Spring 2016

VitisGen Voice

Mapping the way to the next generation of grapes






Highlights from the 5th Annual VitisGen Project Meeting

By Elizabeth (Takacs) Demmings and Bruce Reisch

We hosted the fifth annual *Vitis*Gen meeting of Project Directors, Collaborators, and Advisory Panel Members January 7–8, 2016 in San Diego, California. Over the span of the two-day meeting, 29 *Vitis*Gen participants engaged in discussions, presentations, and plans for the future. The purpose of the annual meeting was to review the previous year's progress; to identify and prioritize action plans for the remainder of the funded project; and to encourage continued collaborations, dialogue, and feedback from our Advisory Panel. Additionally, we scheduled time to finalize plans for a *Vitis*Gen2 proposal, which builds upon the work accomplished during the *Vitis*Gen project. We will submit the *Vitis*Gen2 proposal to the USDA-NIFA Specialty Crops Research Initiative in mid-March.

On the first day of the meeting, Project Directors, Collaborators, and the Advisory Panel reviewed project accomplishments and discussed immediate future goals. Team reports and discussions (in breeding, genotyping, phenotyping, trait economics, and extension) focused on major accomplishments to date and underscored applied as well as more fundamental scientific impacts. Listed below are some of the key *Vitis*Gen achievements.

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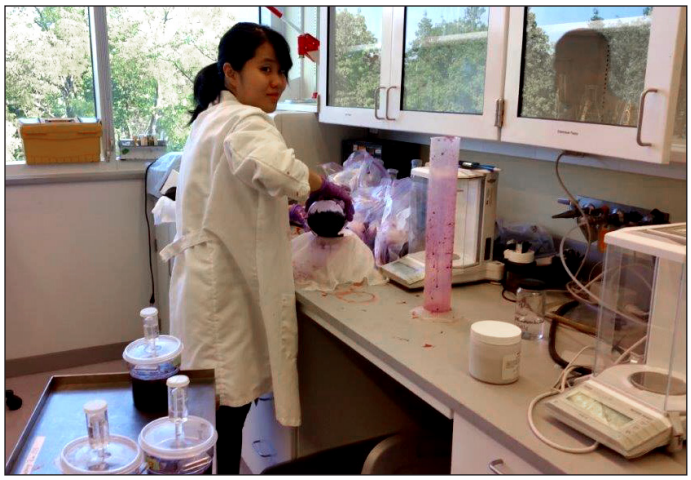
Funding for *Vitis*Gen "Accelerating grape cultivar improvement via phenotyping centers and next generation markers" is provided by a Specialty Crop Research Initiative Competitive Grant, Award No. 2011-51181-30635, of the USDA National Institute of Food and Agriculture.



Lei An Chen juicing samples in Gavin Sacks' lab.

- Implemented marker-assisted selection and marker-assisted parent selection in U.S. breeding programs (processing more than 16,000 seedlings and more than 2,000 genotypes representing germplasm and parental material)
- Identified more than 70 marker-trait associations by integrating data from the genotyping center, the three phenotyping centers, and from the local phenotyping effort within *Vitis*Gen breeding programs
- Demonstrated that juice protein concentration can model low tannin extractability during fermentation
- Produced economic models for benefits from trait improvements such as the adoption of powdery mildew resistant cultivars
- Evaluated the environmental costs of disease management
- Produced and released 5 YouTube videos:
  1) "*Vitis*Gen: How Grape Breeders Make Crosses,"
  2) "*Vitis*Gen: Researching Powdery Mildew Resistance,"
  3) "Industry Voices In Support of *Vitis*Gen,"
  4) "It's All About the Genes: Wine Flavor," and
  5) "It's All About the Genes: Color"
- Published 15 refereed journal articles
- Trained more than 60 visiting scientists, undergraduates, graduate students, and postdoctoral associates

