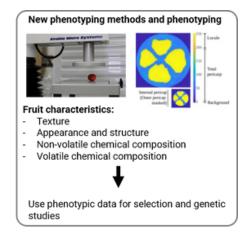


>>> ISSUE 13 | AUGUST 2025

CRANBERRY PHENOTYPING, GENETICS, DNA MARKERS AND BREEDING

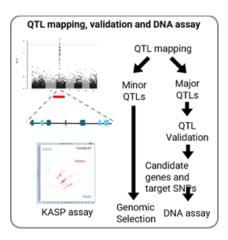
VacCAP Objective 2-3 aimed to establish phenomics and DNA-based strategies to select for improved fruit quality and yield in cranberry



Phenotyping Fruit Characteristics

>>> Phenotyping Team

Develop new and more accurate phenotyping methods with improved accuracy and efficiency to evaluate fruit characteristics (FC) and yield- related traits. Use phenotyping data to perform genetic studies and select breeding lines with improved FC.



Genetics and DNA Markers for Fruit Characteristics

>>> Statistical Genetics Team

Perform marker-trait association analysis to understand the genetic mechanisms controlling fruit characteristics. Based on results of genetic studies, initiate efforts to develop DNA assays.

>>>

ACCOMPLISHMENTS IN GENETIC DISCOVERY AND PHENOTYPING

- Developed quantitative, multi-trait phenotyping methods to evaluate fruit characteristics efficiently and accurately.
- Elucidated the genetic architecture underlying key fruit characteristics and yield.
- Advanced selection of cranberry breeding lines with improved fruit quality and yield.

ADVANCING PHENOTYPING FOR FRUIT CHARACTERISTICS

- Developed accurate, high-throughput phenotyping methods for cranberry fruit, covering external appearance (color, size, shape, uniformity) and texture.
- Shared these methods with the cranberry community; some are already in use or being piloted by public- and private-sector stakeholders.

>>> TEXTURE

Cranberry texture is a critical component for the production of sweetened dried cranberries (SDCs), the most profitable product in the cranberry industry. However, texture is difficult to measure accurately, and previously implemented techniques have given inconsistent results.

A standardized method for evaluating cranberry texture was developed to help breeders obtain consistent results in marker-trait association analyses, enabling selection of lines with superior texture for release to market. This in turn, will help farmers grow higher-quality fruit and maximize profits, while providing processors with a more consistent product. Optimal conditions for texture testing were determined, and we compared methods and traits at fresh harvest and postharvest.

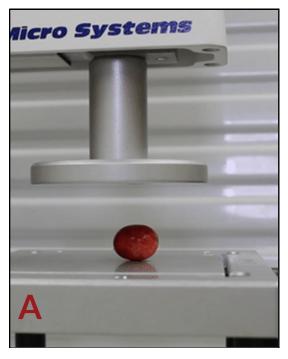
We utilized five methods - double compression, single compression, puncture, shearing, and Kramer shear cell - resulting in 47 textural features related to cranberry fruit flesh, structure, and skin (Lopez-Moreno et al.,2023; 2024). At least 25-30 fruit per genotype constitutes an optimal sample size across methods and traits. Firmness measurements were stable during the first 30 days of cold storage. The greatest changes in texture were observed after 30 days indicating that firmness analysis should be conducted within the first month if fruit is destined for the fresh market. We also found that certain conventional measurements (e.g., maximum force) were highly biased by fruit size and shape, whereas other measurements (e.g., apparent modulus of elasticity, maximum contact pressure) were less biased.

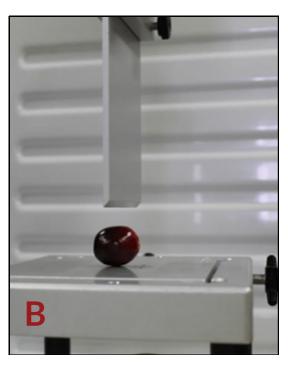
The puncture and double compression methods were identified most effective for texture analysis in cranberry, as they differentiated cultivars at harvest, after refrigeration storage, and after defrosting. The following tests were selected and fine-tuned for cranberries: single compression test (10% strain); C_h1, C_MCP, C_AMOE, and P_R1 and puncture test; P_W, P_DR.

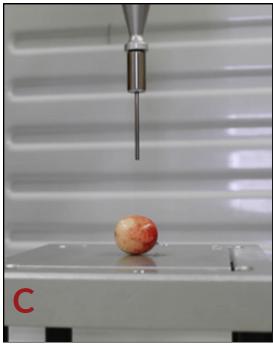
Overall, a multi-trait approach was developed to characterize cranberry texture using the most descriptive methods and traits for both fresh and postharvest conditions. The complementary use of methodologies enables efficient, reliable texture evaluation in breeding programs and industry settings. These methods have been shared at meetings, workshops, and webinars with growers, processors and scientists.

