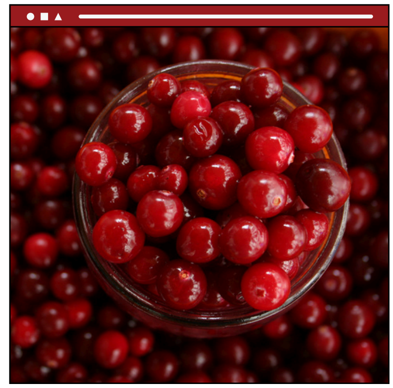
VacTrait

Cranberry Organic Acids

WHY IS THIS TRAIT IMPORTANT?

Probably the first thing that comes to mind when describing cranberry flavor is the inherent tartness that causes puckering. This is largely due to three organic acids: citric, malic and quinic. But what about the taste of various cranberry cocktail beverages or the sweetened dried cranberries? They're sweet because they have added sugar. Consumers have a growing concern over added sugar in food products, including cranberry. Ocean Spray has been actively marketing alternative sugar-free products and so the question remains; can cranberry fruit be made sweeter with a minimal or even no sugar? It is hypothesized that if the organic acid levels in cranberry fruit are lowered (to be more palatable to humans), then less sugar would be needed to mask the tartness.



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WHAT DO WE KNOW ABOUT THE TRAIT IN TERMS OF DIVERSITY AND GENETICS?

The amount of sugars, as measured by Brix (% soluble solids), that accumulate in the cranberry fruit is very low (~8.5%) more than half as low as table grapes (~17-19%).At fruit maturity, there is little variation found for sugars; however, malic and citric acid do vary (4-8 mg/g; 4-12mg/g respectively), therefore providing variation to be selected for in breeding schemes. A mutant accession, (NJ91) was identified to possess low citric acid (~ 4 mg/g), while another native germplasm accession collected from Long Island, New York (NJ93), was found to have low malic acid (4 mg/g). In addition, a third source of lower acidity was found in a selection from the Rutgers breeding program, CNJ98.

DID YOU KNOW?

- Citric acid (CA) is responsible for the sour taste in cranberry fruit, and like its name suggests, is the main organic acid in citrus fruits.
- Malic acid (MA) also contributes to sour taste, and is found in fruits like apple, blueberry and cherry.
- Quinic acid (QA) contributes to some of the bitterness in cranberry fruit, also found in coffee berries.
- The Rutgers cranberry breeding program, previously under the direction of Dr. Nicholi Vorsa for ~30 years, has put an emphasis on breeding for low acidity in fruit.

Through three generations of breeding for low fruit acid, approximately 900 crosses and thousands of progeny, genotypes with low acidity (TA = 0.7 – 1.5%) have been generated and are now under evaluation. Work done in VacCAP assessed the genetic mechanisms controlling organic acids accumulation in cranberry and advancing selection for the low organic acid lines.

HOW DO WE PHENOTYPE THIS TRAIT?

Titratable acidity is measured by quantitative chemical analysis (Metrohm Ti-Touch 916) of cranberry puree using 0.1 N NaOH to an endpoint pH of 8.2 (with % acidity calculated based on citric acid equivalents). This provides an overall acidity measurement useful for screening large numbers of cranberry samples (Vorsa & Johnson-Cicalese, 2012). Organic acids are quantified with ultra high-performance liquid chromatography (UHPLC) using a gradient method described in Wang et al. 2017 with modifications, and C18 reverse-phase column. Identification is based upon retention times of their respective standards and quantified based on standard curves. Phenotyping, and more recently genotyping, are performed while plants are still in the greenhouse. Progeny with lower acid content are then planted in the field for evaluation of yield and other fruit quality traits.

WHAT IS VACCAP DOING TO WORK ON, SOLVE, OR IMPROVE THIS ASPECT?

VacCAP is working to understand the underlying genetic architecture of organic acids in cranberry by identifying stable marker-trait associations in breeding families and germplasm. This will provide genomic tools for making precise selection decisions, which in turn will provide the industry with a more palatable cranberry food product.

So far two genetic studies for organic acid content in cranberry have been completed and others are being completed. In a first study, Fong et al. 2020 used both bulk segregant analysis, and genotyping-by-sequence for fine mapping, in bi-parental populations and identified the cita locus to have Mendelian inheritance on the distal end of linkage group 1. Two molecular markers including a SSR and a KASP (targeting a SNP) were developed (Table 1). The homozygous recessive state (GG) is responsible for low CA (< 2.5mmg/g).