We also hosted a series of short presentations to provide a more in-depth look at some of the research taking place within the scope of the *Vitis*Gen project. Drs. Craig Ledbetter and Rachel Naegele from the USDA-ARS at Parlier, CA gave a presentation detailing traits they evaluate for raisin and table grapes. A graduate student from the University of Minnesota, Soon Li Teh, presented on his dissertation research using "SNP"-based chromosome maps in interspecific half-sib grapevine families to look at the genetics of powdery mildew resistance. Konstantin Divilov, a graduate student from Cornell University, described an imaging approach to automate the evaluation of downy mildew resistance. Dr. Shanshan Yang, a former postdoctoral associate from Cornell University (now at Arizona State University), presented on how amplicon sequencing can be used as a novel approach for marker-assisted selection. Connor Fortin, a graduate student from the Rochester Institute of Technology, presented on advances he has made building a breeding management system or "toolbox" for grapevine breeders. Dr. Elizabeth (Takacs) Demmings, a postdoctoral associate at Cornell University and *Vitis*Gen project manager, presented the final talk on how *Vitis*Gen has helped to train more than 60 researchers over the past four-and-a-half-years.

The second day of the meeting started with discussions on developing the *Vitis*Gen2 proposal and finalizing the new objectives that build on the foundational research accomplished in *Vitis*Gen. To conclude the two-day meeting, the Advisory Panel provided feedback and delivered closing remarks. Following the end of the meeting, project participants gathered for informal discussions on finalizing *Vitis*Gen objectives and resolving plans for *Vitis*Gen2.

Overall, our fifth annual meeting proved to be an excellent opportunity to share ideas and reflect on the progress made over the past four-and-a-half years. We spent much time, both last year and this year, mapping out plans to continue to build upon the advances of the past few years. Our ultimate goal remains to make available to U.S. grape producers and processors new varieties with traits they value and would ultimately lower the cost of production; reduce the environmental impact; and increase the quality of products produced. ■

Deciphering the Mysterious Language of Color and Flavor

By Shanna Moore

Grape and wine flavor and color are important quality traits to growers, producers and consumers. *Vitis*Gen scientists are currently looking closer at color (anthocyanins) and flavor and mouthfeel (tannins) as a way to gain new insights into improving fruit quality.

Color

- The genetic makeup of a grapevine is the primary factor in the final color of the fruit, juice or wine.
- Anthocyanin compounds are the principle pigments responsible for the red color in certain fruit, and the juices and wine made from them.
- European grapes *(Vitis vinifera)* contain almost exclusively one type of anthocyanins (monomeric), and this type can react to make stable wine pigments that deliver the intense red wine color preferred by most consumers. Hybrid varieties, however, have lower proportions of this type of anthocyanins and thus have more variation in color.

Humans are highly visual, and the color of any product can greatly impact consumers' preferences. In red grapes and wines, pigmentation is primarily provided by anthocyanin compounds found in the thin skin of the grape. Anthocyanins are water soluble compounds that protect plants from damage due to ultraviolet light, and result in a wider range of colors including red, purple and blue. *Vitis vinifera* species (e.g., Cabernet Sauvignon) are generally characterized by a deep, rich red, purple, or burgundy color. However, hybrid grapes, which are valued for their adaptive growth habits, low temperature tolerance and broader pest and disease resistance, can often produce wines with more unusual colors—younger wines that are closer to deep purple that then become pale reddish brown after a few years. Colors like these can often diminish the appeal of these wines to consumers if they are not used to them.

"Humans are highly visual, and wine color greatly impacts consumer preferences in wine."

It has been long established that V. *vinifera* species have a high proportion of monoglucosides, or monomeric anthocyanins, which is unusual among grape species. During winemaking, especially in the first two years, monomeric anthocyanins undergo a wide variety of reactions and new pigments are formed. These new, or polymeric colors, act in stabilizing wine color, allowing richer colors to be maintained over time. Previous research has focused on color development and aging, and it was generally assumed that color in hybrid grapes would act similar to V. *vinifera* species. Hybrids, however, have more diglucosides. *Vitis*Gen scientists have shown that these compounds are unable, or less efficient, in forming polymeric pigments, and thus wines from hybrids have a broader range of colors and these may be less stable and change over time.