>>> 3 | NEWSLETTER ISSUE 13, AUGUST 2025

All three public cranberry breeding programs in the country have implemented the methodology for selecting superior cultivars for SDC production. Additionally, the methods were presented and made available to cranberry fruit processors, including Mariani Packing Company (Wisconsin), Graceland Fruit Inc. (Wisconsin), and Ocean Spray (U.S. and Canada).



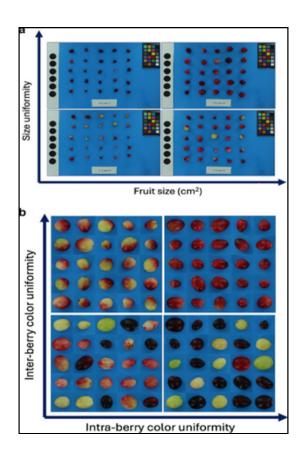






Cranberry fruit evaluated with double compression probe (A), chisel probe, puncture probe (C), and shearing probe (D).





>>> EXTERNAL APPEARANCE

Measuring external cranberry fruit characteristics is critical for the production of sweetened dried cranberries (SDCs), a key product for the industry.

We developed a high-throughput image analysis software, BerryPortraits, rapidly detects and segments berries to extract morphometric data on fruit quality traits such as color, size, shape, and uniformity (Loarca al., 2024). et BerryPortraits has potential applications for other specialty crops, such as blueberry, lingonberry, caneberry, grape, and more.

LINK TO IMAGE PAPER

As an open-source phenotyping tool based on widely-used Python libraries, BerryPortraits allows users to modify, optimize, and integrate it into other image analysis pipelines. The software was developed to be user-friendly and implemented through a partnership with VacCAP scientists and Breeding Insight. Based on this work, the cranberry industry commissioned the development of a large-scale imaging data collection system specific to cranberries that is now deployed across the major cranberry handlers. This method increased the accuracy and efficiency of color evaluation. Also, a different research group leveraged these imaging techniques to develop an app that allows growers to measure fruit color and harvest their fruit at peak color.

Finally, phenotyping and data collection efficiency and accuracy are the biggest challenges in a breeding program. BerryPortraits has opened up the opportunity to easily measure cranberry external appearance and is now being implemented by all cranberry public breeding programs for genetic mapping. These advances allow researchers to take measurements of thousands of berries more expediently.

ADVANCING GENETIC DISCOVERY AND BREEDING FOR FRUIT CHARACTERISTICS

- Completed 10 genetic studies on fruit characteristics (FC), fruit rot resistance (FRR), yield, and vegetative yield-related traits. Key findings include:
 - Yield traits were positively correlated with vegetative upright traits.
 FRR was not correlated with yield or FC, suggesting that improvement in FRR can be made without compromising other traits
 - Yield data collected at plot level, rather than on individual number of upright per plant, were predictive and could be more amenable to high-throughput phenotyping (e.g. by imaging)
 - QTLs were identified for berry shape/size, yield, anthocyanins (TAcy), titratable acidity (TA), Brix, flavonols, texture, proanthocyanidins-PAC, epicuticular wax, FRR, and vegetative upright traits
 - At least 25 QTLs were validated across different populations, making them stable targets for designing DNA assays for marker-assisted selection strategies
 - Yield, FRR, texture, fruit size and fruit shape showed moderate-to-high heritability with complex genetic control, suggesting that genomic and phenomic selection are the best strategies for selection.
 - Stronger-effect QTLs were identified for anthocyanin accumulation/color, organic acids and, flavanols, which are more promising targets for marker assisted selection.

	Trait	#QTL/SNPs	#Major and Stable QTLs	# Validated QTLs	DNA markers	Reccomended MAB strategy and traits
Cranberry						
FC-Chem and Appearance	Anthocyanins and color	119	6	2	1	MAS, Tacy and color
	Proantocynins	93	4	3	-	MAS, total PAC
	Flavonol (quercetin 3-rhamnose)	1	1	-	-	MAS
	Organic acids (malic and citric)	3	2	1	2	MAS, citric and malic
	Titratable acidity	115	-	-	-	GS
	Brix	54	-	-	-	GS
	Size and weight	423	17	7	-	MAS
	Shape	651	8	3	-	MAS
	Epicuticular Wax	3	1	1	1	MAS
FC-Texture	Texture	285	12	4	-	GS, MAS
FC-Disease Appearance	Rot	110	2	2	1	GS, MAS
Yield-related	Yield	488	2	2	-	GS, MAS
Vegetative (e.g. upright length, # leaf)	Vegetative	184	-	-	-	GS
Upright Flowering and Fruit Set	Flowering	399	-	-	-	GS
Misc Fruit Characteristics	Other	479	-	-	-	GS
Total		3407	55	25	5	

