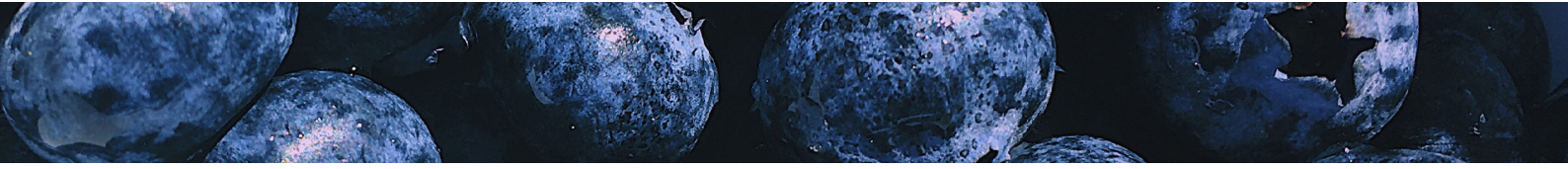

VacCAP

Improving Fruit Quality

Student Spotlight: Özgecan Yalçın \ 4

Breeder Spotlight: Dr. Ebrahiem Babiker \ 5

Issue 8 | August 2023

VacCAP Objective

The Vaccinium Coordinated Agricultural Project (VacCAP) is a nationwide project aimed at developing new genetic tools to enhance breeding for improved fruit quality of cranberries and blueberries.

VacCAP is a nationwide coordinated transdisciplinary project focused on addressing major bottlenecks limiting the growth of the U.S. *Vaccinium* industry by developing and implementing marker assisted selection (MAS) capacity in breeding programs.

This will enable breeders to select and pyramid fruit characteristics that positively contribute to fruit quality and market value.

Long term, the scientific resources developed will increase production of fruit with improved characteristics that meet ever-changing industry, market, and consumer preferences.

A Deeper Look Into Blueberry Cell Wall Composition and Fruit Firmness Phenotypes

Ripe blueberry fruit vary widely in perceived firmness depending on cultivar and species, and consumers pick up on these differences. Our consumers want fresh market blueberries to be consistent from berry to berry in both firmness and flavor. Since blueberry sugars do not increase after harvest, the fruit must be harvested near or at full ripeness. Additionally, blueberry firmness usually decreases with storage, but the rate of loss can depend on the cultivar as well as storage temperature.

Introduction

Several factors have been related to blueberry phenotypic firmness such as fruit morphology, turgor pressure, enzyme activity, internal cell structure, and cell wall changes. We know that plant cell walls are highly complex and can differ in both types and amounts of polysaccharides (cellulose, hemicellulose, and pectin) (Figure 1). Changes and variability in fruit firmness have been linked to cell wall polysaccharides via three main causes: 1) loss of cell wall polysaccharides, 2) changes in cell wall neutral sugar composition and 3) breakdown of the middle lamella.

It has been challenging to link blueberry cell wall composition to perceived firmness traits. Total cell wall polysaccharides have not been well correlated with blueberry firmness, and both the peel and pulp of a blueberry can contribute to firmness. Although the types of cell wall polysaccharides and their linkages are important, establishing these relationships is laborious and difficult. Understanding the quantitative cell wall sugar composition and polysaccharide linkage residues

in blueberry peel and pulp is needed to understand how polysaccharides are arranged and find how these cellular level molecules contribute to blueberry firmness and texture.

In this study, blueberry cultivars representing firm, soft, and crisp phenotypes were used. Based on other published research, we predicted that crisp and firm blueberries would have more hemicellulose and cellulose, as these polysaccharides are less likely to depolymerize during ripening. The objective of our work was to study the quantitative cell wall differences between the peel and pulp of three highbush blueberry cultivars at harvest.