Accurately measuring anthocyanin compositions greatly improves the ability to select new grape vari-

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"By increasing our understanding of tannin production and retention, breeders can work to combine desirable growth and flavor characteristics in new varieties in a faster targeted manner."

eties that will result in robust, consumer pleasing wine color. The kinds and amounts of anthocyanins in red grapes largely depends on the grape varieties themselves. However, the growing conditions, such as viticulture practices and the regional weather, as well as factors of the winemaking process (such as pH, storage temperature, light exposure and the broader sugar, acid, and sulfite profiles), also ultimately contribute to wine color. By combining all of this information with other traits of interests, such as disease resistance and low temperature tolerance, growers can select the best new varieties for long-term production and consumer success.

Flavor

- Tannins are an important component of wine flavor, producing bitter and astringent notes, and contribute to a wine's "mouthfeel."
- Red wines produced from wild species and interspecific hybrids have significantly less tannins than red wines from V. *vinifera* grapes.
- *Vitis*Gen scientists have discovered that grape tannins bind to defense proteins during fermentation, and thus the tannin concentration measured in the final wines can be predicted by the tannins and defense proteins measured in the fruit prior to fermentation.

Tannins are naturally occurring compounds found in bark, wood, leaves, roots, seeds and fruit. They bind to other compounds—proteins, cellulose, and starches—to protect plants against infection and herbivore consumption. Tannins have historically been used in photography, fabric dyeing, medicine, and leather making. In addition, they are an important component of flavor, supplying bitter and astringent notes in black tea, dark chocolate, pomegranate, and red wine.

Tannins are found in grape skins and seeds, and, are extracted into wine during fermentation. However, the correlation between grape tannin and wine tannin can be very poor. Grape tannins can vary up to four times across cultivars, and in a recent study, wine tannin ranged over 50-fold, even when the same winemaking protocol is used. So, a high tannin grape may not result in a similar high tannin wine and thus far, a reasonable explanation for this has been elusive.

One focus of *Vitis*Gen's phenotyping team is to understand the factors that control tannin production and impact tannin concentration in wine. Recently, Lindsay Springer and Gavin Sacks demonstrated that red wines produced from wild species and interspecific hybrids (e.g., wild North American grapes crossed with European V. *vinifera*) have significantly lower tannin concentrations than red wines from V. *vinifera* grapes. Interestingly, these differences are only partially explained by the tannin concentration measured in the grapes themselves. Sacks and Springer discovered that, in wild grapes, and related crosses, a large amount of the tannins bind to proteins, which are often found in higher concentrations in wild species. Thus, by measuring the tannins *and* the defense proteins in fruit, the tannin concentration in a resulting wine can be accurately predicted.

This information allows for new avenues for increasing tannin concentrations in hybrid wine. It provides a target for both breeders and winemakers to measure, informing both new crosses for breeding and wine choice. For example, interspecific hybrids often have desirable growth properties, such as pest resistance or low temperature tolerance, but may also have low tannins resulting in low body or thin mouthfeel. By increasing our understanding of tannin production and retention, breeders can work to combine desirable growth and flavor characteristics in new varieties in a faster and more targeted manner. In addition, it may also lead to new approaches for protein removal for winemaking—both pre and post fermentation—to improve tannin retention.

