

Summary of the 2021 Fourth Annual *VitisGen2* Project Meeting

The annual *VitisGen2* project meeting connects members of the project including the advisory panel, scientists, collaborators, students, and guest speaker(s) for discussion and review of project progress. Due to travel restrictions related to the COVID-19 pandemic, the 2021 project meeting was held online between January 6 – 8, 2021 from 11:30 am to 3:30 pm ET each day. Fifty-eight members of which thirteen were advisory panel members, two external invited speakers, and other members of the project took part in the online meeting. The two invited speakers and a member of the advisory panel gave keynote presentations. Team leaders presented progress reports, and there were presentations from students and postdoctoral associates in the project. This year, there was a follow-up discussion on a successor project followed by feedback from advisory panel members and the invited speakers.

Despite the drawbacks of the COVID-19 pandemic, significant progress was recorded by all project teams. The genetics team continued to support marker-assisted selection in various breeding programs using the newly developed rhAmpSeq markers. The team supported quantitative-trait-loci (QTL) analyses for various traits across different breeding programs. This year, the genetics team tested the KASP marker platform for traits where strong marker-trait associations were previously known. Twenty-three KASP markers were tested for the following traits: powdery mildew (PM) resistance; fruit chemistry (including muscat and diglucosides); seedlessness and seed trace size; and flower sex. The project recorded breakthroughs in laboratory and vineyard trait evaluations using computer vision and advanced analytical methods especially for fruit quality and PM assessment. Additional phenotyping tools, hyperspectral and thermal sensors, are being tested for disease and quality trait evaluations. There were new developments towards the promotion of consumer, industry, and market preference-based breeding strategies for the genetic improvement of grapes. The ex-ante analysis of consumer preferences for specific varietal traits from different breeding methods was completed and the result showed that consumers are willing to pay differently for fruit quality traits based on conventional or gene-editing improvement methods. Sweetness, flavor, crispness, berry color, and berry size were the traits most desired by consumers irrespective of the breeding method. Finally, the Extension and Outreach team focused on knowledge dissemination and communication of project innovations using various channels targeting both scientific and non-scientific grape audiences.

Follow-up discussions on various issues raised during the presentations were related to factors that contributed to the reported poor conversion rate of KASP markers. This was attributed to the lack of specific causal alleles for various traits in grapes where there are multiple alleles affecting trait variations. Other discussion points include the advantage of PacBio sequencing over other platforms that include the generation of long single sequence reads, high accuracy, and the flexibility for haplotype assembly. The lethality of leaf variegation gene was raised and reports indicate that QTL for this trait has not been identified yet and total lethality may not always result from seedlings carrying the variegation gene although they generally express undesirable phenotypes such as shriveled and succulent leaves, less cold-hardy, and weak flowering phenotypes. There was a call for more investment in computational biology, diverse population assembly for model training, and the adoption of strategies for developing a selection index in

preparation for the implementation of genomic selection (GS) and genome-wide association studies (GWAS), especially in table grapes. Also, the use of reverse engineering and machine learning techniques to capture retrospective selection in long-established breeding programs while making room for novelty received some attention.

The accumulation of malate in the vacuole and the lack of transferability of identified malate QTL across families were discussed. The importance of releasing varieties with more than one resistance gene was a dominant topic during the meeting. It was concluded that the combination of resistant genes in a single variety is ideal since a single resistant source could be more easily compromised. Also, there was a suggestion for more experiments to decide best practices for conserving the durability of resistant genes. Such practices may include the optimum number of sprays that could help attain maximum resistance in a variety. The distinctive role (pros/cons) of thermal imaging and hyperspectral (HS) imaging were also discussed. It was acknowledged that pathogen- and stress-induced localized temperature changes often occur before their effects can be visually observed. Thermal imagers are early detection devices, particularly useful for pathogens that are not easily observed, while HS sensors provide much more information including specific biochemical changes, although they are usually expensive. Interestingly, USDA-ARS and Cornell University currently have the two devices and offered to help anyone that has an experiment or would like to image samples to see the capabilities of both devices (please contact Lance Cadle-Davidson).

There were suggestions for the adoption of a pan-genome in grape; the evaluation of the role of crassulacean acid metabolism (CAM) in malate accumulation and possible drought resistance in grapes; understanding the correlation between leaf and berry malate accumulation; between red rachis and berry color; and between laboratory leaf disk assays and PM epiphytotics in the field; the use of hyperspectral sensors in leaf malate evaluation; the possibility of using the Blackbird high-throughput imaging platform for Botrytis evaluation; and the use of berry or flower samples instead of leaf disks for PM evaluations. There were interests in the identification of QTL regulating red rachis; the evaluation of the individual contribution of REN loci in REN stacked genotypes; and the design of experiments such as bulk genome sequence analysis of the locus; and bulk RNA-seq, useful for identifying the underlying genes behind each observed resistance in the project. The protocol for the trait economics survey findings was reviewed; sweetness and flavor ranked among traits of highest interest with no significant difference in preference from whether varieties were developed from traditional or gene-edited breeding approaches.

