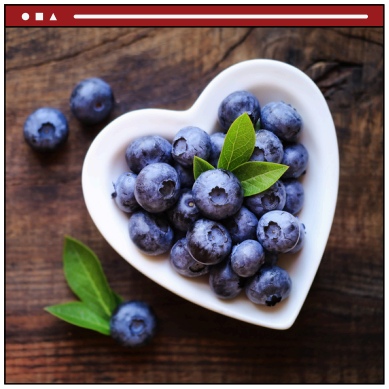
VacTrait

Blueberry Chlorogenic Acid

WHY IS THIS TRAIT IMPORTANT?

Blueberry is well-recognized for its health protective properties with functionality derived largely from bioactive compounds, in particular anthocyanin and chlorogenic acid (CGA). The health benefits associated with blueberry consumption made it very popular among consumers which contributed to the rapid increase in consumption and production in the US and globally during the last 20 years. CGA is a small phenolic compound and one of the predominant compounds in blueberries thought to have a string of biological activity. Phenolics in general are of broad interest due to their color and known benefits to the plant in response to stresses such as UV light and pests (Kundu and Vadassery, 2019; Ngadze et al., 2012).



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Studies have shown that phenolics may also impart health benefits to humans eating them, with CGA providing potentially numerous health protective-benefits. These include antioxidant activity and potential protection against a variety of diseases, including obesity, diabetes, cancer, inflammation, and cardiovascular disease (Naveed et al., 2018). Rigorous human studies are being conducted to verify these health claims. CGA content varies greatly in blueberry due to differences in cultivars and environmental conditions (e.g., sun, rain, soil, pests, etc.). As the benefits of CGA in both plants and humans become better understood, phenotyping the CGA content of berries is becoming increasingly important to inform breeding efforts with downstream impacts on the food chain and consumers.

DID YOU KNOW?

- While blueberries are reported to be one of the best dietary sources of CGA (Table 1), this depends on cultivar and species (e.g., highbush, rabbiteye, etc.).
- Studies show CGA content of blueberries ranges from 4-170 mg/100 FW (Herniter et al., 2023; Mengist et al.,2021). However, Mengist et al. (2022) observed in a biparental population that the maximum CGA content could reach up to 253 mg/100 g FW.
- These studies provide strong evidence that the range of CGA that can be delivered in the diet through blueberry consumption can reach high levels if the fruits are from certain genotypes.

Dietary Sources of CGA | Table 1

Food	Avg. (mg/100 g/mL)
Sunflower seed meal	454
Artichoke heads	202
Red chicory	168
Highbush blueberries	131
Arabica coffee	117
Green chicory	101
Lowbush blueberries	87
Robusta coffee	76
Loquat fruits	55
Rowanberry juice	54

http://phenol-explorer.eu

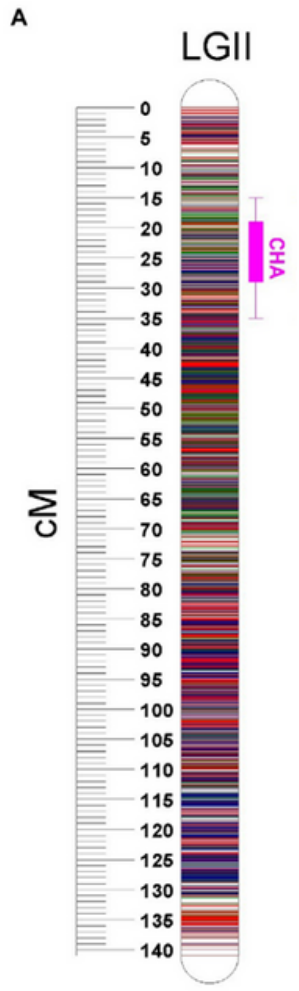
WHAT DO WE KNOW ABOUT THE TRAIT IN TERMS OF DIVERSITY AND GENETICS?

Previous studies also pointed out that while CGA content is affected by environmental factors, the trait has moderate-to-high heritability. This implies that DNA markers or genes controlling CGA could be identified by 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. The level of statistical significance (LOD score) for each QTL can indicate if the QTL has a major or minor effect. Major effect QTLs that are stable and consequently detected across multiple years are the most suitable for DNA-assisted breeding. Work done in VacCAP assessed the genetic mechanisms controlling CGA accumulation in blueberry.

HOW DO WE EVALUATE THIS TRAIT?

CGA and other similar phenols are most often measured using a technique called "liquid chromatography" (Wianowska and Gil, 2019). Liquid chromatography involves pumping a liquid (like water or methanol) in which the compounds to be measured are dissolved through a tube filled with a solid, porous material composed of surfaces. The compounds interact differently with these surfaces based on their distinct chemical properties. Based on these interactions with the surfaces, compounds flow out of the porous material at different rates, which separates the compounds and allows them to be measured individually. Various detectors are then used to verify the identities of the target compounds and determine how much is present. Liquid chromatography involves complex, expensive laboratory instruments and highly trained operators. These instruments are typically found in places like university research labs, pharmaceutical R&D labs, and commercial or government testing facilities. The cost per samples for analysis varies greatly based on the type of instrument used and type of facility. Advancements in analytical technologies continue to decrease the cost and time required for analysis. High-throughput phenotyping of hundreds of genotypes is becoming more feasible and cost-effective.

There are simpler analytical methods, such as the Folin-Ciocalteu method, that require less time and specialized equipment (Perez et al., 2023). However, this method is non-specific and measures hundreds to thousands of different compounds that can react with a dye that changes color. Liquid chromatography is the "gold standard" for phenotyping. Given the cost of evaluating CGA, identifying genes that contribute to CGA accumulation in blueberry fruits could lead to the use of DNA-based assays to indirectly estimate CGA content which could lower the cost to select for this health-related metabolite.



