Received: 14 October 2024 | Accepted: 10 February 2025

DOI: 10.1002/tpg2.70014

The Plant Genome

ORIGINAL ARTICLE

Special Section: Multi-Omics Prediction in Plant Breeding

# Variation for QTL alleles associated with total dissolved solids among crop types in a GWAS of a *Beta vulgaris* diversity panel

**Audrey Pelikan** | **Irwin L. Goldman**

Department of Plant and Agroecosystem Sciences, University of Wisconsin-Madison, Madison, Wisconsin, USA

**Correspondence**
Irwin L. Goldman, Department of Plant and Agroecosystem Sciences, University of Wisconsin-Madison, 1575 Linden Drive, Madison, WI 53706, USA.
Email: ilgoldma@wisc.edu

Assigned to Associate Editor Manish Pandey.

**Funding information**
USDA-Hatch

## Abstract

Sweetness is a main component of the table beet (*Beta vulgaris* L.) flavor profile and a key determinant of its market success for fresh consumption. Total dissolved solids (TDS) is a proxy for sugar content in produce and are easily measured through a refractometer, making TDS valuable in breeding programs focused on increasing sweetness. A diversity panel of 238 accessions from the *Beta vulgaris* crop complex and wild relatives was assembled and genotyped using genotyping-by-sequencing, yielding 10,237 single nucleotide polymorphisms (SNPs) from 226 full panel accessions and 9,847 SNPs from table beet only accessions after filtering. The panel was phenotyped in field trials over 2 years and mean values were adjusted using best linear unbiased estimates. TDS levels varied among crop types and a broad-sense heritability of 0.90 indicated that phenotypic differences can be attributed in large part to genetic variation. A genome-wide association study (GWAS) uncovered four quantitative trait loci (QTLs) identified across multiple models to significantly associate with TDS. A QTL on chromosome 2 was consistently identified among GWAS models, explaining 12.1%–62.6% of the phenotypic variation in the full panel. *Bevul.2G176300*, a gene directly involved in the sucrose biosynthesis pathway, was located downstream the significant marker. A second QTL identified on chromosome 7 revealed QTL alleles that may differentiate between table beet accessions, explaining nearly half the phenotypic variation, and is the first QTL reported in association with TDS unique to table beet. The QTL described can be used to efficiently breed for higher TDS levels in *Beta vulgaris*, avoiding intercrop type crosses and linkage drag.

**Abbreviations:** AARS, Arlington Agricultural Research Station; ANOVA, analysis of variance; BLINK, Bayesian information and linkage disequilibrium iteratively nested keyway; BLUE, best linear unbiased estimate; FarmCPU, fixed and random model circulating probability unification; GAPIT, genomic association and prediction integrated tool; GBS, genotyping-by-sequencing; GLM, general linear model; GWAS, genome-wide association study; HARS, Hancock Agricultural Research Station; LD, linkage disequilibrium; LOCO, leave one chromosome out; MLM, mixed linear model; NPGS, National Plant Germplasm System; PC, principal component; PEV, prediction error variance; PVE, percent variance explained; QTL, quantitative trait locus; SNP, single nucleotide polymorphism; SPS, sucrose-phosphate synthase; TDS, total dissolved solids; USDA, United States Department of Agriculture; UW, University of Wisconsin; WBDP, Wisconsin Beta Diversity Panel.

**Plain Language Summary**

Sweetness is a key factor in table beet (*Beta vulgaris* L.) flavor and influences the crop's success in the consumer market. Total dissolved solids (TDS), a measure of sugar content, is a major breeding target for improving beet root sweetness. To identify areas of the genome associated with TDS, a diverse set of *Beta vulgaris* accessions was studied. By combining phenotypic and genotypic data, a genome-wide association study was conducted and identified four genetic markers associated with TDS. Notably, a region on chromosome 2 was found to play a significant role in TDS levels, as it is nearby a gene directly involved in sugar production. Another genomic region was found on chromosome 7, which may help distinguish TDS levels between beet varieties. These findings are useful for breeding sweeter beets while avoiding undesirable traits, ultimately enhancing the crop for fresh market consumption.

## 1 | INTRODUCTION

*Beta vulgaris* L. encompasses a diverse set of cultivated crops (table beet, fodder beet, Swiss chard, and sugar beet), originating from the crop wild relative *Beta vulgaris* subsp. *maritima*. Table beet (*Beta vulgaris* subsp. *vulgaris*) is often characterized by its swollen, fleshy hypocotyl and root containing betalain pigments along with its distinguishingly sweet, yet earthy flavor profile (Goldman & Navazio, 2003). In recent years, beet production has increased, and consumers have expressed greater interest in its use and consumption. This vegetable has, in fact, acquired a wider following in both the gourmet and health food spaces (Damrosch, 2016), particularly in the fresh market. Beets are associated with a number of direct consumer benefits, especially in terms of their vitamin and mineral content, antioxidants, and anti-inflammatory effects (Ceclu & Nistor, 2020). The crop types included in this complex have been adapted for specific end uses and thus exhibit pronounced phenotypic differences. This diversity aids in the plant's appeal for culinary uses and provides a unique opportunity for improvement through collaboration of breeding techniques and culinary expertise (Beans, 2017).

When a consumer bites into a table beet, they may experience various sensory attributes related to its main flavor components: geosmin, oxalic acid, and sucrose. The distinctive "earthy" or "dirt-like" aroma of geosmin is a signature flavor that uniquely characterizes the table beet from other vegetables (Jiang et al., 2007; Liato & Aïder, 2017). While some consumers appreciate this earthy aroma, it often deters others. Geosmin has proven difficult to remove from water sources, and its removal is even more challenging in table beet due to its endogenous production within the plant (Hanson & Goldman, 2019; Maher & Goldman, 2018). Oxalic acid often causes a burning sensation and, although beneficial to plant defense (Fasset, 1973), can be harmful to humans as the main component of kidney stones and driver of decreased bioavailability of other nutrients, notably calcium (Altamirano et al., 2018; Brogren & Savage, 2003; Freidig & Goldman, 2011; Noonan & Savage, 1999). Oxalic acid can be reduced by cooking the raw material; however, challenges arise for the fresh eating market (Yadav & Sehgal, 2003). Sucrose, the focus of this study, is one of the main compounds associated with sweetness in a variety of fruits and vegetables and a main sensory attribute of the table beet (Hanson & Goldman, 2019). Total dissolved solids (TDS) is commonly used as a proxy for sucrose and is expressed as degrees Brix through a refractometer, which corresponds directly to percentage of TDS (Feller & Fink, 2004). Although small amounts of fructose and glucose are present (Bach et al., 2014), sucrose makes up the vast majority of table beet solutes, making TDS an effective proxy (Wolyn & Gabelman, 1990). High sucrose concentrations are desirable for the fresh eating beet, to create the crisp sweet bite recently desired by consumers. TDS levels are collected in many crops, notably tomato (*Solanum lycopersicum* L.). Processing tomatoes exhibit high TDS levels to increase the efficiency of producing concentrated products like tomato paste. Certain fresh market types have also been bred for high levels of TDS to attract consumers. Selection for TDS has been particularly effective in tomato in part because the trait possesses strong genetic effects and can be measured reliably (Merk et al., 2012; Sánchez et al., 2020).

The sugar beet, characterized by accumulation of sugar in the root, is thought to be selected from a fodder beet population, which was created by the hybridization of chard and table beet (Dohm et al., 2014; McGrath & Panella, 2018). With this hypothesis, researchers attempted to resynthesize sugar beet by crossing chard and fodder beet and selecting for sugar beet shape and composition. Recurrent selection for sugar content did not increase levels in the progeny (Fischer, 1989), but it is possible that the duration of this experiment was too short.

Narrow-sense heritability for sugar content (%) was estimated at 0.60 in a large testcross population of sugar beet genotypes (Würschum et al., 2011) and multiple quantitative trait loci (QTLs) have been identified in association with sugar content in sugar beet (Reif et al., 2010; Würschum et al., 2011). Wolyn and Gabelman (1990) found nonsignificant changes in TDS after three cycles of half-sib selection in table beet; however, these populations were also undergoing selection for elevated betalain pigment concentration. They also reported low realized heritability for TDS at 0.25 and 0.27 for the two populations, respectively. More recently, repeatability for TDS in table beet was reported at 0.43, indicating a moderate role of genotype in the total variation (Hanson & Goldman, 2019).

Hanson and Goldman (2019) found that TDS content in table beet was influenced by both genotype and genotype × environment interactions. Year × location interactions were important contributors to variance in TDS levels, but genotype was considered the primary influence in a 2-year table beet field study, alluding to the genetic control over TDS content (Hanson & Goldman, 2019). D'Angelo and Goldman (2023) found that the accumulation of TDS in table beet roots was lowest early in the growing season, increasing until 12 weeks after planting. Diurnal sampling showed fluctuations of up to 4°Brix during a 12-h period, suggesting additional localized influences on TDS levels (D'Angelo & Goldman, 2023). Genotype × environment interactions may make recurrent selection more difficult, indicating that control of environmental factors and identification of key genomic regions are crucial to the improvement of the table beet flavor profile (Hanson & Goldman, 2019).

One approach to breeding for flavor traits involves the identification and utilization of QTLs, which can be facilitated through both linkage and association mapping. A genome-wide association study (GWAS) has its benefits in QTL identification over traditional gene mapping due to higher resolution and the opportunity to use natural populations to capitalize on historic recombination (Brachi et al., 2011). However, GWAS studies are often most useful when paired with linkage mapping, to mitigate each other's limitations (Korte & Farlow, 2013). Many QTLs related to flavor traits in crop species have been identified including volatile organic compounds in blueberry (*Vaccinium corymbosum* L.) (Ferrão et al., 2020) and soluble solids and sugar content in watermelon (*Citrullus spp*. L.) (Fall et al., 2019; Katuuramu et al., 2023). Specifically, QTLs corresponding to sugar content have been of interest as an important factor in deploying molecular-assisted breeding for flavor. Research in this domain has extended to root crops like carrot (*Daucus carota* L.) where investigations have identified specific genetic loci, such as the reducing sugar (Rs) locus, which is a single major gene determining sugar type (Freeman & Simon, 1983). Researchers developed a polymerase chain reaction based marker to score the status of the Rs locus accurately in 1 week old seedlings, providing a useful and efficient tool for breeders (Yau et al., 2005).