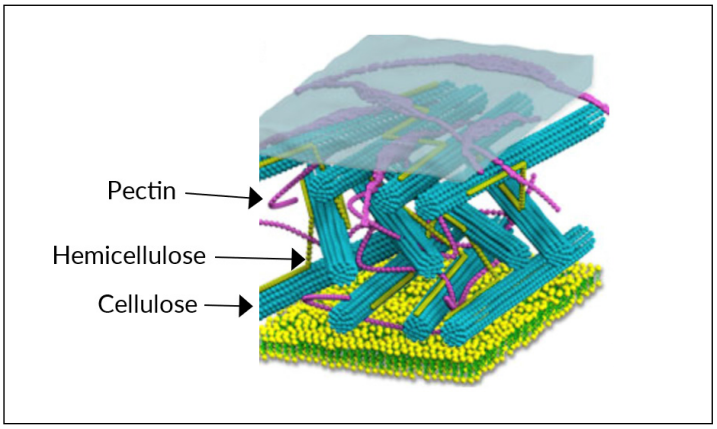

Figure 1. The plant cell wall and polysaccharides of pectin, hemicellulose and cellulose. Figure provided by Dr. Marlee Trandel-Hayse.

Selection of the cultivars

Using 2020 firmness/texture data for blueberry fruit, we found clear firmness differences among crisp, firm and soft cultivars. We selected 'Indigocrisp' (crisp), 'Emerald' (firm, industry standard), and 'Jewel' cultivars (soft) for this study (Figure 2, marked in blue).

Preparation of peel and pulp cell wall tissue

Frozen whole blueberries of 'Indigocrisp', 'Emerald', and 'Jewel' were packed into test tubes with six steel balls and cold ethanol. Tubes were immersed in liquid nitrogen (N) then samples were ground to detach peel particles from intact pulp. After peel separation, pulp was removed then transferred to a new set of test tubes containing a second set of steel balls. Pulp samples were flash frozen in liquid N and further ground. Then, hot water extraction of the blueberry peel and pulp was done using a series of organic solvents to yield peel and pulp cleaned cell wall tissues (Figure 3).

Quantitative cell wall extraction of blueberry peel and pulp

In order to quantitate cell wall neutral sugars and linkage residues, extensive wet chemistry was performed following Trandel et al. (2020) and Pettolino et al. (2012). All samples went through a series of carboxyl reductions, methylation for linkage residue quantitation, cell wall hydrolysis, derivatization and acetylation and then were ran on a GC-MS. Nuetral sugars of glucose, galactose, arabinose, ribose, xylose, mannose, fucose and rhamnose were quantitated using sugar calibration curves. Uronic acids were calculated and cell wall linkage residues were identified using partially methylated sugar standards and mass spectral data.

Linkage analysis and polysaccharide classification

Linkage analysis is a critical component of quantitative cell wall work as it allows for the determination and estimation of specific polysaccharide classes. Linkage analysis provides information about how the single sugar units (monosaccharides) are linked together through chemical bonds. For example, linkage analysis showed us that carbon 1 on rhamnose was linked to carbon 4 on glucose. By knowing the various linkage types, we can piece together the polysaccharide classes. We can then estimate the amount of specific polysaccharides by looking at the relative proportion of linkages within a polysaccharide. We used the classification calculations provided by Pettolino et al. (2012), who used Arabidopsis as the model crop.