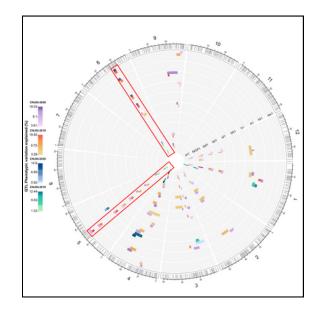
ADVANCING GENETIC DISCOVERY AND BREEDING FOR FRUIT CHARACTERISTICS

- Found that specific organic acid profiles, rather than juice pH and TA measurements, were more effective at detecting QTLs associated with fruit acidity
- Integrated QTL data with new, high-quality reference genomes to identify genetic loci contributing to variation in fruit organic acids, anthocyanins, flavonols, epicuticular wax, fruit rot resistance, and textur
- Developed the first set of DNA markers for FRR, fruit color, organic acids and epicuticular wax. These markers were tested in mapping populations and are currently being tested across a wider set of cranberry germplasm

>>> TEXTURE

We used 10 texture measurements that allowed us to assess the firmness and elasticity of cranberry fruit flesh in two biparental breeding populations [CNJ02 (n=168) and CNJ04 (n=67)], derived from crosses among three cranberry cultivars (Stevens, Mullica Queen, and Crimson Queen).

Red boxes (right) highlight example of stable QTLs identified for texture in two cranberry mapping populations.



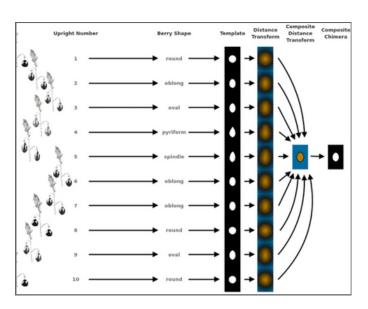
We used the data to conduct the first genetic study implementing a compression method to elucidate the genetic basis of cranberry texture (<u>Lopez Moreno</u>, <u>2025</u>). Some of the traits were strongly related to each other and showed similar phenotypic and genetic patterns. We found that siblings from the two different crosses had a wider array of firmness levels from softer to firmer than their parents.

Textural traits that were adjusted for fruit size and shape stood out and proved more reliable from year to year and across different populations. Thus, adjusting for size and shape made the measurements more consistent and less affected by other factors and resulted and better genetic signal detection. Genetic mapping revealed polygenic architecture of this trait. Twelve QTLs located on chromosomes 1,2,3,4,5,8,9, and 12 were stable and/or of major effect.

>>> 7 | NEWSLETTER ISSUE 13, AUGUST 2025

These genomic regions explained, on average, 5.8-13.6% of the phenotypic variance in CNJ02 and 3.2-10.6% in CNJ04. Four QTLs were validated across the two populations.

Overall, the study indicates that although some QTLs were stable across years and populations, they explain limited amounts of phenotypic variation, suggesting that a combination of genomic selection and marker-assisted selection (MAS) may be the best approach for texture traits. These findings provide insights into the genetic architecture of texture in cranberry, informing breeders about the best DNA-based strategy to select for this important trait. Furthermore, we anticipate that these results will guide future studies aimed at optimizing postharvest fruit handling and management practices.



FRUIT SIZE, SHAPE, AND WEIGHT

Berry size and shape are important for the processing industry because large and round berries are preferred for producing sweetened dried cranberries (SDCs).

Example showing the methodology for generating representative genotype shape, or berry chimera, from 10 upright samples.

Previous studies have identified QTLs for size and shape, but the data had not been integrated. A new study was conducted to identify and integrate QTLs for size, weight and shape across three populations (Maule et al., 2024). All data were collected using high-throughput and accurate phenotyping methods. In total, 151, 266, and 651 SNPs were associated with size, weight, and shape, respectively. Across these analyses, 25 QTLs were stable across years and 10 were validated across studies. For example, multiple meta-QTL associated with round, spherical berries, berry length:width ratio, and shape eccentricity were identified on chromosomes 2, 11, and 12. The QTLs can be targeted to narrow regions of interest for these traits and to develop DNA markers for MAS.

>>> YIELD

Yield is the most important characteristic for cranberry production. Cranberry yield traits, including total fruit mass and number, have traditionally been measured on a per-upright basis. However, given the substantial cost and time required to accurately phenotype these traits, yield evaluation is now done at the plot-level (or per-unit-area) basis.