**Core Ideas**

- A diversity panel of 238 *Beta vulgaris* accessions, comprised of six crop types, was phenotyped for total dissolved solids (TDS).
- A chromosome 2 quantitative trait locus (QTL) is 332 kb upstream a candidate gene directly involved in the sucrose biosynthesis pathway.
- A QTL on chromosome 7 revealed QTL alleles that may differentiate among table beet accessions for sweetness.
- These QTLs may be useful in breeding for TDS within each crop type to avoid wide crosses and genetic drag.

Due to its economic importance, sugar beet has received more attention from plant breeders than the table beet. Sugar beet acreage exceeds table beet acreage in the United States by more than 100-fold, and substantial efforts in both the public and private sectors have addressed the development of improved sugar beet cultivars. Numerous QTLs have been identified in sugar beet, particularly for resistance to Cercospora leaf spot, powdery mildew, and rhizomania (M. Wang et al., 2019), along with important agronomic traits such as yield, potassium, sodium, and sugar content (Schwegler et al., 2014; M. Wang et al., 2019). QTLs associated with sugar content have been identified on all nine *Beta vulgaris* chromosomes. Previous GWAS in sugar beet have revealed four markers on chromosomes 3, 4, and 5 to be associated with sugar content (%) and validated by prior reports in the literature (Reif et al., 2010; Schneider et al., 2002; Weber et al., 2000; Würschum et al., 2011). The genetic control of sugar content in many minor vegetable crops has yet to be elucidated, and no QTLs associated with sugar content in table beet have yet been reported. Crop improvement in fresh-eating produce has largely depended on conventional breeding approaches; however, application of marker-assisted selection has the ability to unlock a "transformative era" for the fresh-eating table beet (Shahwar et al., 2023).

Despite the success of QTL identification in sugar beet and other high value crops, the genetic architecture of key traits in table beet leaves much room for discovery. Hanson et al. (2021) identified two regions on chromosome 8 in table beet that showed significant association with the flavor trait geosmin in an $F_{2:3}$ mapping population. The first table beet reference genome has been sequenced and assembled from the inbred line W357B, through contributions of

Wigg et al. (2023) and Dorn (2022). Using this new reference genome and linkage mapping, Wigg et al. (2023) identified QTL on chromosome 2 associated with resistance to *Rhizoctonia solani*, the fungi responsible for root and crown rot. Most recently, Dixon and Goldman (2024) reported QTL associated with response to Cercospora leaf spot, caused by the fungal pathogen *Cercospora beticola*, in table beet, using similar germplasm. To date, neither comprehensive GWAS nor linkage mapping studies for many important traits have been conducted in table beet.

Understanding the genetic architecture of the compounds that affect flavor and eating quality will help breeding programs aimed at enhancing crop quality and meeting culinary preferences. Identification of QTL can also assist in the deployment of breeding tools, such as marker-assisted selection, which allows breeders to select for flavor traits on a genetic basis, negating the costly consumer trials and better allocation of resources (Klee, 2010). The objective of this work was to identify regions of the genome associated with TDS through a GWAS of a *Beta vulgaris* diversity panel, with an emphasis on understanding if different crop types possess unique QTL for this trait.

## 2 | MATERIALS AND METHODS

### 2.1 | Plant material and experimental design

The germplasm used in this study was comprised of 238 unique accessions from the *Beta vulgaris* L. crop complex, collectively referred to as the Wisconsin Beta Diversity Panel (WBDP, Table S1). The WBDP (Dixon & Goldman, 2024) consisted largely of table beet accessions but encompassed other subspecies, including Swiss chard, fodder beet, sugar beet, and wild relative *Beta vulgaris* subsp. *maritima*. Seed was obtained from various sources and chosen upon crop type and availability. These sources included United States Department of Agriculture (USDA) laboratories, USDA National Plant Germplasm System (NPGS) collections, commercial sources, and the University of Wisconsin (UW) table beet breeding program. Many of the accessions are cultivars currently grown by producers all over the world.

In general, crop types were assigned in accordance with standard NPGS assignments (https://www.ars-grin.gov/npgs/). Comprehensive recategorization occurred using genotypic clustering and visualization, crop use, root imagery, and knowledge of cultivar origins. Some accessions did not clearly fit into a crop type category and were denoted as mixed. It is worth noting that research has suggested certain *Beta vulgaris* subsp. *maritima* accessions utilized in this study may in fact be *Beta vulgaris* subsp. *adanensis* (Sandell et al., 2022). This underscores the complexity of categorization within the germplasm and highlights the importance of further genetic and taxonomic investigations. Regardless, these accessions are still considered wild relatives of the *Beta vulgaris* L. cultivated crops.

Accessions were grown in 2022 and 2023 in a randomized complete block design with three blocks. A plot consisted of a single row, 1.8 m in length and 46 cm row spacing. Cultivars whose seed was abundant were planted by a hand-propelled Planet Jr. planter with a cone seeder attachment for the entirety of the plot. Due to limited quantities of seed obtained for the USDA-NPGS accessions, these seeds were manually sown in the center 0.6 m of the row, while the remaining sections of row were hand planted with beet cultivar Ruby Queen in 2022 and Bull's Blood in 2023. Immediately after sowing, all fields were treated with pre-emergent herbicides Dual Magnum (Syngenta) and Nortron SC (Bayer Crop Science), which are metachlor and ethofumesate based, respectively.

This experiment was conducted at the UW Arlington Agricultural Research Station (AARS) in Arlington, WI, and Hancock Agricultural Research Station (HARS) in Hancock, WI during the 2022 and 2023 growing seasons. The WBDP was grown at Arlington on a Plano silt loam soil (fine-silty, mixed, superactive, mesic Typic Argiudolls). In 2022, the field was planted on May 25, harvested on August 15, and received 30.8 cm of precipitation during the growing season. In 2023, the Arlington field was sown on May 24, harvested on August 22, and received 25.0 cm of rainfall (National Weather Service, 2022–2023). Drought during critical early growth stages necessitated supplementation with irrigation in 2023.

A subset of the WBDP, comprised mainly of commercial cultivars and UW table beet germplasm (G113–G238, Table S1), was planted on Plainfield sand and Pearl and Friendship loamy sand soils (mixed, mesic Typic Udipsamments; loamy, mixed, superactive, mesic Arenic Oxyaquic Hapludalfs; mixed, frigid Typic Udipsamments) at Hancock. In 2022, the field was planted and harvested on May 17 and August 17, respectively, and received 35.2 cm of rainfall. In 2023, the experiment was sown on May 25, harvested on August 15, and received 16.0 cm of precipitation (University of Wisconsin-Madison, 2022–2023). Hancock fields received consistent irrigation and nitrogen applications for the duration of the growing season.

### 2.2 | Phenotypic data collection

At harvest, 10 roots were selected from the middle third of the row, and aboveground tissue was removed. All roots from a row were bulked and placed in a labeled mesh harvest bag for transportation and storage. Roots were stored at 6.0°C until sampling occurred. At the time of sampling, six representative roots were chosen and bisected. The roots were sampled by removing a cylinder 1 cm in diameter, totaling 15 cores, containing both epidermal and root tissue. Cores were placed

in a labeled polyethylene bag and stored at −20°C until further processing.

To prepare beets for flavor analysis, frozen cores were placed in a NutriBullet Personal Blender (Model NBR-0801; Nutribullet) and ground into a paste on a 600-W motor base for 30 s. Samples were standardized to include 5% epidermal tissue by weight to account for varying levels of flavor compounds in epidermal and fleshy root tissue (Albihn & Savage, 2001; D'Angelo & Goldman, 2023; Lu et al., 2003). A subset of the ground tissue was used to collect TDS measurements immediately by squeezing 10 μL of liquid through a Kimwipe (Kimberly-Clark Professional) onto a refractometer (ABBE-3L; Thermo Fisher Scientific). Six refractometer measurements were recorded for each sample.

## 2.3 | Phenotypic data analysis

The experimental unit was the mean of the six subsamples from each row. All further data analysis and visualization were conducted in R statistical software v4.3.1. Each year–location combination was treated as a separate environment due to changes in field location and weather conditions, resulting in four environments (Arlington 2022, Arlington 2023, Hancock 2022, and Hancock 2023). Accessions were excluded from analysis if they (1) did not exhibit at least one observation in each of the four environments, (2) were excluded during the genotypic data analysis for poor quality, or (3) roots were too fibrous to sample. Additionally, individual sample outliers were eliminated if they fell beyond three standard deviations from the mean. In Hancock 2022, inadvertent chemical sprays occurred, leading to the removal of 10 row values. Various models were employed to analyze the data, each selected according to the specific objectives of the analysis. A fixed effect model analysis of variance (ANOVA) was performed to assess Brix variation between and within three balanced groups of data (Arlington 2022 and 2023 [$n = 167$], Hancock 2022 and 2023 [$n = 106$], and Arlington and Hancock 2022 and 2023 [$n = 99$]).

Broad-sense heritability ($H^2$) was estimated according to Holland et al. (2002) and Vega and Goldman (2023). A completely random effects model was fit for variance component estimation using the "lme4" package (Bates et al., 2015). Entry-mean broad-sense heritability was estimated using the following equation:

$$H^2 = \frac{\sigma_G^2}{\sigma_G^2 + \text{PEV}}$$

where $\sigma_G^2$ is the variance associated with genotype and PEV is prediction error variance. PEV can be defined as the following:

$$\text{PEV} = \frac{\sigma_{GE}^2}{e} + \frac{\sigma_\epsilon^2}{be}$$

where $\sigma_{GE}^2$ and $\sigma_\epsilon^2$ are variance components associated with genotype × environment interactions and the residual error variance, respectively. The denominator terms $e$ and $b$ correspond the number of environments (four) and replications (three), respectively.