QTL for CGA detected in RxA population in chromosome 2. Extracted from Mengist et al., 2022. (top)

Liquid chromatography mass spectrometry (LC-MS) system (bottom)

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

VacCAP is working to understand the relationship between CGA and other fruit quality traits and determine the genetic mechanisms controlling CGA variation in blueberry.

So far in the VacCAP project, two genetic studies for CGA have been completed. The first study was performed in a tetraploid population named "DSxJ" derived from the cross between Draper-44392 and 'Jewel' (Mengist et al., 2022). Across 190 siblings, CGA value ranged from 2.6-253 mg/100 g FW. One major QTL region in chromosome 2 associated with CGA content was detected for three years. These QTLs explained about 20% of the phenotypic variance.

The second study used a diploid population named "BNJ16-4" derived from the cross between V. corymbosum var. caesariense and V. darrowii. The study evaluated CGA (indicated as 5-O-caffeoylquinic acid, 5-CQA), acetylated caffeoylquinic acids (ACQA1 and ACQA2), and caffeoylarbutin (CA). Among 230 siblings, CGA ranged from 50–126 mg/100g FW, ACQA1 ranged from 2–40 mg/100g FW, ACQA2 ranged from 4–25 mg/100g FW, and CA ranged from 0–179 mg/100g FW. In total, 11 QTLs were identified for CGA, ACQA1, ACQA2, and CA. Six QTLs, two for each CGA, ACQA1, and ACQA2, overlapped in a region of chromosome 2 and were stable across the years and explained 35.6%, 48.7%, and 44.4% of the observed phenotypic variation, respectively. For CA, QTLs in chromosomes 5 and 7 were stable across the years. These QTL explained 9.7% of the observed phenotypic variance. The QTLs for CGA, ACQA1, and ACQA2 mapped in chromosome 2 span the same chromosome region where QTLs for CGA were mapped in the DSxJ population.

These results establish the foundation to initiate further efforts to identify candidate genes controlling the accumulation of CGA which is the first step to develop DNA markers for CGA. In the future, blueberry breeders can use these resources to select blueberry genotypes that have much higher CGA.

Material	Growing Location	Metabolite	# QTL	# Stable QTL/ Years	Chr.	Reference
BNJ16-4 (N=230)	Chatsworth, NJ	Chlorogenic acid (CGA or 5-CQA)	2	1/2 Yrs	2	Herniter et al., 2023
		Acetyl-caffeoylquinic acid iso 1 (ACQA1)	2	1/2 Yrs	2	
		Acetyl-caffeoylquinic acid iso 2 (ACQA2)	2	1/2 Yrs	2	
		Caffeoylarbutin (CA)	5	2/2 Yrs	7, 12	
DSxJ (N=190)	Corvallis, OR	Chlorogenic acid	3	1/3 Yrs	2	Mengist et al., 2022

Summary of QTLs for chlorogenic acid, acetyl-caffeoylquinic & caffeoylarbutin identified in blueberry

OTHER RESOURCE AND REFERENCES:

- Herniter, I., Kim, Y., Wang, Y., Havill, J., Hohnson-Cicalese, J, Muehlbaur, G.J., Iorizzo, M., Vorsa, N. (2023). Trait mapping of phenolic acids in an interspecific (Vaccinium cyrmbosum var. caesariense x V darrowii) diploid blueberry population. Plants, 12(6), 1346 https://doi.org/10.3390/plants12061346
- Kundu, A. & Vadassery, J. (2019). Chlorogenic acid-mediated chemical defense of plants against insect herbivores. Plant Biology, 21 (2) 185-189. https://doi.org/10.1111/plb.12947
- Mengist, M.F., Bostan, H., Young, E. et al. (2021). High-density linkage map construction and identification of loci regulating fruit quality traits in blueberry. Hortic Res, 8, 169. https://doi.org/10.1038/s41438-021-00605-z
- Mengist MF, Grace MH, Mackey T, et al. (2022) Dissecting the genetic basis of bioactive metabolites and fruit quality traits in blueberries (Vaccinium corymbosum L.). Front Plant Sci, 13, 964656. https://doi.org/10.3389/fpls.2022.964656
- Naveed, M., Hejazi, V., Abbas, M., Kamboh, A., Khan, G., Shumzaid, M., Ahmad, F., Babazadeh, D., FangFang, X., Modarresi-Ghazani, F., WenHua, L., & XiaoHui, Z. (2018), Chlorogenic acid (CGA): A pharmacological review and call for further research. Biomedicine & Pharmacotherapy, 97, 67-74. https://doi.org/10.1016/j.biopha.2017.10.064.
- Ngadze, E., Icishahayo, D., Coutinho, T. & van der Waals, J. (2012), Role of Polyphenol Oxidase, Peroxidase, Phenylalanine Ammonia Lyase, Chlorogenic Acid, and Total Soluble Phenols in Resistance of Potatoes to Soft Rot. Plant Disease. 96(2), 186-192. https://doi.org/10.1094/PDIS-02-11-0149
- Wianowska, D., and Gil, M. (2019). Recent advances in extraction and analysis procedures of natural chlorogenic acids. Phytochem Rev, 18, 273–302. https://doi.org/10.1007/s11101-018-9592-y
- Pérez M, Dominguez-López I, Lamuela-Raventós RM (2023) The Chemistry Behind the Folin-Ciocalteu Method for the Estimation of (Poly)phenol Content in Food: Total Phenolic Intake in a Mediterranean Dietary Pattern. J Agric Food Chem, 71(46), 17543-17553. https://doi.org/10.1021%2Facs.jafc.3c04022

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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