A study was conducted to evaluate the relationship between upright yield-related traits and plot-based yield (Maule et al., 2024). The genetic mechanisms controlling these traits were also evaluated. The results indicated that several plot yield traits are correlated with upright yield-related traits. For example, plot-level mean fruit mass (MFM) was strongly correlated with upright mean fruit mass (UMFM). Total yield measured at the plot level correlated moderately with upright berry width, upright berry mass, upright mean fruit mass, and upright number of mature berries. In total, 488 markers were associated with yield-related traits. Multiple QTLs for upright and plot-level yield traits colocalized, further supporting their relationships. A region of chromosome 11 harbored several QTLs for plot-level total yield and multiple upright yield traits.

Overall, results indicated that evaluating yield at the plot level is accurate and could be more amenable to future high-throughput phenotyping (e.g., imaging). Although promising QTLs were detected for yield, given the complexity of the traits, future work will focus on testing genomic selection and assessing the integration of associated markers into the models.





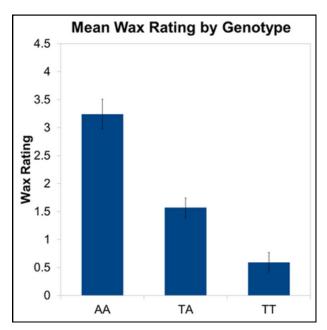
Example of plot level phenotyping. This method is accurate and more amenable to high-troughput phenotyping than uprights based phenotyping. The upright based phenotyping is the traditional method of evaluation in blueberry and require the breeder to randomly collect fruits from 10 uprights.



EPICUTICULAR WAX

A study was conducted to understand the effect of epicuticular wax (ECW) on light and heat tolerance and evaluate the genetic mechanisms controlling ECW (Erndwein et al., 2023).

radiation solar fruit Intense causes sunscald across cranberry growing regions, reducing yield. The study indicated that genotypes with more ECW had lower berry mass percent loss (i.e., less water loss) and maintained cooler berry surface temperatures relative to genotypes with less ECW. QTL mapping identified a QTL on chromosome 1 that explained over 36% of the phenotypic variation.



Segregation of cranberry ECX at QTL loci. Allele AA has significantly higher wax than allele TA or TT. LINK TO IMAGE PAPER

A second QTL study in a different mapping population identified two QTLs for ECW and further validated the QTL on chromosome 1 (Kawash et al., 2024). Within the chromosome 1 QTL interval, 11 potential gene sites were identified. Within this region a SNP at position 38,782,094 bp (chr01 38,782,094; using the 'Ben Lear' reference genome) was used to develop a PACE genotyping assay. The assay was used to genotype 34 individuals chosen for high, medium, and low ECW phenotypes. Genotyping results demonstrated that for this marker, there were significant differences in wax ratings among genotypes. The results suggest that while this marker can be used to reliably predict high or low ECW phenotypes among cranberry seedlings (i.e. homozygous CC vs. homozygous TT), phenotypic predictions for individuals that are heterozygous TC are less reliable. Future work should narrow the region associated with ECW and validate the marker across the cranberry germplasm collection.

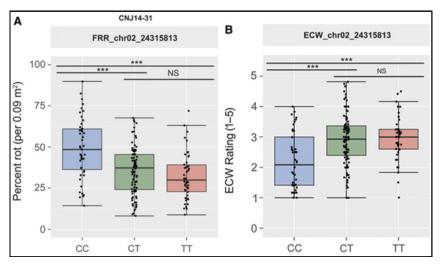


>>> FRUIT ROT RESISTANCE

Two studies evaluated the genetic mechanisms controlling fruit rot resistance (FRR). Fruit rot is a fungal disease complex that threatens cranberry yields in North American growing regions. Control methods heavily rely on fungicide applications, a practice tht may be increasingly restricted in the long term. Breeding for FRR is essential for sustainable production.

The first study evaluated the genetic mechanisms controlling FFR and its relationship to ECW (Kawash et al., 2024). Correlation analysis between ECW and FRR indicated a significant negative relationship between these two traits, suggesting that individuals with higher ECW tend to exhibit lower fruit rot. Significant QTLs for FRR were located on chromosomes 2, 3, 10, and 11. The combined QTLs accounted for 43.67% of the FRR variance. Interestingly, a QTL on chromosome 2 associated with FRR was found to overlap with one associated with fruit ECW. A PACE genotyping assay was designed for the QTL marker identified on chromosome 2, position 24,315,813 bp ('Ben Lear' reference genome) and used to genotype cranberry accessions with varying levels of FRR and ECW. Genotyping results demonstrated that, for marker FRR_chr02_24315813, there were significant differences in percent fruit rot among genotypes and ECW classes. The DNA assay successfully identified accessions that exhibit the desired phenotypes (i.e., lower rot and higher ECW), thus making it a useful tool for marker-assisted selection. Possible functional genes were identified in associated genomic regions that may contribute to FRR and ECW.