Wilcoxon signed rank and Spearman rank correlation tests were performed on all pairwise combinations of environments to evaluate the significance of change in rank and repeatability. An overall nonsignificant rank change warrants the generation of a single genotype mean value across all environments. Single genotypic values were estimated for each accession through combining phenotypic data across replications and environments. Values were estimated according to the following mixed effects model,

$$Y_{ijkl} = \mu + G_i + E_j + B_{j(k)} + (G \times E)_{ij} + \epsilon_{ijkl}$$

which was built upon experimental design parameters. Genotype ($G$) was modeled as a fixed effect; environment ($E$), block ($B$) nested within environment, and the genotype × environment ($G \times E$) interaction were modeled as random. Best linear unbiased estimates (BLUEs) were subsequentially calculated for each accession using the package "lme4" (Bates et al., 2015), with $G_i$ as the fixed effect.

## 2.4 | Genotyping-by-sequencing

Whole leaf tissue from up to 15 plants for each accession was collected during the 2022 field season. Tissue disks approximately 1 $cm^2$ in size were pooled and placed in a microtube plate. The plate was then frozen at −80°C and subsequently lyophilized. Several accessions were germinated in an incubator, and etiolated petiole tissue was utilized. DNA extraction and genotyping-by-sequencing (GBS) was completed using the Elshire et al.'s (2011) method by the UW Biotechnology Center (Madison, WI). Genotyping was conducted in accordance with Wigg et al. (2023). Briefly, DNA libraries were prepared by dual digestion with *Nsi*I and *Bfa*I restriction enzymes. These enzymes were selected upon prior optimization of table beet (Hanson et al., 2021). Paired end reads (2 × 150 bp) were generated on an Illumina NovaSeq6000 sequencer (Illumina). Quality control, sequence alignment, and single nucleotide polymorphism (SNP) calling were completed on the GBS data by the UW Bioinformatics Resource Center (Madison, WI). The Tassel v2 GBS Pipeline (Glaubitz et al., 2014) and Bowtie2 alignment software (Langmead & Salzberg, 2012) were used to align demultiplexed forward reads to the W357B table beet reference genome (Dorn, 2022; Wigg et al., 2023). The Tassel v2 Discovery and Production

SNP Caller system was used to identify 753k unfiltered variants, returned in a variant call format file, as outlined in Wigg et al. (2023).

## 2.5 | Genotypic data analysis

Four accessions were identified as poor quality, based on demultiplexed reads per sample, and removed from any further analysis, along with accessions where no phenotype was present. Bcftools v1.17 was used to filter the data to include only bi-allelic SNPs, excluding multi-allelic SNPs and indels, with a read depth of 10 for at least 90% of the samples and no missing data. Furthermore, markers that did not have at least one individual in each genotype call were removed and subsequentially filtered for a minor allele frequency greater than 0.05. Genotype calls were converted to their corresponding dosages: 0/0 to 0 (homozygous reference), 0/1 to 1 (heterozygous), and 1/1 to 2 (homozygous alternate). Markers exhibiting zero variance were removed. Scaffolds of the W357B table beet reference genome were converted to chromosomes based on the sugar beet reference genome's (RefBeet 1.2.2, Dohm et al., 2014) chromosomal alignment, as described in Wigg et al. (2023). After applying these filtering criteria, a final SNP set containing 10,237 SNPs from 226 individuals was used for final analysis. A table beet only file was also created based on the same filtering criteria, yielding 9,847 SNPs from 165 individuals.

Principal component analysis was conducted using the R package "prcomp" for full panel and table beet only genotype files. Linkage disequilibrium (LD) was estimated on a genome-wide basis using GWASPoly software (Rosyara et al., 2016), which internally estimates the spline using the "scam" package in R. A threshold of $r^2 = 0.1$ was used to assess LD decay and determine linkage block sizes.

## 2.6 | Genome-wide association study

BLUE values served as the phenotypic response variable for association mapping, where SNPs underwent testing for significant association with the phenotype. GWAS was performed using both full panel (WBDP) and table beet only genotype matrices in the GWASpoly (Rosyara et al., 2016) and Genomic association and prediction integrated tool (GAPIT) (version 3, J. Wang & Zhang, 2021) software packages. For GWASpoly, population structure was controlled using a kinship ($K$) matrix, calculated through the leave one chromosome out (LOCO) method (Yang et al., 2014). Two principal components (PCs) were included in analysis of the full panel and excluded when examining table beets only. Additive (additive) and simplex dominant (1-dom) marker effect models were tested. In the GAPIT software analysis, population structure was controlled using a $K$ matrix calculated through the VanRaden method (VanRaden, 2008), with two PCs for the full panel and none for the table beet only panel. Four models were used to test for associations: general linear model (GLM); mixed linear model (MLM); fixed and random model circulating probability unification (FarmCPU); and Bayesian information and linkage disequilibrium iteratively nested keyway (BLINK).

For all models, the discovery threshold was set as a Bonferroni threshold with a significance level of $p < 0.05$, in relation to the total number of marker tests, corresponding to 5.31 for the full panel and 5.29 for the table beet only panel. Manhattan and effect plots were generated to display significant SNPs and their respective dosages relative to TDS in genetic models. Quantile–quantile (QQ) plots of the observed versus expected $-\log_{10}(P)$ values were used to evaluate the efficacy of population structure control. QTLs were determined by filtering the output to include only the most significant marker within a specified window, corresponding to the LD block size. Percent variance explained (PVE) for each marker was internally calculated using each GWAS software program. It may be noted that a significant SNP identified in a GWAS analysis could be described as an SNP-trait association or a QTL. In this study, the regions found to significantly associate with TDS levels are referred to as QTL to maintain consistency with current literature.

## 2.7 | Candidate gene search

A candidate gene search was conducted for the QTL identified across multiple GWAS models. Annotated genes from the *Beta vulgaris* EL10.2 sugar beet reference genome (McGrath et al., 2023) were transferred by sequence homology to the table beet W357B reference genome using the software package "Liftoff" (Shumate & Salzberg, 2021), to create a table beet annotated gene file. The search window upstream and downstream of the QTL was determined by the linkage block size, corresponding to the genomic distance at which the LD decay curve intersected with $r^2 = 0.1$. Integrated Genomics Viewer was used to identify genes within the search window using the W357B Liftoff annotation (Thorvaldsdóttir et al., 2013). Phytozome v13, the Plant Comparative Genomics portal of the Department of Energy's Joint Genome Institute, was used to identify candidate genes based on predicted function and their relevance to TDS (Goodstein et al., 2012).

# 3 | RESULTS AND DISCUSSION

## 3.1 | Phenotypic data analysis

Brix values at AARS ranged from 5.08 to 22.4 in 2022 and 8.4 to 22.3 in 2023, with mean values of 11.8 and 13.1, respectively. At HARS, Brix values ranged from 5.1 to 18.0

in 2022 and 6.8 to 21.3 in 2023, with mean values of 10.6 and 12.2, respectively. Brix values, on average, were 1.5 Brix greater in 2023 when compared to 2022 across both locations, likely attributable to varying moisture conditions among years. Accessions also exhibited higher Brix values in Arlington, WI when compared to Hancock, WI. ANOVA was conducted separately on three balanced sets of observations: Arlington 2022 and 2023 (167 accessions), Hancock 2022 and 2023 (106 accessions), and Arlington and Hancock 2022 and 2023 (99 accessions). Overall, each ANOVA revealed significant effects for genotype, environment, and their respective interaction (Table S2). A significant block effect was observed among the two AARS environments, but not among the two HARS environments. Many of the accessions in the full panel, grown at AARS, were gene bank accessions that have not undergone intense selective pressure and therefore exhibited higher intra-accession heterogeneity. The accessions grown at HARS were mainly cultivars developed for growers, though these accessions still varied due to the presence of both open-pollinated cultivars and F1 hybrids. Many table beet F1 hybrids are produced using open-pollinated cultivars as the male parent and are thus less uniform than F1 hybrids where both parents are highly inbred.

ANOVA identified significant differences between environments (Table S2), although accession rank was generally conserved. Spearman rank correlation analysis between all pairs of environments for TDS revealed significant correlations between the environments. The correlations were strong to moderate, ranging from 0.71 to 0.39. The Spearman rank correlation coefficients fell into two categories: 0.39–0.42 and 0.69–0.71. The lower range of coefficients always contained Hancock 2022 as one of the paired environments, alluding to its deviation from the other three environments. This might be attributed to unintentional chemical sprays to the plots, leading to an overall reduction in TDS levels. Severe outliers were removed, but differences persisted. Despite variations in strength, Wilcoxon rank tests revealed nonsignificant rank changes for all pairwise environmental comparisons, justifying the combination of Brix values across years and locations to create a single BLUE value. The correlation analysis indicated that genotype trends remained consistent across environments, regardless of statistically significant genotype × environment interactions. Variability observed could be due to previously reported diurnal fluctuations in TDS, with changes up to 4°Brix over a 12-h period (D'Angelo & Goldman, 2023). Both cultivar and environment remain important determinants of TDS, further supporting the claims of D'Angelo and Goldman (2023).

Despite the influence of growing environment, genotype constituted a primary source of variation (Table 1). Similar results have been reported for TDS in table beet and other crops (Hanson & Goldman, 2019; Merk et al., 2012). Entry-mean broad-sense heritability ($H^2$) was estimated as 0.90 for the full panel and 0.80 for the table beet only panel. These heritability levels highlight the strong contribution of variance components associated with genotype relative to variance components associated with genotype × environment interactions and residual error. Heritability for the full panel was larger than that for the table beet only panel, likely due to more pronounced genotypic differences among accessions in the full panel. Heritability estimates reported in this study were larger than previously reported (Hanson & Goldman, 2019), but a larger panel with more accessions was used here.