This information also adds to our mechanistic understanding of *terroir*—the taste and character of a wine resulting from the environment in which it

Want to learn more about VitisGen work on flavor and color? Check out two new videos:

"It's All About the Genes: Wine Color"—Dr. Anna Katharine Mansfield discusses why wine color is so important to the perception and pleasure we have of wine. http://www.vitisgen.org/videos.html

"It's All About the Genes: Wine Flavor"—Dr. Gavin Sacks explains important flavor traits and how our knowledge of them can improve wine flavor and grape breeding. http://www.vitisgen.org/videos.html

is produced. It is well known that excessive rainfall during a growing season can impact red wine quality including aspects like mouthfeel, but a biochemical explanation for this phenomenon has been lacking. Disease pressure is greater under rainy conditions and disease pressure increases production of grape defense proteins, thus this higher level of protective proteins in the fruit will bind with more tannins in the must and therefore, decrease tannin extraction during fermentation.

Finally, this is an excellent example of the utility of a multifaceted project such as *Vitis*Gen. Tannin data can now ultimately inform both disease resistance and fruit quality, strengthening linkages in the broad platform of information that supports grape and wine research.

What comes next?

*Vitis*Gen 1 allowed for the study of quality traits in populations for a single harvest time point grown at a single location. While we developed great tools for breeders and genetic scientists, many key fruit quality traits—including the aforementioned tannin-binding proteins, as well as several vegetal off-aroma compounds and malic acid ("sourness")—change over the course of fruit ripening, and with different growing locations. Thus, *Vitis*Gen 2 will present the opportunity to study these traits of interest throughout ripening and for the same populations grown at multiple sites. Not only will this yield new genetic insights, it will also help breeders to better understand the genetic control of these traits. For example, a unique genetic variation may result in a grape variety that produces lower grape acidity only in hot environments or in long growing seasons, and this valuable variety would not be identified (or useful) in a shorter, cooler growing season. Hence, *Vitis*Gen 2 will provide the opportunity to look more in depth at traits of interest and build on the platform of information and resources established by *Vitis*Gen 1. ■

Genetic Marker Paves Way for New Cold-Climate Wines With Tamer Acidity and Drier Style

By Sarah Thompson

Excessive acidity is the Achilles heel of winemakers using cold-hardy hybrid grape varieties, requiring additional steps in the winery to make these wines palatable. Now, a team of scientists has identified a section of grape DNA that appears to be responsible for high accumulation of malic acid in these hybrid grapes before harvest. Further research is needed, but this initial discovery opens the door for breeders to use this marker to develop new cold-hardy grape varieties that can produce lower-acid, drier wines requiring less winemaking intervention.

This new trait-associated marker, as well as nearly 75 others identified over the past five years by the collaborative *Vitis*Gen research project, also allows grape breeders in cold- and short-season growing regions to increase the rate of innovation—circumventing delays caused by winter injury, and ensuring that their breeding pipeline is filled only with seedlings combining good growing and flavor properties.

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"We lose a lot of plant material early on, so we could never do these kinds of analyses. *Vitis*Gen has changed how we approach the breeding program, allowing us to commit to keeping experimental populations in place for longer to do genetic testing. There are so many questions but so many opportunities now. It's very exciting," said Matthew Clark, assistant professor, grape breeding and enology at the University of Minnesota (UMN) and head of UMN's grape breeding program.

The discovery of the malic acid marker was made possible by Professor Anne Fennell at South Dakota State University, who had the rare opportunity to maintain and study one experimental second generation (F2) breeding population for nearly a decade. As *Vitis*Gen team members, Fennell and her longtime collaborator James Luby, professor of horticulture at UMN, recognized the value of the genetic diversity in their F2 population—foresight that led to its inclusion in the *Vitis*Gen project.

"Short-season viticulture and winemaking is challenged on two ends: ripening grapes sufficiently and ensuring grapes survive the winter so they can make wine."

The First Rule of Viticulture

For breeders like Clark, and the wine regions that rely on the cold-hardy grape varieties his program develops, the ability to control malic acid levels before harvest would be a huge boon. Short-season viticulture and winemaking is challenged on two ends: ripening grapes sufficiently and ensuring grapes survive the winter so they can make wine.