A second study evaluated the genetic mechanism controlling Malic acid (MA). Fong et al. 2021 have shown that MA is controlled by a single locus mala with an additive gene action. Genotypes with a heterozygous state (AC) will have a lower MA, while genotypes with a homozygous state (AA) tend to possess an even lower MA, however, the plants are vegetatively dwarf and therefore not commercially viable (Figure 1). Two molecular markers using KASP technology (targeting a SNP) were developed (Table 1). The marker can explain low (MA < 2.5mmg/g) vs moderate (MA 3.5-5 mg/g) vs high Malic Acid (MA >5 mg/g). Selection for the A allele will make the low MA genotype possible (Figure 2).

Work to validate the result of these previous genetic studies on CA and MA and understand the genetics of QA is currently ongoing. With the development of the 17K cranberry array, high quality SNPs have been applied to multiple cranberry breeding families and the germplasm collection for genotyping. Our research group has measured CA, MA, and QA with UHPLC, and TA for these populations. Currently, we are performing genetic analyses on these datasets to confirm marker-trait associations (MTA) for CA and MA by fine-mapping. Potentially we will discover additional MTA for QA and the genes controlling these loci. With developed CA and MA markers, we are now able to perform large-scale screening of cranberry seedlings in the greenhouse for low fruit acidity.

Sensory studies have been initiated and will continue so we can better understand consumer preferences of common cultivars and advanced low acid selections, and how these likings correlate with organic acid levels in the cranberry fruit. With this information, we will learn the "ideal" organic acid composition. Thus, cranberry researchers are closer to bringing a low-acid cranberry cultivar to the market. This will enable the reduction of added sugars to cranberry food products and therefore benefit human health.

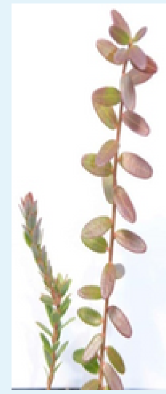

Figure 1.
Dwarf phenotype of putative Ma*lm*/Ma*lm* genotype (left) versus standard (right).

See next page for Table 1

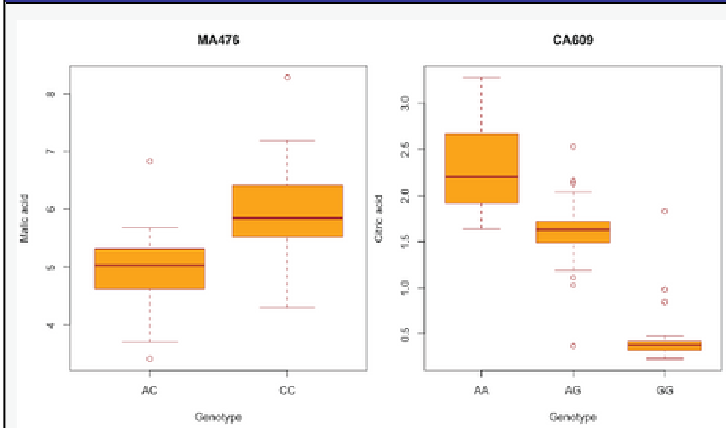

Figure 2.
Results from KASP genotyping low acid population CNJ15-55 (n = 121)
The A allele is causative for low malic acid, while selection for G allele will result in low citric acid.

Table 1. Summary of citric and malic acid markers in cranberry.

Traits	Marker ID	Marker Type	Material Tested	Sensitivity	Specificity	Reference
Low vs Moderate vs High Citric Acid Low CA < 2.5mmg/g; Moderate CA 3-6 mg/g; High CA >6 mg/g	scf258d	SSR	Biparental populations	0.94	0.99	Fong et al. 2020
	SNP CA_609	KASPs		0.91	0.98	
Low vs Moderate vs High Malic Acid Low MA < 2.5mmg/g; Moderate MA 3.5-5 mg/g; High MA >5 mg/g	SNP MA_271	KASPs	Biparental populations	0.94	0.81	Fong et al. 2021
	SNP MA_476	KASPs		0.94	1	

OTHER RESOURCE AND REFERENCES:

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The Vaccinium Coordinated Agricultural Project (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

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