BLUE values ranged from 7.85 to 20.97, with an average of 11.93 (Figure 1; Table S1). Many of the larger values originated from, but were not limited to, sugar beet, *Beta vulgaris* subsp. *maritima*, and chard crop types. Lower to average values were typically observed in fodder and table beet types. It is worth noting that TDS can be defined as the concentration of solutes dissolved in a particular amount of water. In this case, TDS measurements depend on both the amount of solutes and the volume of water used for their dissolution in the roots. *Beta vulgaris* subsp. *maritima* and some chard crop types possess roots with lower levels of moisture; while sugar beets contain high levels of sucrose; table beets contain high levels of moisture with varying levels of sucrose; and fodder beets have a high moisture content with lower sucrose levels. These solute:moisture ratios directly influence TDS measurements and could be interpreted as indicators of the root's water content. However, sugar beets challenge this notion, as they have high moisture content for dissolution yet also exhibit high TDS levels. Comparisons within crop groups may be more meaningful, although larger numbers of crop group accessions will be needed to make definite conclusions.

Not only does *Beta vulgaris* subsp. *maritima* possess low moisture, but it also has a fibrous, woody root that is challenging to sample. To extract TDS measurements, the root cell walls must be ruptured through the grinding process. Breaking down the lignin in cells present in many *Beta vulgaris* subsp. *maritima* and some Swiss chard accessions proved difficult, leading to the exclusion of certain samples from the analysis. For instance, PI 604518 was removed from the analysis due to the difficulty in assessing true TDS levels from pieces of fibrous tissue. Further investigation is needed to determine the true sucrose content and perceived sweetness in the roots of *Beta vulgaris* subsp. *maritima* and Swiss chard varieties.

## 3.2 | Genotypic data and population structure

The average read depth per marker across all individuals was 25. This is sufficient depth to run GWAS. However, due to *Beta vulgaris* L. being an outcrossing species and the read depth of 25, it is challenging to distinguish between true

**TABLE 1** Source of variation, variance estimate for total dissolved solids (TDS), and proportion of variance for the full Wisconsin Beta Diversity Panel and a subset of the table beets only, grown in four Wisconsin field environments (Arlington 2022, Arlington 2023, Hancock 2022, and Hancock 2023).

| | Full panel | | | Table beet only | | |
|---|---|---|---|---|---|---|
| Source | Estimate | Proportion of variance | Significance | Estimate | Proportion of variance | Significance |
| Genotype ($\sigma^2_G$) | 3.14 | 0.445 | *** | 0.88 | 0.250 | *** |
| Environment ($\sigma^2_E$) | 1.15 | 0.163 | *** | 0.66 | 0.187 | *** |
| G × E ($\sigma^2_{GE}$) | 0.79 | 0.111 | *** | 0.32 | 0.090 | *** |
| Block/E ($\sigma^2_{BE}$) | 0.03 | 0.005 | *** | 0.006 | 0.002 | NS |
| Residual ($\sigma^2_\epsilon$) | 1.94 | 0.276 | | 1.67 | 0.471 | |

Abbreviation: NS, nonsignificant.
***Significant at $p \leq 0.001$.

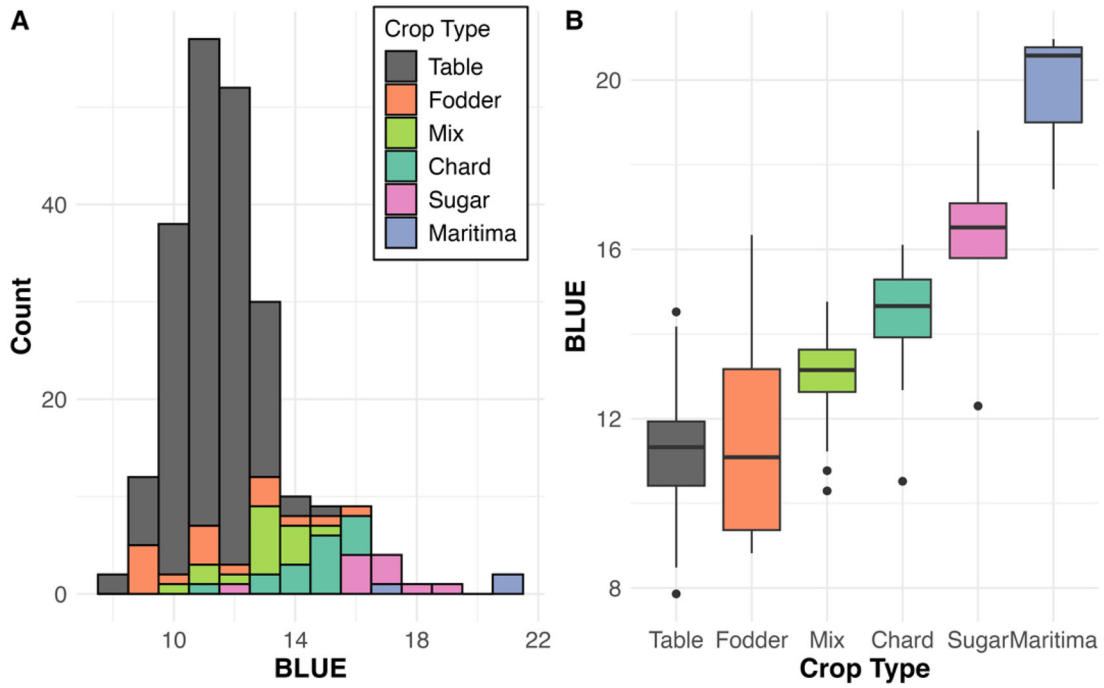


**FIGURE 1** Distribution and variation of total dissolved solids (TDS) as best linear unbiased estimates (BLUEs) from the Wisconsin Beta Diversity Panel grown as a replicated field experiment in four Wisconsin environments. The stacked histogram (A) and boxplot (B) exhibit variation within and between crop type group categorization.

heterozygosity and intra-accession heterogeneity. Thus, the heterozygote state should be interpreted with caution as it may represent a true heterozygote state or accession heterogeneity and was not heavily considered in the effect plots.

Galewski and McGrath (2020) reported the mean expected heterozygosity ($2pq$) within table beet to be 0.147, with even larger diversity contained in other crop type groups, although sample sizes were relatively small. Techniques that utilized bulked tissue from multiple accessions for GBS have proven useful in such situations (Anand et al., 2016). Alternatively, by sequencing at a higher depth, it is possible to utilize continuous measures of alternate allele frequency (Dixon & Goldman, 2024), instead of categorical allele dosages used here.

Population structure was evaluated from the genotype matrices of the full panel ($n = 226$) and table beet only panel ($n = 165$), respectively (Figure 2). The first three PCs explained 19.5% of the total genotypic variation. PC1 explained 12.0% of the variation and distinguished the table beet group. PC3 explained 3.3% of the variation and differentiated among fodder beet, sugar beet, and chard crop types. TDS was found to significantly correlate with PC1 ($r = -0.54$, $p < 0.001$) and PC2 ($r = -0.30$, $p < 0.001$), but not PC3 ($r = -0.04$, $p = 0.57$). Thus, the first two PCs were included as covariates in the full panel association analysis. No PCs were included in the table beet only association analysis, as no clustering patterns or correlations were observed, suggesting little