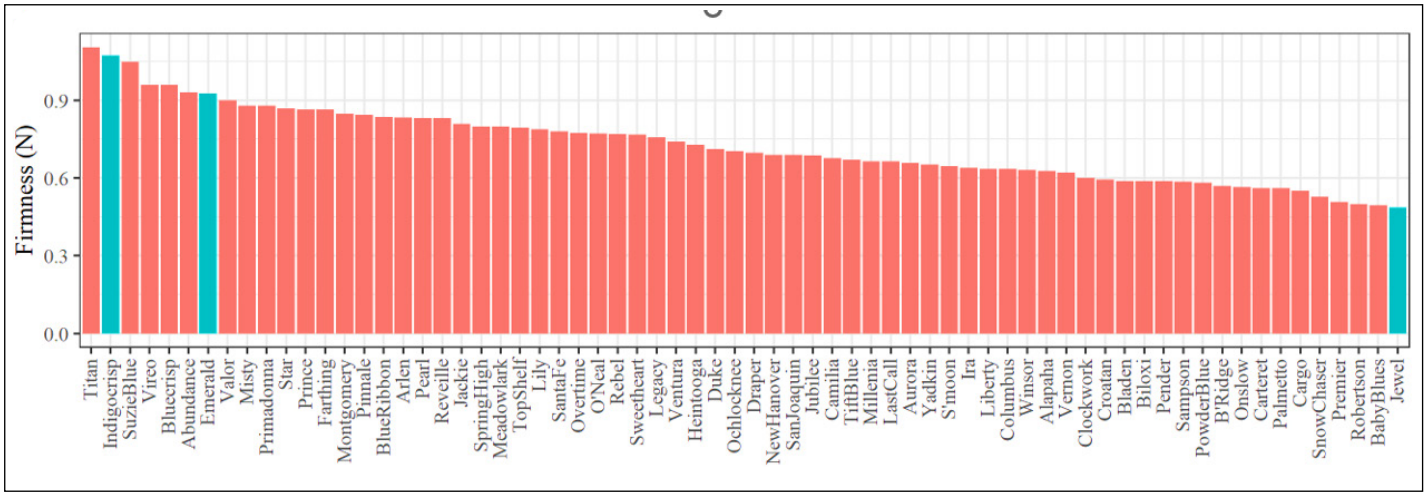

Figure 2. Preliminary tissue firmness data of 68 cultivars indicating phenotypic firmness differences between crisp, firm and soft blueberry cultivars. Figure provided by Dr. Marlee Trandel-Hayse.

Key results and discussion

Neutral sugar composition

Hemicelluloses were found in the peel and pulp of all three blueberry cultivars, although the type of hemicellulose appears to differ with location and cultivar. 'Indigocrisp' peel was high in glucuronic acid and contained xylose. In contrast, 'Jewel' peel contained more mannose and arabinose. Arabinose is used to build hemicelluloses like arabinan and type I arabinogalactan, while mannose has been linked to heteromannan and galactoglucomannan (Mariettes et al., 2021; Dheilly et al., 2016). The pulp of 'Emerald' blueberries had the most glucose, which is predominantly found in hemicellulosic xyloglucans (Amos et al., 2019).

Unfortunately, these neutral sugar assessments did not fully explain the phenotypic texture differences among cultivars. Identification of cell wall linkage residues was pursued in order to determine the cell wall polysaccharide classes.

Blueberry cell wall linkage residues and differences in pulp and peel among cultivars

In this experiment a total of 45 linkage residues were identified in blueberry peel and pulp. Using this method, 'Indigocrisp' pulp was found to be higher in hemicellulose cell wall neutral sugars and linkage residues of xyloglucan, heteromannan, and arabinan. In several fruit crops, hemicelluloses have been most correlated with increased firmness phenotypes, and the type of hemicellulose differentiates crisp and firm phenotypes. In contrast, 'Jewel' pulp was high in type I arabinogalactan, and the linkages identified indicated a high percent methylation. Higher percent methylation has been previously related to increased softening during crop storage (Lurie et al., 2003), suggesting cell wall interconnections between arabinogalactan and pectin may degrade at a faster rate in phenotypically soft blueberry cultivars, leading to decreased firmness.

In blueberry peel, 'Indigocrisp' had lower concentrations of heteromannan and xyloglucan than the peel of the other cultivars. However, 'Indigocrisp' peel had the highest amounts of heteroxylan and this polysaccharide has been associated with increased firmness in eggplant and apple (Mariettes et al., 2016).

The peel of 'Jewel', the softer cultivar, had higher concentrations of type I arabinogalactan and heteromannan. Although rhamnogalacturonan amounts did not differ in 'Jewel' peel relative to 'Indigocrisp' or 'Emerald', the increased ratio of Ara/Rha suggests an increased presence of hairy side branches, which could lead to increased softening due to pectin's susceptibility to depolymerization.

How does phenotypic blueberry firmness relate to cell wall polysaccharides?