This is why Gavin Sacks said that, regardless of climate, the first rule of viticulture is always "don't kill the grapevine."

According to Sacks, associate professor of food science at Cornell University, winemakers would ideally start with fruit having malic acid concentrations similar to those found in grape varieties in the species *Vitis vinifera*—the European grapes that account for more than 99 percent of wine production worldwide. But *vinifera* grapes are very sensitive to cold and have little to no natural resistance to the native pests and diseases of North America. Thankfully, there are wild varieties within the 60-odd species of the *Vitis* (grape) family that can tolerate cold and diseases; many of these are native to North America and several have formed the basis for cold-hardy hybrids—like the red grape 'Marquette', released in 2006 by UMN—and new wine industries in Minnesota, Wisconsin, and Vermont.

Yet keeping grapevines alive is only half the battle for Clark, who said that even though current cold-hardy hybrids survive winter, many are just one step removed from wild grapes and not ideal for winemaking because of their high acidity. For Sacks, Clark, and the industry, this downside is a major deterrent to adoption.

"The wild varieties that can do this [tolerate cold and disease] are not palatable for consumers. Wild grapes have acid concentrations similar to lemons or cranberries—sometimes three to five times the total acid found in *vinifera*. If the fruit is unacceptably sour, there won't be broad acceptance of wines made with these grapes," Sacks said.

Malic Acid Headache

Even though tartaric and malic acid are the predominant acids in grapes and wine, the sourness of cold-hardy hybrids is due mainly to excess malic acid. Grapes will metabolize malic acid during ripening, but in cooler regions with shorter growing seasons, there may not be sufficient time for hybrid varieties to lose enough malic acid.

"High malic acid in grapes is a headache. High tartaric acid isn't a huge problem because of the poor solubility of 'cream of tartar' (potassium bitartrate) in wine, which results in a very small range of tartaric acid levels in finished wines. But malic acid is more soluble and creates the same levels of sourness in finished wines," said Sacks.

Malic acid is largely unaffected by yeast during alcoholic fermentation, leaving winemakers few options for adjusting the sourness of high malic-acid wines. They can put these wines through a secondary, malolactic fermentation (MLF), during which lactic acid bacteria convert malic acid to the less acidic lactic acid. However, lactic acid is still an acid, and the resulting wine may still be too sour. Another option

is for winemakers to adjust drinkers' *perception* of sourness by making finished wines sweeter. But winemakers interested in producing a dry wine are limited to other approaches for reducing sourness that are typically expensive, less effective, or may have unintended consequences. For example, they could add bases, like potassium bicarbonate, to either the juice or wine—a step that might slightly reduce the sourness of the wine but not its pH level.

"So winemakers using high-malic acid hybrids can be stuck with the worst of both worlds—a wine that has excess sourness and too high a pH to prevent microbial spoilage. Under these circumstances, solving one problem just exacerbates the other," Sacks said.

Changing the Game

Sacks said that since grape acid levels are so crucial to the quality of finished wine, most breeders have screened for acidity by tasting grapes in the field. But as UMN's Clark attests, this method is often inaccurate because tasters get fatigued and don't perceive high acidity against a backdrop of high grape sugars, measured in degrees Brix.

"Some hybrids are at 28° Brix with measured acidity of 19 grams per liter, but they tend to be balanced so we don't taste this big difference," Clark said.

Testing in the lab is highly accurate but also more expensive and time consuming, with a lag time of at least three years for new hybrid crosses to bear fruit—assuming they survive the winter. This is where new tools, like the genetic marker for malic acid accumulation, could drastically alter the pace of development for Clark and other breeders challenged by the growing season and environmental stresses. Clark may soon be able to run a simple DNA test on seedlings just a few months old, screening them for markers associated with low malic acid accumulation, early-ripening, disease resistance and cold-hardiness traits—before anything even leaves the greenhouse.