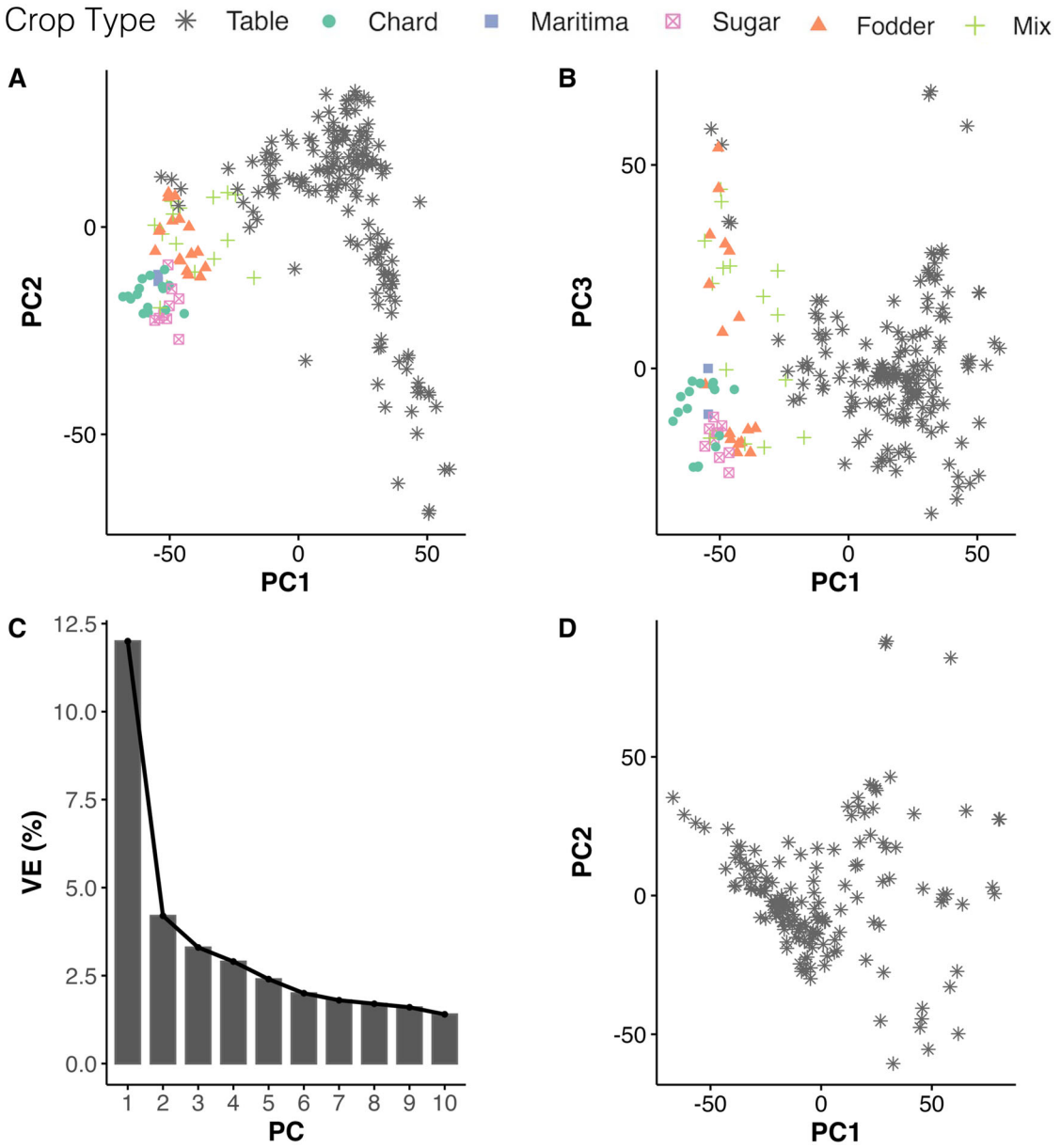


**FIGURE 2** Principal component (PC) analysis conducted on genotype matrices of full ($n = 226$) and table beet only ($n = 165$) subsets of the Wisconsin Beta Diversity Panel. The full panel PC plots (A–B) show clustering patterns among different crop type groups, designated by color and shape. The bar plot (C) illustrates the percent variation explained for the first 10 PCs of the full panel. Table beet only panel PC plots (D) show no apparent clustering patterns.

population structure is present in the table beet germplasm studied.

## 3.3 | Linkage disequilibrium

LD was estimated to be 0.45 Mb in the full panel and 1.1 Mb in the table beet only panel (Figure 3). The average inter-marker distance across all chromosomes was calculated to be 61,255 and 63,726 bp, for full and table beet only, respectively. As the average distance between SNPs was found to be considerably smaller than the average linkage block size, it was concluded that the SNP set provided sufficient genome-wide coverage (Figure S1). The differences observed between the subsets of accessions can be attributable to the increased diversity in the full panel. More diversity from the subpopulation crop types is often associated with increased historic recombination, different allelic frequencies, and the mixing of genetic backgrounds, which influence correlations and ultimately LD decay (Gaut & Long, 2003).

Various approaches exist to estimate LD decay. The mathematical trend line and thresholds can differ, and multiple

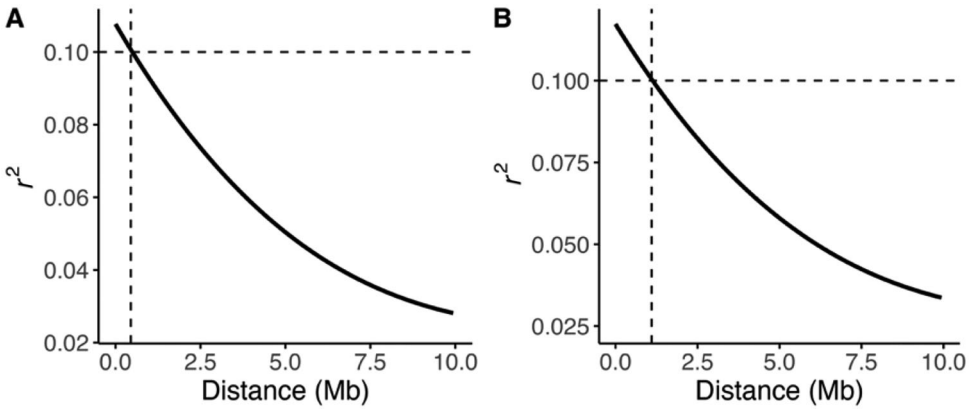


**FIGURE 3** Linkage disequilibrium (LD) decay curve calculated through GWASpoly software. Squared Pearson correlations between marker pairs from a diversity panel of (A) 226 *Beta vulgaris* accessions using 10,237 single nucleotide polymorphisms (SNPs) and (B) 165 *Beta vulgaris* table beet accessions using 9,847 SNPs. Linkage block size was determined as the chromosomal distance where $r^2 = 0.1$.

methods are widely accepted (Vos et al., 2017). A nonlinear regression line (Dixon & Goldman, 2024), a LOESS function (Esteras et al., 2013), and a spline function (Rosyara et al., 2016) have been used to estimate decay. The most commonly used threshold to determine linkage block size is an $r^2$ of 0.1, which is why it was chosen for this study, but an $r^2$ of 0.2 is often used as well. Another possibility to define the LD decay rate is the chromosomal distance where the $r^2$ falls to half its maximum value or by simply following the shape of the curve (Vos et al., 2017), which would have yielded a significantly larger linkage block size. Combinations of trend line functions and LD thresholds, as described above, result in variations in LD decay estimates. This variability may hinder comparisons to this study and pose even greater challenges when dealing with germplasm not included in the WBDP. As whole genome sequencing becomes more affordable in the future, researchers will be able to observe true haplotype switches and more precisely define the sizes of linkage blocks.

## 3.4 | Genome-wide association study

Four SNP markers, identified by multiple models, were found to significantly associate with TDS content ($p \leq 4.88 \times 10^{-6}$) in the full panel and were located across four of the nine *Beta vulgaris* chromosomes (Figure 4; Table 2). A SNP marker located on chromosome 2 (Chr2_55158230) was uncovered by all models and explained 12.10% (GWASpoly), 21.30% (BLINK), 27.19% (FarmCPU), and 62.60% (MLM) of the total phenotypic variation observed for the respective models. Markers Chr6_80434475 and Chr9_7424769 were also found to significantly associate with TDS. They explained 26.54% and 33.59% of the variation using the FarmCPU model, and 3.94% and 4.51% of the variation using the BLINK model, respectively. The remainder of the SNPs confer smaller genetic effects, with PVE ranging from 1.30% to 12.80%. Two statistically significant markers ($p \leq 5.08 \times 10^{-6}$) were identified in association with TDS in the table beet only panel using the BLINK model (Figure 4; Table 2). No associations were found through the MLM or FarmCPU for these markers. PVE for markers Chr7_57824948 and Chr8_42836376 was 45.28% and 9.02%, respectively. Although no marker consistencies were observed between models in the table beet only panel, marker Chr7_57824948 was significantly associated with TDS in both the full and table beet only panels.

Severe *p*-value inflation was observed in the GLM (Figure S2), where population structure control was limited to the *Q* matrix (Price et al., 2006). Although the PC adjustment accounted for some variation, it was insufficient, particularly when TDS was associated with the crop type groups (Figure 1). Inflation was also observed in the table beet only panel, even where only one crop type was represented. Consequently, many markers identified (Tables S3 and S4) are likely false positives and have been excluded from the reported analysis.

Inevitably, certain accessions group together because they are part of the same breeding program. The kinship matrix is used to quantify this genetic relatedness or similarity and is the most common type of control for identity by descent. Relatedness was controlled in all models (Figure S3) except GLM and proved to be the most effective control of relatedness, exhibited by the severe inflation of GLM *p*-values (Figure S2A–D). VanRaden (VanRaden, 2008) and LOCO (Yang et al., 2014) methods were both sufficient at controlling *p*-value inflation, shown through the GWASpoly and GAPIT MLM comparison (Figure 4A,B, Figure S2E–H). Using the WBDP to develop a core group of accessions, based on genetic diversity and representativeness of the collection, could be valuable for future studies.

In contrast, the single SNP (Chr2_55158230) signal conferred by the MLMs demonstrates a clear reduction in *p*-value