Although blueberry cultivars vary greatly in fruit firmness, the underlying causes of this variation have been difficult to discern in previous cell wall studies. In our study, we found the pulp of the crisp cultivar 'Indigocrisp' had higher amounts of hemicelluloses, specifically arabinan, heteromannan and xyloglucan, and cellulose, than 'Emerald' or 'Jewel'. These finding suggest increased hemicellulose content in the pulp may lead to higher phenotypic firmness in blueberry.

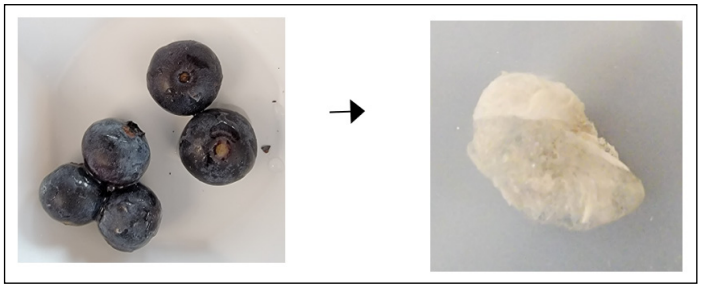

Figure 3. Whole blueberry samples (left) went through a series of grinding and extraction via organic solvents to yield blueberry pulp cell wall tissue (right). Images provided by Dr. Marlee Trandel-Hayse.

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Student Spotlight: Özgecan Yalçın

In our Student Spotlight Series, we want to introduce you to the students who help make VacCAP possible through their passion and hard work. In this segment, get to know ***Özgecan Yalçın****, a graduate student of Horticulture, Plant Breeding and Genetics at Oregon State University with advisors Dr. Nahla Bassil and Dr. Claire Luby.*

What is the project you're working on for VacCAP about?

My study mainly focuses on understanding the genomic regions affecting fruit firmness in blueberry. My project was to identify Quantitative Trait Loci (QTLs) for plant development and fruit quality traits in blueberry plants from a bi-parental population (Draper-Selection _44392 and 'Jewel'), and comparing phenotyping data with genotyping data of the same population using QTL analysis to understand the regions controlling fruit quality traits in blueberry.

What is something you like or find most interesting about your work?

The possibility of finding a major genomic region confirming fruit firmness and identifying the causal genes in blueberry is inspirational. This locus could be used by other scientists to understand, study further and improve fruit firmness in blueberry in the future.

What do you hope to do in the future after your work here?

I am planning to pursue a PhD in molecular studies in my home country, Türkiye, preferably on blueberries.

Anything else you would like to add?

I would like to thank Dr. Chad Finn, who initially accepted me to this project, and my advisors Dr. Nahla Bassil and Dr. Claire Luby for their expert guidance and mentorship through this project, and the VacCAP team for their support.

Breeder Spotlight: Dr. Ebrahiem Babiker

In our Breeder Spotlight Series, we interview blueberry and cranberry breeders to learn more about their roles, challenges in their breeding programs, and have them highlight some of their favorite new cultivars. In this spotlight, we spoke to ***Dr. Ebrahiem Babiker****, a Research Plant Geneticist at USDA-ARS Thad Cochran Southern Horticultural Laboratory.*

Please describe your role in the blueberry industry.

My expertise is in plant breeding and genetics and the application of molecular tools to speed up the breeding cycle. Blueberry consumption has increased markedly in the past few decades and, as a result, demand for high quality berries has also increased. Developing adapted southern highbush cultivars with improved berry qualities necessitates evaluation of a large and diverse germplasm collections in replicated field trials across years for different traits.

Since I joined the USDA-ARS Southern Horticultural laboratory in 2016, I have been involved in research projects aimed at improving the blueberry breeding procedures through utilizing the advances in molecular genetics and high throughput phenotyping to accelerate the breeding process.

Cultivar Highlight - Please tell us about some top cultivars you're excited about and why you chose them.

'USDA-Spiers' is an early rabbiteye cultivar that produces an abundance of medium to large attractive and firm berries. It is a new public domain that was released in 2022 by the USDA-ARS for the USDA hardiness zones 8a thru 9a. Mature 'USDA-Spiers' bushes are moderately spreading with narrow crowns, and compared to most rabbiteye blueberry cultivars, require little pruning to manage excessive vegetative growth.

