VacTrait

Titratable Acidity (TA) and pH

WHY IS THIS TRAIT IMPORTANT?

Developing blueberry cultivars with improved fruit quality is a high breeding priority. Currently, the industry is challenged by highly variable blueberry fruit quality and flavor across cultivars. In blueberry, titratable acidity (TA) and pH are used as a proxy of the acidity or organic acids (OA) content which affect taste. High TA and lower pH have been correlated with higher fruit sourness and lower overall liking. TA and pH are also used to measure maturation indexes, as organic acid content in blueberry fruit reduce during ripening. Since extracting and quantifying individual organic acids is time consuming and expensive, measuring TA and pH has become a widely used assay to estimate the field quality of produce, especially relative to sourness.

A refractometer used to measure TA on blueberry juice.

Date: 1/23/2024

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

Multiple studies have evaluated TA and pH in blueberry. Genetic and environmental factors were found to affect TA and pH values, moderate to high hereditable traits. This implies that DNA markers or genes controlling TA and pH can be identified and used in blueberry breeding employing quantitative trait loci (QTL) mapping. A QTL represents one or more DNA markers spanning a region of the genome that is significantly associated with a given phenotype. Statistical significance (LOD score) for each QTL can indicate if it has a major or minor effect. Major effect QTLs that are stable and detected across multiple years are the most suitable for DNA assisted breeding. Previous work identified one QTL for pH and this QTL was not tested for stability across years.

DID YOU KNOW?

- An adequate sugar to acid balance is important for blueberry flavor perception, and a SSC/TA ratio between 10-33 and a pH of 2.2 – 4.2 is recommended for a good commercial quality.
- The pH measures the alkalinity of a food or solution using a numerical scale between 1 and 14. A pH value of 1 is most acidic, 7 is neutral, and values above 7 are referred to as basic or alkaline.
- Usually, pH is measured electronically using a pH meter and electrode suitable for water or diluted purees. The pH can be measured by placing the electrode in the solution and waiting until the reading is stable.

HOW DO WE PHENOTYPE THIS TRAIT?

TA is determined by neutralizing the acid present in a known quantity of food sample using a standard base. The endpoint for titration is usually either a target pH or the color change of a pH-sensitive dye, typically phenolphthalein. The volume of titrant used, along with the normality of the base and the volume (or weight) of sample, is used to calculate the TA, expressed in terms of the predominant organic acid. For blueberry, TA is typically expressed as percentage of citric acid (wt/wt) per 1 g fresh weight and is determined by titrating a diluted sample of puree or juice to an endpoint of pH 8.2 with 0.1N sodium hydroxide. Acid refractometers that determine TA quicker are now available. These utilize light, similar to SSC refractometers. The refractometer, which can be pocket-sized, handheld, or bench top, uses a light beam to pass through the juice sample. The light beam bends more as the amount of dissolved organic acid increases. For TA, the meter needs to be matched to the fruit of interest, e.g. blueberry, where the primary organic acid is citric acid. A juice or puree sample diluted at 1:50 (1 g material and 49 ml distilled water) is mixed and an aliquot is placed on the refractometer. An instructional video on how to measure TA in blueberry is available here.

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

VacCAP is working to understand the relationship between TA, pH and organic acids, how TA and pH contributes to flavor/taste, if TA and pH are stable during storage, and determine the genetic mechanisms controlling TA and PH variation in blueberry.

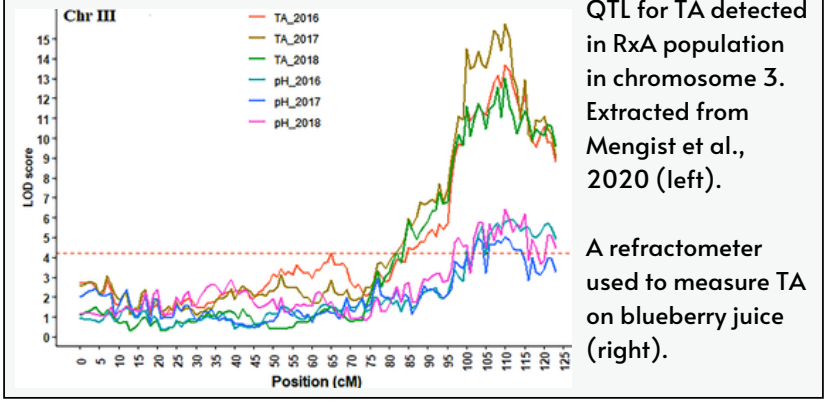
So far in the VacCAP project, two genetic studies for TA and pH in blueberries have been completed. The first study was performed in a population derived from the cross between 'Reveille' and 'Arlen'(RxA) Across 287 siblings, TA value ranged from 0.2 to 2 (% citric acid per 1 g FW). In total, seven QTLs were identified for TA. Six QTLs mapped in chromosome 3 and 5 were stable across three years. These QTLs explained phenotypic variance ranging from 6.8 to 28%.

pH value in the RxA population ranged from 2.2 to 4.8. In total, ten QTLs were identified for pH. QTLs mapped on chromosome 3, were stable across three years, QTLs mapped on chromosome 5 were stable across two years. These QTL explained phenotypic variance ranging from 8 to 13%. As expected the study also determined that pH had strong negative correlation with TA and a moderate negative correlation with citric and quinic acids. In contrast TA had a positive correlation with citric and quinic acids, in particular with citric acid.