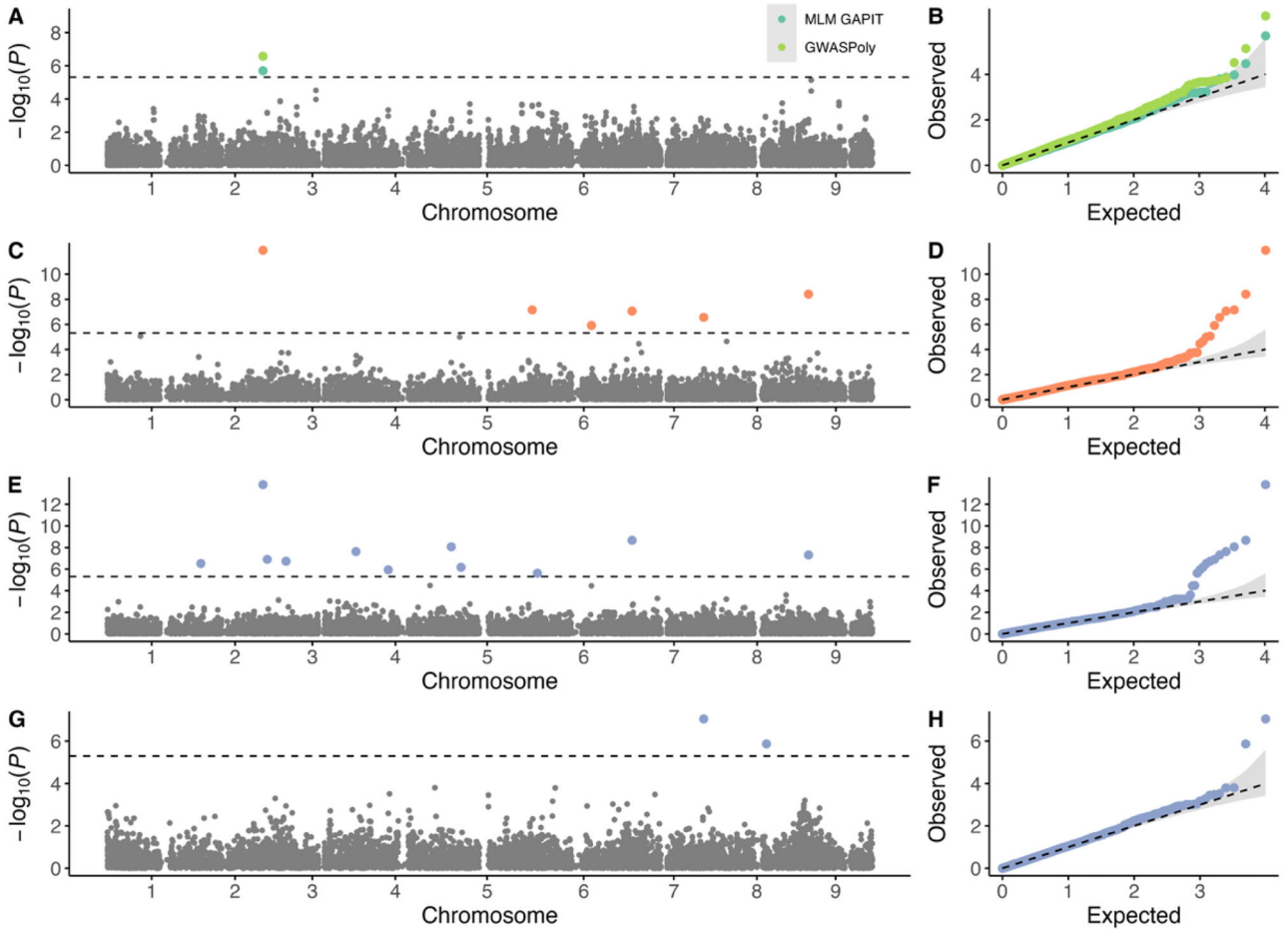


**FIGURE 4** Manhattan and quantile–quantile (QQ) plots for total dissolved solids (TDS) based on (A–B) mixed linear models in GWASpoly and genomic association and prediction integrated tool (GAPIT) software, (C–D) fixed and random model circulating probability unification (FarmCPU), and (E–F) Bayesian information and linkage disequilibrium iteratively nested keyway (BLINK) models across 226 *Beta vulgaris* genotypes; (G–H) Manhattan and QQ plots for TDS based on the BLINK model across 165 table beet only accessions. All accessions were grown in 2 years at two locations and genome-wide association studies (GWAS) were conducted using best linear unbiased estimates (BLUEs). The dashed horizontal line is a Bonferroni cutoff of $\alpha = 0.05$, and markers, color coded by model, above this threshold are identified as significantly associated with TDS. The observed $-\log_{10}(P)$ marker scores returned from the respective GWAS model are plotted against expected $-\log_{10}(P)$ marker scores.

inflation, again highlighting the necessity of a kinship matrix. This control of confounding factors may lead to undetected associations (Figure S2E–H), necessitating that true associations demonstrate strong signals, as seen with SNP marker Chr2_55158230. The kinship matrix in MLM was calculated using all available SNPs, which can also reduce power (Yu et al., 2006).

The detection of SNPs is influenced not only by the presence of kinship control but also by the method used to derive it, as demonstrated by the FarmCPU and BLINK models. These models reveal more associated SNP markers, which can be attributed to their unique methodologies for calculating relatedness. FarmCPU calculates kinship from markers associated with the trait of interest, combining features of both random and fixed effect models (Liu et al., 2016). BLINK further refines this approach by selecting significant markers and then removing those in LD with the most associated marker (Huang et al., 2019). Both methods aim to reduce false positives and negatives, thereby improving the statistical power. Despite these methodological differences, the consistency among models for identification of the QTL on chromosome 2 underscores the statistical significance of this genomic region and its importance for the TDS flavor trait in *Beta vulgaris*.

Allelic effects were assessed for the SNPs that were found to significantly associate with TDS across multiple models (Figure 5; Figure S4). For each copy of the alternate allele, TDS values increased by an average of 1.17 across all models that identified the Chr2_55158230 marker as significantly associated with TDS. At marker Chr6_80434475, each copy of the alternate allele decreased TDS levels by −0.94, on average. At marker Chr9_7424769, each copy of the alternate allele decreased TDS levels by −0.56, on average. For the Chr7_57824948 marker, each copy of the alternate allele

**TABLE 2** Markers found to significantly associate with total dissolved solids (TDS) from a full *Beta vulgaris* diversity panel ($n = 226$) and a table beet only panel ($n = 165$) grown in two locations over 2 years.

| Marker | Chr | Pos (bp) | MAF | PVE (%)[a] | Model[b] |
|---|---|---|---|---|---|
| **Full panel** | | | | | |
| Chr2_4130384 | 2 | 4130384 | 0.05 | 4.30 | BLINK |
| **Chr2_55158230** | 2 | 55158230 | 0.08 | 12.10–62.60 | BLINK, FarmCPU, GWASPoly, MLM |
| Chr2_58616449 | 2 | 58616449 | 0.18 | 7.79 | BLINK |
| Chr3_9831300 | 3 | 9831300 | 0.23 | 3.30 | BLINK |
| Chr4_30920895 | 4 | 30920895 | 0.10 | 2.78 | BLINK |
| Chr4_4406794 | 4 | 4406794 | 0.15 | 2.34 | BLINK |
| Chr5_17176273 | 5 | 17176273 | 0.45 | 2.36 | BLINK |
| Chr5_75656162 | 5 | 75656162 | 0.07 | 4.59 | FarmCPU |
| Chr5_9213453 | 5 | 9213453 | 0.05 | 12.80 | BLINK |
| Chr6_2775811 | 6 | 2775811 | 0.19 | 1.30 | BLINK |
| Chr6_47115698 | 6 | 47115698 | 0.13 | 8.35 | FarmCPU |
| **Chr6_80434475** | 6 | 80434475 | 0.08 | 26.54–33.59 | BLINK, FarmCPU |
| **Chr7_57824948** | 7 | 57824948 | 0.25 | 5.78 | FarmCPU |
| **Chr9_7424769** | 9 | 7424769 | 0.30 | 3.94–4.51 | BLINK, FarmCPU |
| **Table beet only panel** | | | | | |
| **Chr7_57824948** | 7 | 57824948 | 0.25 | 45.28 | BLINK |
| Chr8_42836376 | 8 | 42836376 | 0.15 | 9.02 | BLINK |

*Note*: Genome-wide association analyses were performed with genomic association and prediction integrated tool (GAPIT) (mixed linear model [MLM], fixed and random model circulating probability unification [FarmCPU], and bayesian information and linkage disequilibrium iteratively nested keyway [BLINK]) and GWASpoly software using best linear unbiased estimates. The bolded markers were identified across multiple models.

Abbreviation: MAF, minor allele frequency.

[a]Percent variation explained (PVE) by the identified marker for the respective model.

[b]GWAS model that identified marker as significant ($p \leq 4.88 \times 10^{-6}$ and $p \leq 5.08 \times 10^{-6}$, respectively).

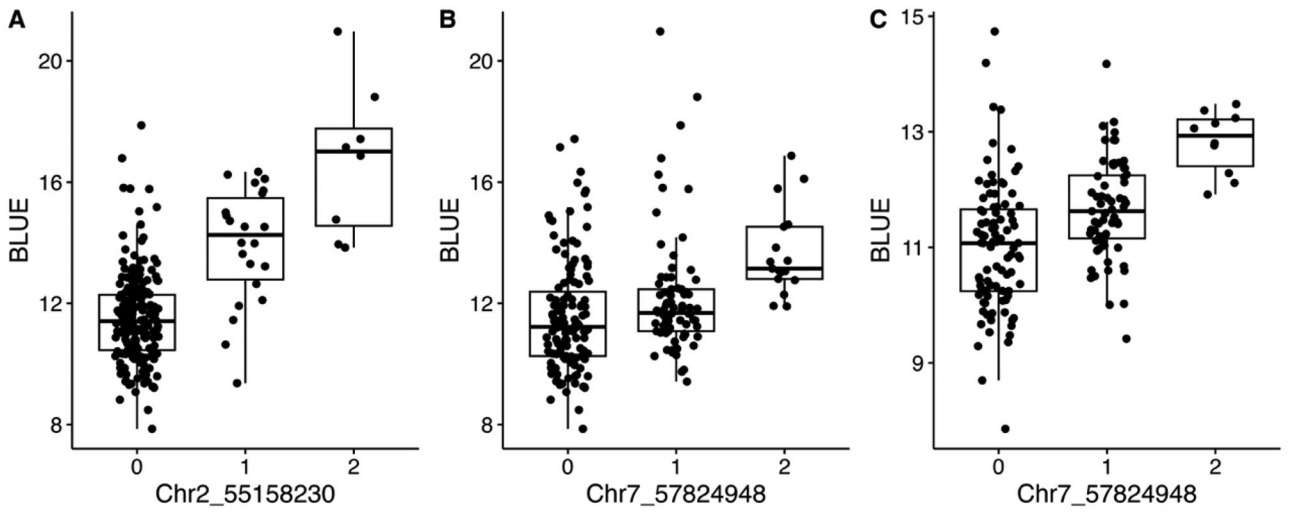


**FIGURE 5** Boxplots showing allelic effects of two single nucleotide polymorphism markers associated with total dissolved solids (TDS) in a genome-wide association study (GWAS). (A) The marker identified as significant in both GWASPoly and genomic association and prediction integrated tool (GAPIT) software located on chromosome 2 at position 55158230. (B) The marker identified as significant in both the full panel ($n = 226$) and (C) table beet only panel ($n = 165$) located on chromosome 7 at position 57824948. BLUE, best linear unbiased estimate.