What are some challenges in the breeding program?

Heterozygosity, polyploidy, and genome complexity, impede progress in improving valuable traits through conventional breeding methods. Further, most of the fruit quality traits are under polygenic control displaying a continuous phenotypic expression, moderate heritability values, and are subject to significant genotype × environment interaction, making breeding for fruit quality traits a difficult task. In addition, manual phenotyping.

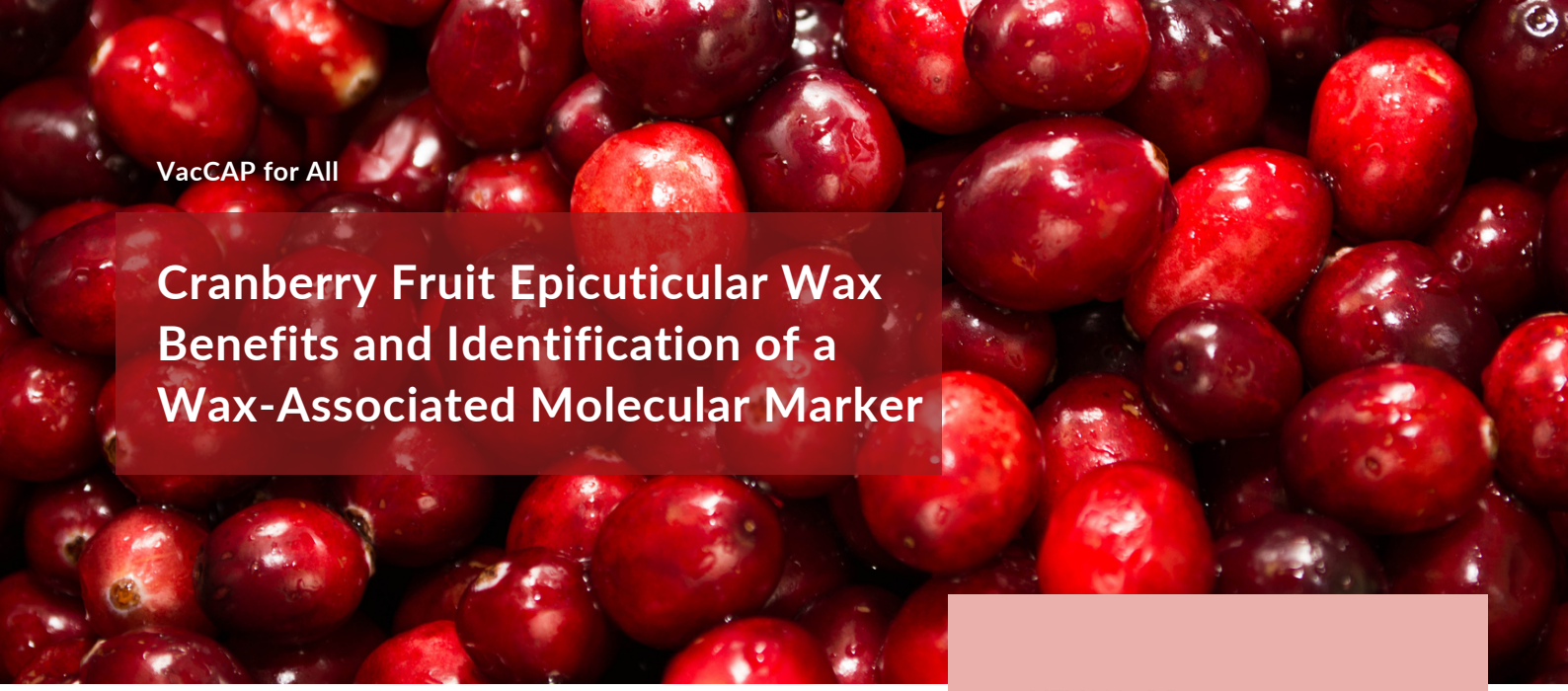
Where do you see the future of* Vaccinium *breeding going in the next 20 years?