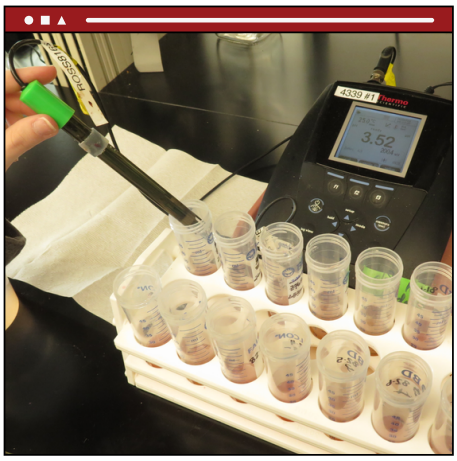
The second study used a population derived from the cross between Draper-44392 and 'Jewel' (DSxJ). Among 190 siblings, TA value ranged from 0.2-1.5 (% citric acid per 1 g FW). In total, four QTLs were identified for TA. For TA, one in chromosome 12 and three in chromosome 3. These QTL explained phenotypic variance ranging from 16 to 22%. The QTLs for TA mapped in chromosome 3 were stable across the years and span the same chromosome region where QTLs for TA were mapped in RxA population.

pH value in the DSxJ population ranged from 3 to 4.8. In total, two QTLs were identified for pH and both QTLs were mapped on chromosome 3 for two consecutive years and explained phenotypic variance ranging from 19 to 21%. The QTLs for pH and TA mapped in chromosome 3 were stable across the years and span the same chromosome region where QTLs for TA and pH mapped in RxA population and a previous QTL identified for pH. Identification of stable QTLs cross studies provide the foundation to continue working on identifying genes underlying this QTL to develop marker assisted selection strategy. On the other side, identification of few non-stable QTLs indicates that in blueberry environmental factors have an influence on TA and pH.

VacCAP plans to conduct additional genetic studies for TA and pH, and experiments to better understand the contribution of TA and pH to consumer preferences and its contribution with OA content. Additionally, advances in molecular resources and improved phenotyping techniques for TA and pH will be evaluated to make the development and application of marker-assisted selection increasingly feasible and cost-effective. In the future, blueberry breeders can use these resources to select blueberry cultivars that have much higher and more consistent fruit quality.



QTL for TA detected in RxA population in chromosome 3. Extracted from Mengist et al., 2020 (left).



A refractometer used to measure TA on blueberry juice (right).

QTLS FOR TA AND PH IDENTIFIED IN BLUEBERRY

Summary of QTLs for pH identified in blueberry

Material	Growing Location	pH value range (°Brix)	# QTL	# Stable QTL/Years	Chr.	Reference
Diversity Panel (N=1575)*	Citra, FL	2.5 - 5	1	N/A	3	Ferrao et al., 2017
RxA (N=287)**	Ivanhoe, NC	2.2 - 4.8	5	1 - 3 Yrs	3	Mengist et al., 2021
DSxJ (N=190)**	Corvallis, OR	3 - 4.8	2	1 - 2 Yrs	3	Mengist et al., 2022

* Previous study ** VacCAP study

Summary of QTLs for TA identified in blueberry

Material	Growing Location	TA value range	# QTL	# Stable QTL/Years	Chr.	Reference
RxA (N=287)**	Ivanhoe, NC	0.2 - 2	7	2 - 3 Yrs	3, 5	Mengist et al., 2021
DSxJ (N=190)**	Corvallis, OR	0.2 - 1.5	4	1 - 3 Yrs	3	Mengist et al., 2022

** VacCAP study

OTHER RESOURCE AND REFERENCES:

- Gilbert, J. L. et al. Identifying breeding priorities for blueberry flavor using biochemical, sensory, and genotype by environment analyses. PLoS ONE 10, 1–21 (2015).
- Colantonio V, Ferrão LFV, Tieman DM, et al. Metabolomic selection for enhanced fruit flavor. Proc Natl Acad Sci U S A. 2022;119(7):e2115865119. doi:10.1073/pnas.2115865119
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- Mengist MF, Grace MH, Mackey T, et al. Dissecting the genetic basis of bioactive metabolites and fruit quality traits in blueberries (Vaccinium corymbosum L.). Front Plant Sci. 2022;13:964656. Published 2022 Sep 2. doi:10.3389/fpls.2022.964656

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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VacCAP
IMPROVING FRUIT QUALITY

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