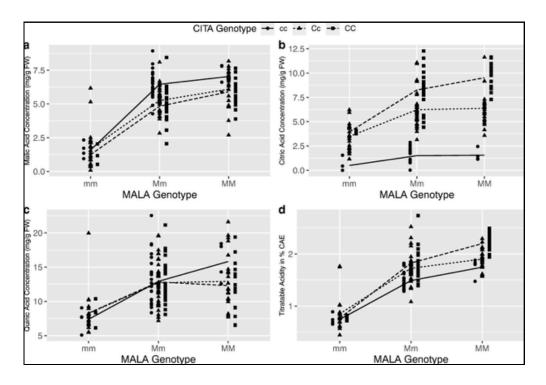
A second study integrated multiple available QTL studies for FRR evaluated as field fruit rot (Maule et al., 2024). The study identified 108 SNPs associated with field fruit rot, and one QTL located on chromosome 12 ('Stevens' reference genome) was validated across studies. Future work will evaluate the marker across the cranberry germplasm collection and assess interactions between markers developed for ECW and FRR, and explore the region identified on chromosome 12 for additional DNA assay development. We will also evaluate genomic prediction models will be evaluated. This work will expedite breeding for improved cranberry fruit quality.



PCR allele competitive extension (PACE) genotyping assays for FRR and ECW. LINK TO IMAGE PAPER

>>> ORGANIC ACIDS AND SUGARS

Cranberry fruits contain very high level of organic acids relative to sugars, about five times that of other fruits, which contribute to high titratable acidity (TA)-the tartness of cranberries.



The effect of mala and cita on organic acid concentration and titratable acidity. Non dwarf lines that are Mm and cc in panel d can reach TA value as low as 1.1-1.3. LINK TO IMAGE PAPER

To offset the tartness for consumers, higher amounts of sugar is added to cranberry products, such as sweetened dried cranberries (SDCs) and juices. As a result, consumers have a negative perception of cranberry products (see Obj. 4 newsletter) despite the health benefits of cranberries. Reducing cranberry acidity would allow less sugar to be added to cranberry products. Previous work evaluated Brix, TA, and organic acids in the Rutgers cranberry germplasm collection. Brix is traditionally used as a proxy of sugar content while TA is used as a proxy of organic acids. Results indicated that variation for Brix is limited in the cranberry germplasm while higher variation exists for TA. Lines with lower organic acids and TA were identified.

During the VacCAP project, these materials were characterized, genetic studies were conducted, and the low acid lines were used to develop new cranberry selections. Genetic studies indicated that two major QTLs control low acidity in the low acid lines (Fong et al. 2020; Fong et al. 2021). The two QTLs named cita and mala, control the level of citric and malic acids, respectively. Ongoing work in a diversity panel and biparental populations validated the QTL for citric acid.



The low citric acid QTL can reduce TA to 1.6%. The low malic QTL can decrease TA to below 1%, which is within the range of fruits that are consumed fresh. However plants with very low acidity have a dwarf-like growth habit which is likely not commercially viable. Interaction analysis between the cita and mala loci indicated genotypes heterozygous for mala and homozygote recessive for the cita locus that are non-dwarf (commercially viable) and can bring the TA value down to 1.5, which is significantly lower than the normal TA value in current cranberry cultivars (>2). These studies demonstrated that lowering acidity in cranberry cultivars is possible and the DNA markers developed can help accelerate this process. This has allowed the cranberry breeding program at Rutgers University to select lines with lower acidity before field evaluation. This material will be used to assess SDCs production and reduce the amount of added sugars. Ongoing work focuses on developing high-throughput SNP markers for the cita and mala loci.

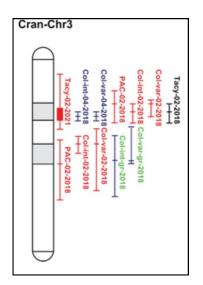
>>> HEALTH RELATED BIOACTIVE COMPOUNDS

Four studies evaluated the genetic mechanisms controlling the accumulation of anthocyanin, proanthocyanidins and flavonols in cranberries (Maule et al., 2024, Diaz Garcia et al., 2021, Albert et al., 2023, Jiménez et al., 2025).