**TABLE 3** Accessions that are homozygous alternate (dosage = 2) at single nucleotide polymorphism marker Chr2_55158230, and their respective information. This marker was identified as having a statistically significant association with total dissolved solids (TDS) in a genome-wide association study (GWAS) of 226 *Beta vulgaris* genotypes through GWASpoly and genomic association and prediction integrated tool (GAPIT) software.

| Name | Type | TDS |
|---|---|---|
| PI 518303 | *Beta vulgaris* subsp. *maritima* | 20.97 |
| F1042 | Sugar | 18.81 |
| PI 504245 | *Beta vulgaris* subsp. *maritima* | 17.42 |
| KDH13 | Sugar | 17.15 |
| EL10 | Sugar | 16.87 |
| OG MacGregor's favorite | Mix | 14.76 |
| Perpetual spinach | Chard | 13.95 |
| Vulcan | Chard | 13.84 |

increased TDS levels in individuals by 0.51 and 0.52 in the full and table beet only panels, respectively, yielding nearly the same effect.

Differences in significant SNPs observed between the full panel and the table beet only panel suggest the presence of novel QTL within different crop types. The clear separation of most table beets from other crop types in hierarchical clustering (data not shown) implies that alleles at marker Chr2_55158230 may not be present in the table beet population studied here. Marker Chr2_55158230 effects can be observed in eight individuals of the WBDP, including *Beta vulgaris* subsp. *maritima*, sugar beet, Swiss chard, and a mixed crop type (Figure 5; Table 3). Individuals containing the chromosome 2 marker hierarchically cluster into three distinct groups, defining the distribution of these alleles. Alternatively, the marker could have been originally present in historic *Beta vulgaris* populations and retained through selective breeding in sugar beets and other high TDS relatives but not in table beet. While table beets have been selected for various traits such as pigments and shape, sugar beets have primarily been selected for sucrose content. Sugar beet was selected from a fodder beet population, which is thought to have originated from a chard-table beet hybridization (Ford-Lloyd & Williams, 1975). This could explain why some accessions exhibit this phenotype and contain these marker alleles, while others, more similar to table beet, do not.

The SNP marker Chr7_57824948 was identified as significant in both the full and table beet only panels. Seventeen individuals aligned with a homozygous alternate dosage for this marker, with the majority being table beet (Figure 5). This QTL allele is present in certain table beet accessions, among other crop types, but is not found in the majority of table beets. This locus could be highly valuable for table beet breeding efforts, as the alleles are already present in table beet populations, along with sugar beet and Swiss chard. Breeders can make crosses within crop types to introduce the allele, without the need for wide crosses and genetic drag. The remaining small effects of significant SNPs found highlight a complexity of the genetics underlying the TDS trait.

## 3.5 | QTL and candidate genes

All four SNPs identified in both WBDP subsets across multiple models were located on separate chromosomes, each representing an independent QTL. Although these QTLs were significantly associated with TDS, they need to be interpreted with differing levels of caution. SNPs were filtered based on the presence of at least one of each genotype call. Fewer individuals in a genotype call allow for larger biases from a single individual. This was observed for significant SNPs in the homozygous alternate state (Figure S4). Read depth was also analyzed for the markers identified across multiple models and is summarized in Table S5. Mean read depth across these four SNPs ranged from 20 to 35. The number of individuals with a read depth less than 10 ranged from 2 to 21. The number of individuals with a depth less than 10 in the homozygous alternate genotype ranged from 0 to 3 (Table S5). The homozygous alternate category contains less individuals than the other genotype calls, and sufficient read depth is notable here.

QTLs have been previously identified on all nine *Beta vulgaris* chromosomes for sugar content in sugar beet, including some located on the chromosomes identified in this study. Reif et al. (2010) identified a QTL with significant association to sugar content (%) on chromosome 2, which was found to co-locate with previous QTL identified by Weber et al. (1999, 2000) and Schneider et al. (2002). These QTLs are located on the distal end of the long arm of chromosome two in sugar beet, which appears to be the same as those QTLs identified in this study. Reif et al. (2010) also identified two QTLs 23.7 cM apart on the long arm of chromosome 7, which is where Chr7_57824948 is generally located (McGrath et al., 2023). Beyond this information, the lack of a universal map hinders precise validation of QTL, making it impossible to determine if they are the same QTLs. Furthermore, in an outcrossing species, like beet, there is an expectation of inversions, translocations, and duplications, which can even extend between arms of a chromosome. This has been observed in carrot between two genotypes of the same species (Y. H. Wang et al., 2023), and the case may be more accentuated for table and sugar beet genomes. QTLs for other sugar-related traits, such as white sugar content (%), sugar yield (Mg $ha^{-1}$), and white sugar yield (Mg $ha^{-1}$) have also been reported to be located on chromosome 2, among other chromosomes (Reif et al., 2010). Multiple QTLs have also been identified on chromosomes 6 and 9, as reported here. The current

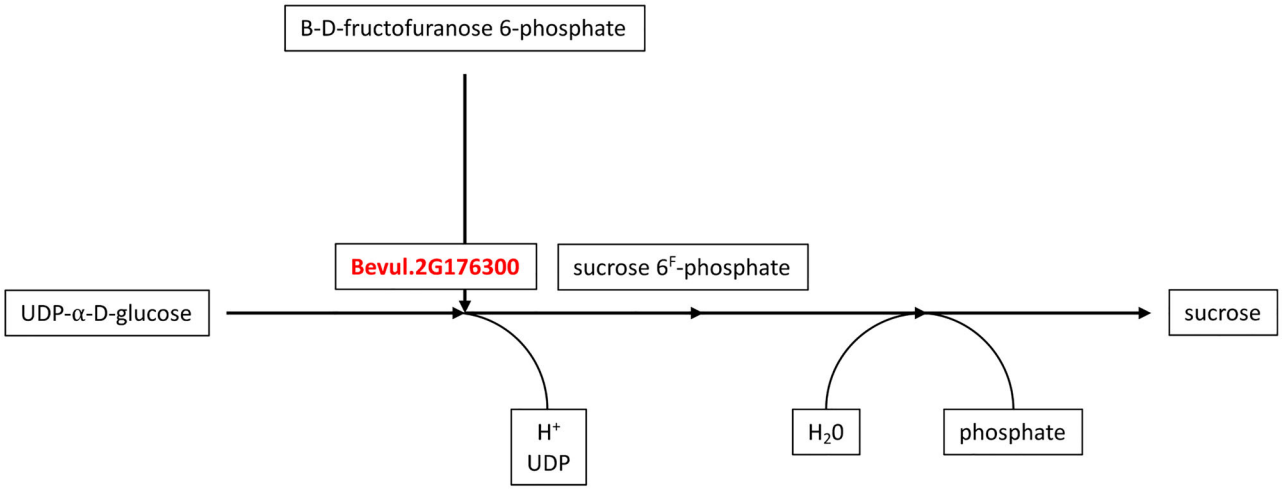


**FIGURE 6** The final steps of the sucrose biosynthesis pathway in plants. *Bevul.2G176300*, the gene indicated in red, encodes a sucrose-phosphate synthase enzyme (SPS, 2.4.1.14). This candidate gene is located 332 kb downstream of marker Chr2_55158230, which was identified to be significantly associated with total dissolved solids (TDS) in a genome-wide association study of the Wisconsin Beta Diversity Panel.

study adds to the evidence that chromosome 2 is an important genomic region for TDS and sugar control, and points to the unique presence of QTL alleles for TDS in certain table beet accessions. This is the first report of QTL associated with TDS, or sucrose, content in table beet.

Direct sugar content is commonly reported for sugar beet because the measurements are used for processing sugar, rather than as a proxy for perceived sweetness in the fresh eating market like table beet. However, QTLs for TDS have been identified in other crops and are commonly used as a proxy for sucrose levels. Katuuramu et al. (2023) identified five QTLs across four chromosomes associated with soluble solids content in citron watermelon (*Citrullus amarus* L.) through a GWAS. The TDS trait has also undergone direct selection, which has proven successful in tomato breeding strategies (Merk et al., 2012; Sánchez et al., 2020). These moderate to large effect QTLs could be valuable for deploying marker-assisted selection.

The reported PVE varied due to the different internal calculation methods used by the GWAS software. GWASPoly calculates $R^2$ values for each significant marker using the likelihood ratio test with backward elimination to determine PVE values. In contrast, GAPIT calculates PVE by dividing the marker's corresponding variance (the sum of residual variance and the variance of the associated marker) by the total variance (J. Wang & Zhang, 2021). These differing methods contribute to the range of PVE observed, alongside the model type and other significant marker effects. Smaller effect QTL can be used in backcrossing and for selection within populations. A biparental linkage mapping study would be valuable to assess the QTL identified in the WBDP and eventually the effectiveness of direct selection for this trait.

A candidate gene search was conducted on the four QTLs identified across multiple models, as these regions were statistically important for the TDS flavor trait. Within each QTL window, numerous genes with diverse predicted functions were identified, ranging from 42 to 111 genes per window. Candidate gene *Bevul.2G176300* was located 332 kb downstream of the chromosome 2 marker (Chr2_55158230) and encodes for a sucrose-phosphate synthase (SPS, 2.4.1.14) enzyme, which is directly involved in the sucrose biosynthesis pathway (Figure 6). This enzyme catalyzes the creation of sucrose $6^F$-phosphate, which leads to the final step involving the removal of phosphate to yield sucrose (Huber & Huber, 1996). SPS in sugar beet is found to be expressed in a tissue-specific manner, being predominately active in the taproot. The sequence of sugar beet SPS exhibits homologies with other crops (quinoa, maize, and spinach), and this likely holds true within the *Beta vulgaris* crop types (Hesse et al., 1995). This major QTL controlling TDS, and possibly sucrose production, may ultimately improve root sweetness desired by consumers and culinary experts if markers linked to it can be effectively employed in a breeding program.