We will continue to deliver elite, region-specific blueberry cultivars and conduct genomics studies to understand the genetic architecture of complex traits via genome-wide association studies, quantitative trait loci mapping, and genomic selection. Markers developed through these activities will be utilized to screen seedling populations at an early stage and discard significant portions of hybrids that do not have the desired combinations of alleles.

Also, we have developed numerous analytical techniques to assess different fruits quality traits. These techniques will greatly enhance the blueberry breeding efficiency and help breeders in selecting superior genotypes for crosses and further testing.

In what way have you used resources from VacCAP to facilitate your work?

In collaboration with VacCAP, we will use the VacCAP genotyping platform to investigate the genetic diversity, population structure and locate genomic regions associated with key traits in a diverse panel. The panel contain 58 released southern highbush cultivars from blueberry breeding programs in Mississippi, Florida, Georgia, and North Carolina and 136 breeding selections from the USDA-ARS blueberry breeding program.



VacCAP for All

Cranberry Fruit Epicuticular Wax Benefits and Identification of a Wax-Associated Molecular Marker

BACKGROUND

As the global climate changes, North American cranberry growing regions will struggle with high temperature extremes and drought conditions. One consequence is sunscald, which damages developing berries and reduces yields. Epicuticular wax may be useful in mitigating sunscald in cranberry. This study assessed the function of Epicuticular wax in cranberries to ease stresses associated with sunscald by subjecting high and low epicuticular wax cranberries to controlled desiccation—extreme dryness—and light/heat exposure.

RESULTS

High epicuticular wax on cranberries imparts protection from heat and desiccation

Cranberries with high epicuticular wax lost less mass percent and maintained a lower surface temperature than fruit with low wax.

QTL Analysis identifies a locus and candidate genes associated with epicuticular wax in cranberry

QTL analysis identified a marker on chromosome 1 at position 38,782,094 bp associated with the epicuticular wax phenotype.

Epicuticular wax phenotypes can be predicted from genotyping assays

Genotyping assays revealed that cranberry selections homozygous for a selected SNP have consistently high epicuticular wax scores. A candidate gene (GL1-9), associated with epicuticular wax synthesis, was also identified near this QTL region.

CONCLUSIONS

High cranberry epicuticular wax load may help reduce the effects of primary contributors to sunscald. The molecular marker identified in this study can be used in marker assisted selection to screen cranberry seedlings for the potential to have high fruit epicuticular wax. This work serves to advance the genetic improvement of cranberry crops in the face of global climate change.

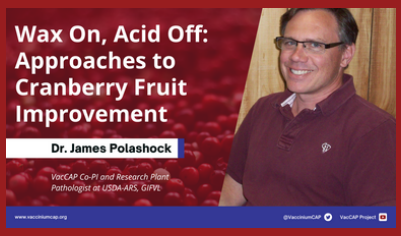
KEY WORDS

Epicuticular Wax

- A waxy coating that forms highly crystalline structures on plant surfaces.
- Functions as a barrier to various environmental stresses in other fruit crops.
- Plants regulate their cuticular wax load and chemical composition in response to water shortage, temperature, and excess heat—three environmental factors associated with sunscald in cranberry.

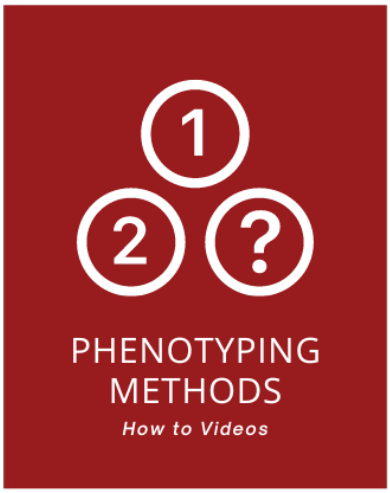
Want to learn more?

Watch Dr. James Polashock's webinar on the importance of epicuticular wax in resistance to heat scald, the value in reducing organic acids in the fruit, and the development of markers for these traits to be used in breeding and selection. Now on YouTube.



Full Article and Citations Here

Check Out These VacCAP Resources



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