These bioactives are associated with health benefits and fruit color in cranberries. Anthocyanin and proanthocyanidins were evaluated using spectrophotometric and color intensity measurements, while flavonols were evaluated using high-performance liquid chromatography.

A major and stable QTL for quercetin-3-rhamnoside was identified in chromosome 3 ('Ben Lear' reference genome). This locus is a potential target for candidate gene discovery and DNA marker development. Ten stable QTLs were identified for anthocyanins and proanthocyanidins. Three QTLs for proanthocyanidins overlapped with QTLs for anthocyanins and measurements of total anthocyanins (TAcy), suggesting interaction

between these metabolites.

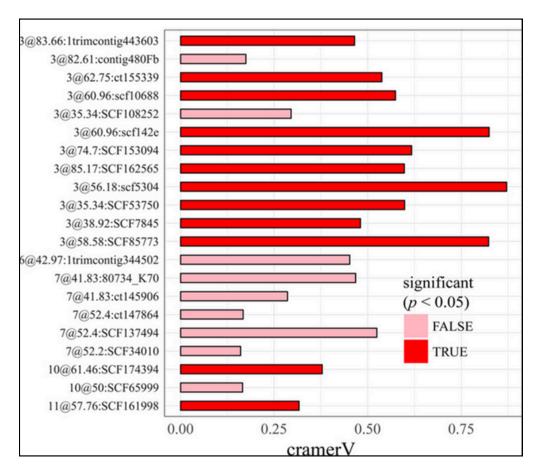


QTLs for anthocyanins and three QTL proanthocyanidins were validated across studies. Two QTLs for color and anthocyanin content (presence vs. absence of red color) mapped to two regions on chromosome 3, including the most significant QTL that was evaluated for candidate genes. A transcription factor anthocyanin accumulation was identified in this region. This QTL region was used as a target to develop a SSR marker for fruit color.

Validated and stable QTLs for anthocyanin and color detected in cranberry chromosome 3. LINK TO IMAGE PAPER

During the VacCAP project, the SSR markers were tested by the USDA-ARS cranberry breeding program at UW-Madison and used to select cranberry lines that lack anthocyanin accumulation in fruit skin (yellow skin color).

This work was done in collaboration with Valley Corporation, a fourth-generation private cranberry breeding company in Tomah, Wisconsin. The selected line was named 'Lemon Drop' (see page 14) and is expected to be released as a cultivar by Valley Corporation. Future work will focus on developing SNP-based markers for the presence/absence of red color and detecting QTLs for color intensity.



Strength of association between SSR markers at a major TAcy/color QTL in chromosome 3 and fruit color phenotype (red vs yellow). LINK TO IMAGE <u>PAPER</u>

ADVANCING BREEDING FOR FRUIT QUALITY TRAITS

• Phenotypic data collected during the project enabled cranberry breeders to advance selections into replicated trials and to make new crosses aimed at combining multiple fruit quality traits. A few examples of selections from two breeding programs are illustrated below.



USDA-24-64. Very large size, firm, early, low rot.



Possible name 'Lemon Drop.' Valley Corporation Inc. (Ed Grygleski). First yellow variety; rich in proanthocyanidin.



USDA-24-29. High yield, low fruit rot



USDA-24-100. Large size, firm, round, high yield, low fruit rot

USDA-WI Zalapa

Pictures by Dr. Juan Zalapa





CNJ12-092-073

A low acid selection. Titratable acidity = 1.8, Yield at 186 barrels/acre, Brix = 9.1, Fruit weight 1.5 g



CNJ12-092-075

A low acid selection. Titratable acidity = 2.0, Yield at 221 barrels/acre, Brix = 9.1, Fruit weight 1.4g. Well liked at a 12.5% sugar reduction in a consumer panel (n=63).



NJ93-117-004

A high flavonol selection. High in quercetin 3-rhamnoside and heat stress responsiveness. A wild selection collected in Cape Henlopen, Delaware.



CNJ14-031-142

A fruit rot resistant selection. Yield at ~255 barrels/acre with two in-bloom sprays and less than 20% rot. Titratable acidity = 2.08, Brix = 8.64, Fruit weight = 1.27g

Rutgers University Sideli

Pictures by Dr. Gina Sideli



IMPACT

The outcomes of Objectives 2-3 provided new phenotyping tools and DNA markers, which will benefit cranberry breeding programs in their development of cultivars with improved fruit quality. Growers and processors seeking to maximize fruit quality within their operations will also benefit from these advances. A number of stakeholders are already starting to adopt these methods.

