Outreach Team produced a video entitled *VitisGen* - Tracking Resistance about how members of the powdery mildew phenotyping team, including Cornell scientist Beth Takacs and USDA scientist Lance Cadle-Davidson, evaluate disease resistance in the *VitisGen* project.

Phenotyping Grape Selections for Powdery Mildew Resistance

VitisGen researchers screen thousands of grape seedlings--progeny of breeding crosses or genetic 'family' studies--each year for powdery mildew resistance. Samples are sent to the genotyping team for genetic analysis and also to the trait analysis team to determine resistance of leaf tissue. Here is how the leaf tissue is evaluated for powdery mildew resistance:

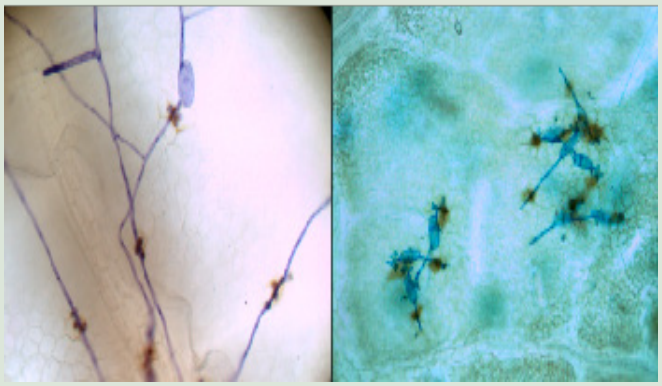
Step 1 *Leaf Disk Cutting*: Tissue samples of the same size are taken from leaves of each grapevine to test for resistance or susceptibility to powdery mildew infection.

Step 2 *Leaf Disk in Pyrex:* Each leaf sample is placed in a specific location in a glass dish, which can be traced to an individual vine in the vineyard.



Step 3 *Spore Suspension***:** Each sample is exposed to the same number of powdery mildew spores to make sure that the powdery mildew 'dose' each sample receives is equal in all samples.

Step 4 *Disease Rating***:** Each leaf disk is evaluated and scored for powdery mildew resistance. More susceptible (left) shows more growth than the leaf disc that is more resistant (right).



How *VitisGen* is helping. *VitisGen* is working to simplify and streamline the grape breeding process by greatly expanding the number of DNA markers for desirable traits available to grape breeders. This expanded range of DNA markers will allow breeders to 'stack' multiple genes for PM resistance into the same vine. A single resistance gene may more likely be overcome by a pathogen mutation, however two or more genes associated with resistance in the same vine should equate to longer-lasting resistance in the vineyard. There is no way to stack genes by simply testing vines for PM resistance as vines with one gene or five genes may appear equally resistant to PM infections. However, molecular markers can identify vines having one, two, three, or more resistance genes. This is a major advantage for a breeding program compared to traditional evaluation techniques. To date, scientists have identified ten different genes that independently confer PM resistance. By incorporating even a few of these genes into single plants, breeders may increase the chance that PM resistance will last for the life of the vineyard. Thus, these varieties could potentially result in a dramatic reduction in fungicide applications, while reducing the overall costs and environmental impacts of grape production.

Just as this technique of identifying DNA markers can be used to develop vines resistant to powdery mildew, additional markers for traits such as cold hardiness and grape quality can be incorporated into improved grapevine varieties at a quicker rate. Ultimately, *VitisGen* holds a promise to accelerate new variety development, leaving everyone from breeder to consumer more time to enjoy the fruits of their labor.

References

VitisGen Project Website: http://www.vitisgen.org

How Breeders Make Grapevine Crosses - https://www.youtube.com/watch?v=z-Pranxd9fw

Tracking Powdery Mildew Resistance - https://www.youtube.com/watch?v=eFSqfL946j4

VitisGen Publications: http://www.vitisgen.org/pubs.html

'*VitisGen* Voice' Newsletter (Issue 2) - http://www.vitisgen.org/docs/newsletter/Winter2013.pdf

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