"It really changes the game, especially for marker-assisted parental selection. We can use these markers first to create enriched breeding populations—selecting good parents to start—and then use them to cull out the 'bad' seedlings from these crosses for whatever reason. It's a numbers game, and you're setting yourself up to be successful," said Clark.

This power in numbers comes from VitisGen, the five-year, $4.5 million project funded by the USDA-NIFA Specialty Crop Research Initiative that's involved more than 20 scientists across 11 academic and governmental institutions since it launched in 2011. By combining advanced genetic sequencing technology with expertise from breeders, geneticists, chemists, and economists, VitisGen has developed genetic maps of 16 different grapevine families, with each map having between 1,000 and 10,000 genetic markers—75 of which have so far been associated with fruit quality, disease resistance, and cold hardiness traits.

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*Vitis*Gen Vocabulary

Anthocyanin

Water soluble flavonoid compounds. They are odorless, nearly flavorless and may appear red, purple or blue.

Tannin

An astringent phenolic compound.

Astringent

A dry, puckering mouthfeel. Astringent compounds bind to salivary proteins, causing a "dry mouth" feeling.

Interspecific Hybrids

A cross between two distinct species (e.g., *Vitis vinifera* and *Vitis riparia*)

Terroir

The unique flavor of a wine imparted by its broad environment, including soil, topography and climate.

Phenotype

An organism's observable traits resulting from the interaction of genotype and environment.

Those dense genetic maps will speed the efficiency of grape breeding programs, allowing breeders to release more new varieties at one time with multiple desirable traits—and get plant material into the hands of growers and winemakers sooner.

"We're moving into a new era of hybrids, with the ability to put out new varieties with a whole suite of better traits in an informed way. The goal is to get *vinifera*-type wine quality out of our breeding program, and let them [winemakers] take it the next step," said Clark.

For short-season and cold-climate viticulture, that next step is one step closer to more sustainable, palate-pleasing hybrid wine grape varieties—ones that may mimic the flavor of consumers' favorite *vinifera* varietals, but also ripen early, require little or no chemical spraying, and survive another year to make wine.

Learn more:

- *Vitis*Gen: http://www.vitisgen.org/
- University of Minnesota Grape Breeding: http://mnhardy.umn.edu/varieties/fruit/grapes
- Cornell University Grape Varieties: http://www.ctl.cornell.edu/plants/plant-varieties.php?plant=Grapes
- Professor Anne Fennell, South Dakota State University: http://www.sdstate.edu/ps/research/vitis/
- Northern Grapes Project: http://northerngrapesproject.org/
- Acidity in Wine: The importance of management through measurement [University of Missouri, Grape and Wine Institute]: http://gwi.missouri.edu/publications/2013spring.pdf ■

Recent Publications and Presentations

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Conference Papers and Proceedings

Cadle-Davidson, L. (2015, August). Strategies for durable resistance to the grapevine powdery mildew fungus, Erysiphe necator. Poster presented at the American Phytopathological Society Annual Meeting, Pasadena, CA. Abstract retrieved from http://www.apsnet.org/meetings/Documents/2015_meeting_abstracts/aps2015abP486.htm

Kovaleski, A., and Londo, J. (2015, August). Deacclimation rate in cultivated and wild grapes is dependent on dormancy stage and temperature. Paper presented at the American Society for Horticultural Science Annual Meeting, New Orleans, LA. Abstract retrieved from https://ashs.confex.com/ashs/2015/webprogramarchives/Paper21817.html Paper presentation retrieved from https://ashs.confex.com/ashs/2015/videogateway.cgi/id/1967?recordingid=1967

Sacks GL. (2015). Getting Consumer-Friendly Wine from Grower-Friendly Grapes: Challenges to Improving the Flavor Chemistry of New Sustainable Grape Varieties. Paper presented at the Proceedings of the American Chemical Society—Northeast Regional Meeting, Ithaca, NY.

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