The genetic studies elucidated the mechanisms controlling several fruit quality traits, laying the foundation to advance the development of molecular breeding strategies for these traits. For some traits, like fruit color, organic acids, and epicuticular wax, it also tested the use of DNA markers for marker assisted selection, providing direct proof of concepts. As a result, research in cranberry is now focusing more on identification and functional characterization of genes controlling fruit quality traits and testing implementation of molecular breeding. Molecular and phenotypic data were also used to advance selections of cranberry breeding lines with improved quality characteristics, which represent new potential cultivar releases.

Further, the VacCAP project fostered active collaboration among Vaccinium research groups. This new dynamic contributed to reducing duplicative work, leveraged the unique expertise of all participants, and tuned research projects to be complementary to each other.

Overall, these outcomes represent a transformative step to advance research and breeding within the large and expanding *Vaccinium* community.

ONGOING RESEARCH

- Additional studies to understand the genetic mechanisms controlling organic acid, flavonoids and texture in cranberry
- Continued development of targeted DNA assays
- Developing the next phase of the VacCAP project (VacCAP 2.0)

Testimonials From the Field

Dr. Jeff Neyhart Research Geneticist, USDA-ARS Genetic Improvement for Fruits and Vegetables Laboratory



Based on what we've learned about the genetics of multiple fruit >>> characteristics, do you think the insights gained from VacCAP will advance molecular breeding efforts for both blueberry and cranberry? In what ways?

Certainly, yes. The genetic studies of fruit quality in cranberry and blueberry from the VacCAP project indicate that many of these traits are not simply inherited (that is, more than a handful of genes are influencing these traits) and that environment plays an important role. This is the case for fruit yield and cranberry fruit rot, but size, shape, color, and texture. With such traits, molecular breeding can switch to utilizing a whole-genome approach, like genome-wide selection, instead of focusing on only a few markers. In other crops, this has led to faster breeding progress, and it could be the same for cranberries and blueberries.

VacCAP has integrated more accurate phenotyping, new genotyping tools, and enhanced genomic resources. As a result of this, research is shifting >>> towards identifying and characterizing candidate genes. Do you think this can benefit your breeding program? Why is this shift important for the future of breeding in blueberry and cranberry?

Identifying candidate genes is a nice form of validation that helps us confirm whether a signal from a genetic mapping study may be biologically real. In that case, a marker tagging that gene can be expected to reliably predict the phenotype of new offspring carrying that marker. This works very well for those traits that are controlled by a few genetic loci with large effects, such as some fruit quality traits or disease resistance that follow a simple gene-for-gene model in pathogen recognition. Characterizing the function of these genes allows us to reliably predict how they will behave in potential new cultivars. In the case of more complex traits like yield, quantitative disease resistance, or abiotic stress tolerance, the genes influencing these traits are likely to be so numerous that characterizing candidates will produce only marginal returns on the investment of time.

So, we need to strike a balance betweencharacterizing genes for "low-hanging fruit" and highimpact simple traits (pun most definitely intended) and improving traditional breeding and selection with phenotyping and genotyping tools for more complex traits.



Has VacCAP provided new tools or opportunities that you plan to incorporate into your breeding program? How do you foresee using the data or resources generated from this project in your future breeding efforts?

Yes, definitely! We are already exploiting the 3K marker array from Breeding Insight (developed using the 17K array from VacCAP) to predict phenotypes and make selections of seedlings before they enter field trials, and wewill continue using this tool for routine genotyping of new offspring from theprogram. We also plan to expand on our collaborative work in image-based phenotyping of fruit quality; we assisted in the development of the "BerryPortraits" software with Dr. Jenyne Loarca of the VacCAP and Breeding Insight, and we are deploying similar software in our custom-made "BerryBox" fruit imaging platform, which allows us to measure fruit size, shape, color, uniformity, and percentage of rotten fruit using only color images and an AI model. Finally, the genomic data in the GenomeDatabase for Vacciniumhas already been immensely usefulfor our pre-breeding work. We have used this data to identify candidate genes for cold tolerance, flowering time, and disease resistance.

In the future, we will take advantage and expand upon genomic resources, including the cranberry pangenome, to more fully inventorythe haplotypes (groupsof genes that are inheritedtogether) present in cranberry germplasm. This will have two direct benefits: first, we can target unadapted germplasm (such as wild or landrace clones) with novel alleles that may confer favorable quality, disease resistance, or stress tolerance traits; second, we can use this haplotype inventory to impute from the high-density pangenome data down to the low-density 3K or 17K marker data. In thisway, we can obtain whole-genome coverage of new offspring for the very modest price of a few thousand markers.



Do you think VacCAP opened opportunities for you to collaborate with the community? Do you see any potential collaborations or partnerships that could emerge from the research and resources developed through VacCAP? How might these collaborations benefit your breeding program?