Lastly, the logistics for conducting a virtual tasting under the present pandemic restrictions were considered. Generally, while there was a consensus that early variety profile and engagement with growers on upcoming products is very important for adoption of these varieties when they are eventually released, it was concluded that virtual wine tastings (while quite complicated) will be easier to organize compared to table grapes and juice. Such industry-based virtual interactions will

be educational with reference to ongoing scientific innovations and potential products expected from the ongoing efforts. Similarly, industry-centered outreach events to address industry-related interests were suggested. The use of short videos to showcase research activities and continual updating of the project website were recommended to increase the impact of the project.

There were five student and postdoctoral presentations covering topics on high-throughput phenotyping, QTL mapping, marker-assisted selection, transcriptomics, and bioinformatics. Some of the tools developed by students and postdocs such as high-throughput phenotyping platforms; a user-friendly pipeline for marker-assisted selection; and ‘HaploSync’ genome processing tools are already in use in the project and are helping improve breeding efficiency.

Three speakers, Dr. Paola Barba, Dr. Marianna Fasoli and Dr. Edward Buckler, presented keynote seminars. Dr. Barba reviewed the current needs in the table grape industry. Having recently convened the 9th International Table Grape Symposium (9itgs), she reviewed the major areas of interest at the conference, include crop physiology, genetics, reproductive biology, and fruit quality. She highlighted the major industry wish list; the current effort of her program; and the potential of using GWAS and GS approaches in meeting the rising demand for new varieties. Discussions after her presentation were on variety licensing protocols, the contribution of yield in variety adoption, the effect of cracking on fruit market value and if there have been some genetic studies on cracking the use of CRISPR, and breeding goals and how often they are reviewed. In her response, good yield is a must-have for any new variety in addition to other qualities such as ease of management due to labor constraints. There was a recommendation for rapid cycling up to 4 cycles before model update in testing genomic selection.

The second guest speaker, Dr. Fasoli underscored the importance of the understanding and modeling of gene expression on fruit quality. In her talk titled “How understanding and modeling Gene Expression will shape the future of Viticulture”, she showed global as well as differential gene expressions as a function of varieties, fruit maturity stage, and vine status or cultural practices. Follow-up discussions after her presentation focused mostly on the effect of circadian cycle on fruit ripening, the incorporation of gene expression in genomic predictions, stability of results across other varieties, industry application of the identified genes, and the translation of the results into vineyard management practices.

Finally, Dr. Edward Buckler, from his over 29 years of grape experience, forecasted the future of grapevine improvement in the coming years, speaking on "After pondering grapes for 29 years, where could grape research go?". He highlighted the efficiency and opportunities of deriving expression, pathways, and deleterious mutations in genomic selection in grapes; genotype-by-environment modeling; diversities in protein structure in disease resistance; and purging the load for temperature tolerance. He commended the current effort in phenotyping and recommended among other things, increasing the diversity of scientists; human and wine sensory biology;

development of a selection index for grape improvement; and maintaining carbon neutrality. The final thoughts following his presentation include maintaining contact with growers amid technological advancement.

A brainstorming and discussion session on successor project(s) was coordinated by Nick Dokoozlian and Lance Cadle-Davidson. The aim of this session was to review suggestions from last year's meeting and set priorities for very important topics to pursue in the next phase. The summary of the exercise is available by request.

In their remarks, the Advisory Panel expressed their excitement and commended the teams for sustaining the objectives of the project even in the face of the pandemic. They expressed their appreciation for the content and the overall organization, the level of collaboration among scientists and the judicious use of funds and resources. Some of their recommendations include maintaining developed populations for future uses, validation of markers across populations, multilocational evaluation of populations, validation of stacked genes, investigating the predictive value of markers, decreasing the turn-around time of variety release, and the commercialization of products from the project. Also, they asked for more effort to reach a larger grower and non-scientific audience.

The closing remarks for the 2021 *VitisGen2* online annual meeting were given by the project directors Bruce Reisch and Lance Cadle-Davidson and Advisory team representatives, Donnell Brown, and Nick Dokoozlian. They extended their warm appreciation to everyone in attendance and acknowledged the various contributions of members of the project and collaborators. They acknowledged the efforts of many people that put the annual meeting together. The leadership of the project is optimistic that the current restriction will be relaxed enabling an in-person meeting next year given the one-year no-cost extension of the project. The tentative venue of the meeting will still be in San Diego, California. Please plan on these dates: January 6 to 7, 2022. More information on this will be communicated in due time.

Ugochukwu Ikeogu
Postdoctoral Associate and *VitisGen2* Project Manager