Absolutely. My first task after starting in my position was to reach out to the community to start establishing collaborations, and VacCAP was a great mechanism to facility those connections. We already have good collaborations with the other cranberry breeders to facilitate joint testing of new populations, alongwith others to investigate diseaseresistance, fruit quality, phenology, and stress tolerance traits. Breeders must wear many hats, but we obviouslycannot do everything alone; these collaborations are critical to provide 1) direct, actionable information to guide the selection program, or 2) inform how we design new evaluation schemes to measure important traits on the large populations needed to sustainbreeding progress.

I have been impressed by how we have been able to quickly use genomic and phenomic tools developed through VacCAP in the breedingprogram. My graduateand postdoc researchwere in small grains (barley and oats), and that community has very impressive genomic resources. I think VacCAP has helped rapidly close the resource gap between the blueberry and cranberry community and those of crops with a longer history of research investment.

Testimonials From the Field

Dr. Gina Sideli Assistant Professor Rutgers University, Department of Plant Biology



Based on what we've learned about the genetics of multiple fruit >>> characteristics, do you think the insights gained from VacCAP will advance molecular breeding efforts for both blueberry and cranberry? In what ways?

Yes, I believe the Vaccinium community has gained substantial insights into fruit traits during the VacCAP project period, which will significantly advance molecular breeding efforts in both blueberry and cranberry. Most importantly, we now have comprehensive genomic resources available, including sequenced genomes, pangenomes, and high-throughput genotyping platforms—all of which provide the foundational infrastructure necessary for advanced genomic studies.

Regarding specific traits, the project has delivered improved phenotyping methods for texture, color, and fruit chemistry, enabling breeding programs to implement both marker-assisted selection and genomic prediction modeling for more effective cultivar development. These combined advances create a comprehensive toolkit that moves breeding programs from traditional phenotypic selection toward precision genomics-based approaches.

VacCAPhas integrated more accurate phenotyping, new genotyping tools, and enhanced genomic resources. As a result of this, research is shifting towards identifying and characterizing candidate genes. Do you think this can benefit your breeding program? Why is this shift important for the future of breeding in blueberry and cranberry?

Absolutely! The transition from broad genetic associations to specific candidate genes allows us to identify the actual genes controlling trait expression, enabling the development of precise molecular markers that remain reliable across diverse genetic backgrounds-unlike broader associations that can fail in different breeding populations. This knowledge also creates opportunities for gene editing applications, particularly for fine-tuning fruit chemistry such as sugar-acid balance to meet specific market requirements.

As both consumer expectations and environmental conditions continue to shift, there's increasing demand for varieties that deliver targeted flavor profiles and nutritional attributes while maintaining performance under climate stress. Understanding the underlying genetic mechanisms of fruit development provides breeding programs with the precision needed to address these complex, interconnected demands



Has VacCAP provided new tools or opportunities that you plan to incorporate into your breeding program? How do you foresee using the data or resources generated from this project in your future breeding efforts?

Yes, we have now genotyped numerous breeding populations and germplasm collections in both my blueberry and cranberry programs, which enables marker validation and implementation as well as genomic prediction modeling. I view this as an opportunity to implement genomic tools that improve selection decisions before field planting, allowing us to make more informed choices about which seedlings merit the investment of field space and evaluation resources.



Do you think VacCAP opened opportunities for you to collaborate with the community? Do you see any potential collaborations or partnerships that could emerge from the research and resources developed through VacCAP? How might these collaborations benefit your breeding program?

Definitely! I really value this collaborative network that promotes open exchange of ideas and resources. It allows me to collaborate with scientists who specialize in domains beyond breeding and genetics, complementing my work and deepening our understanding of the traits I focus on. In turn, these collaborations help expand the range of traits I can evaluate, ultimately leading to better products for the industry. For example, in blueberry, I'm currently collaborating with southern highbush blueberry breeders to obtain, test, and integrate germplasm into my breeding program, introducing genetic variation that we currently lack in our northern materials.



Are there any additional comments or thoughts you'd like to share regarding the value of the VacCAP project's outcomes for your breeding work?

VacCAP consists of a solid group of dedicated researchers. These collaborations have enabled us to advance much further than any single lab could achieve independently, which is tremendously valuable. Regarding practical outcomes, the extension and outreach component has been particularly helpful in disseminating research findings and facilitating meaningful dialogue with growers about implementing these advances.

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MORE RESOURCES

Recordings of all Objective 2 and 3 webinars are available on the <u>VacCAP</u> Project YouTube Channel. Visit www.vacciniumcap.org or follow us @VacciniumCAP on X (Twitter) to stay updated on the latest VacCAP news.