Gene *Bevul.6G006200.1* was located just upstream of significant marker Chr6_80434475 and codes for a nucleotide-diphospho-sugar transferases superfamily protein. This could be a potential candidate for sugar transport, though not directly involved in TDS and sucrose production. No candidate genes were identified in the other repeatable QTL regions, suggesting the presence of novel QTLs. Notably, the significance of SNP marker Chr7_57824948 appears to be a novel finding in table beet. As previously mentioned, QTL alleles at this marker associated with elevated TDS were present only in certain table beet accessions, including

those from the UW table beet breeding program. The UW program made use of desirable sugar beet alleles for male sterility and self-fertility, among others, through a backcrossing approach (Goldman & Navazio, 2003), which might explain the retention of unique QTL alleles for elevated TDS more typically associated with sugar beet. In this hypothetical situation, the QTL alleles would have been eliminated from table beet early on, but non-deliberately reintroduced back to table beet germplasm in modern times, as table beet is a progenitor of the sugar beet (Dohm et al., 2014; Goldman & Navazio, 2003; McGrath & Panella, 2018). This QTL accounted for nearly half (45.28%) of the observed phenotypic variation in table beet and may be particularly valuable for enhancing TDS levels in table beet breeding programs, as no TDS-specific QTL for table beet have been previously reported.

## AUTHOR CONTRIBUTIONS

**Audrey Pelikan**: Conceptualization; data curation; formal analysis; investigation; methodology; project administration; writing—original draft; writing—review and editing. **Irwin L. Goldman**: Conceptualization; funding acquisition; project administration; resources; supervision; writing—review & editing.

## ACKNOWLEDGMENTS

This research work was supported by a USDA–Hatch award administered by the University of Wisconsin College of Agricultural and Life Sciences. The authors would like to thank lab members Dr. Andrey Vega Alfaro, Dr. Madeline Oravec, Dr. Liam Dixon, Audrey Morrison, and Adam D'Angelo for their work in assembling the WBDP and technical support. The WBDP was collectively assembled, grown, and genotyped by the team previously mentioned to be used as a core for *Beta vulgaris* research objectives. Subsets of the WBDP were then separately filtered by each researcher and deployed for analysis of different traits. The authors also thank lab undergraduates for their assistance with field plot maintenance and data collection. A final thanks to the UW research station staff, especially Dan Heider and Paul Systma, for their field trial support.

## CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

## DATA AVAILABILITY STATEMENT

Data supporting the findings of this study are available within the article and its supplementary files. Additional datasets can be accessed at https://doi.org/10.6084/m9.figshare.27207981. Any reasonable inquiries/requests can be made to the corresponding author.

## ORCID

*Audrey Pelikan* https://orcid.org/0009-0001-8907-182X
*Irwin L. Goldman* https://orcid.org/0000-0003-0741-7821

## REFERENCES

Albihn, P. B. E., & Savage, G. P. (2001). The effect of cooking on the location and concentration of oxalate in three cultivars of New Zealand-grown oca (*Oxalis tuberosa* Mol). *Journal of the Science of Food and Agriculture*, *81*(10), 1027–1033. https://doi.org/10.1002/jsfa.890

Altamirano, S. M., Borrelli, N., Benvenuto, M. L., Honaine, M. F., & Osterrieth, M. (2018). Calcium oxalate crystals in plant communities of the southeast of the Pampean Plain, Argentina. In K. Endo, T. Kogure, & H. Nagasawa (Eds.), *Biomineralization from molecular and nano-structural analyses to environmental science* (pp. 303–311). Springer. https://doi.org/10.1007/978-981-13-1002-7

Anand, S., Mangano, E., Barizzone, N., Bordoni, R., Sorosina, M., Clarelli, F., Corrado, L., Boneschi, F. M., D'Alfonso, S., & De Bellis, G. (2016). Next generation sequencing of pooled samples: Guideline for variants' filtering. *Scientific Reports*, *6*, Article 22735. https://doi.org/10.1038/srep22735

Bach, V., Mikkelsen, L., Kidmose, U., & Edelenbos, M. (2014). Culinary preparation of beetroot (*Beta vulgaris* L.): The impact on sensory quality and appropriateness. *Journal of the Science and Food of Agriculture*, *95*(9), 1852–1859. https://doi.org/10.1002/jsfa.6886

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*(1), 1–48. https://doi.org/10.18637/jss.v067.i01

Beans, C. (2017). Vegetable breeders turn to chefs for flavor boost. *Proceedings of the National Academy of Sciences*, *114*(40), 10506–10508. https://doi.org/10.1073/pnas.1714536114

Brachi, B., Morris, G. P., & Borevitz, J. O. (2011). Genome-wide association studies in plants: The missing heritability is in the field. *Genome Biology*, *12*(10), Article 232. https://doi.org/10.1186/gb-2011-12-10-232

Brogren, M., & Savage, G. (2003). Bioavailability of soluble oxalate from spinach eaten with and without milk products. *Asia Pacific Journal of Clinical Nutrition*, *12*(2), 219–224.

Ceclu, L., & Nistor, O. V. (2020). Red beetroot: Composition and health effects—A review. *Journal of Nutritional Medicine and Diet Care*, *6*(1), 1–9. https://doi.org/10.23937/2572-3278.1510043

Damrosch, B. (2016). *Beets are trendy again, for a reason*. The Washington Post. https://www.washingtonpost.com/lifestyle/home/beets-are-trendy-again-for-a-reason/2016/03/28/1f96ace6-f039-11e5-89c3-a647fcce95e0_story.html

D'Angelo, A., & Goldman, I. L. (2023). Temporal accumulation of geosmin, oxalic acid, and total dissolved solids in table beet. *HortScience*, *58*(9), 974–982. https://doi.org/10.21273/HORTSCI17152-23

Dixon, L., & Goldman, I. L. (2024). Genetic architechture of Cercospora leaf spot response in table beet with implications for other *Beta vulgaris* crop types. *Crop Science*, *64*(6), 3352–3371. https://doi.org/10.1002/csc2.21396

Dohm, J. C., Minoche, A. E., Holtgräwe, D., Capella-Gutiérrez, S., Zakrzewski, F., Tafer, H., Rupp, O., Sörenson, T. R., Stracke, R., Reinhardt, R., Goesmann, A., Kraft, T., Schulz, B., Stadler, P. F., Schmidt, T., Gabaldón, T., Lehrach, H., Weisshaar, B., &

Himmelbauer, H. (2014). The genome of the recently domesticated crop plant sugar beet (*Beta vulgaris*). *Nature*, *505*(7484), 546–549. https://doi.org/10.1038/nature12817

Dorn, K. M. (2022). *W357B table beet reference genome* (1.0) [Data set]. Zenodo. https://zenodo.org/record/5911852#.Ypd87uzMI6A

Elshire, R. J., Glaubitz, J. C., Sun, Q., Poland, J. A., Kawamoto, K., Buckler, E., & Mitchell, S. E. (2011). A robust, simple genotype-by-sequencing (GBS) approach for high diversity species. *PLoS One*, *6*(5), e19379. https://doi.org/10.1371/journal.pone.0019379

Esteras, C., Formisano, G., Roig, C., Díaz, A., Blanca, J., Garcia-Mas, J., Gómez-Guillamón, M. L., López-Sesé, A. I., Lázaro, A., Monfortw, A. J., & Picó, B. (2013). SNP genotyping in melons: Genetic variation, population structure, and linkage disequilibrium. *Theoretical and Applied Genetics*, *126*, 1285–1303. https://doi.org/10.1007/s00122-013-2053-5

Fall, L. A., Perkins-Veazie, P., Ma, G., & McGregor, C. (2019). QTLs associated with flesh quality traits in an elite × elite watermelon population. *Euphytica*, *215*, Article 30. https://doi.org/10.1007/s10681-019-2356-y

Fassett, D. W. (1973). Oxalates. In *Toxicants occurring naturally in foods* (2nd ed., pp. 346–362). National Academy of Sciences.

Feller, C., & Fink, M. (2004). Nitrate content, soluble solids content, and yield of table beet as affected by cultivar, sowing date, and nitrogen supply. *HortScience*, *39*(6), 1255–1259. https://doi.org/10.21273/HORTSCI.39.6.1255

Ferrão, L. F. V., Johnson, T. S., Benevenuto, J., Edger, P. P., Colquhoun, T. A., & Munoz, P. R. (2020). Genome-wide association of volatiles reveals candidate loci for blueberry flavor. *New Phytologist*, *226*, 1725–1737. https://doi.org/10.1111/nph.16459

Fischer, H. E. (1989). Origin of the 'Weisse Schlesische Rübe' (white Silesian beet) and resynthesis of sugar beet. *Euphytica*, *41*, 75–80. https://doi.org/10.1007/BF00022414

Ford-Lloyd, B. V., & Williams, J. T. (1975). A revision of *Beta* section *Vulgares* (Chenopodiaceae), with new light on the origin of cultivated beets. *Journal of the Linnean Society*, *71*(2), 89–102. https://doi.org/10.1111/j.1095-8339.1975.tb02448.x

Freeman, R. E., & Simon, P. W. (1983). Evidence for simple genetic control of sugar type in carrot (*Daucus carota* L.). *Journal of the American Society for Horticultural Science*, *108*(1), 50–54. https://doi.org/10.21273/JASHS.108.1.50

Freidig, A. K., & Goldman, I. L. (2011). Variation in oxalic acid content among commercial table beet cultivars and related crops. *Journal of the American Society for Horticultural Science*, *136*(1), 54–60. https://doi.org/10.21273/JASHS.136.1.54

Galewski, P., & McGrath, J. M. (2020). Genetic diversity among cultivates beets (*Beta vulgaris*) assessed via population-based whole genome sequences. *BMC Genomics*, *21*(1), Article 189. https://doi.org/10.1186/s12864-020-6451-1

Gaut, B. S., & Long, A. D. (2003). The lowdown on linkage disequilibrium. *The Plant Cell*, *15*(7), 1502–1506. https://doi.org/10.1105/tpc.150730

Glaubitz, J. C., Casstevens, T. M., Lu, F., Harriman, J., Elshire, R. J., Sun, Q., & Buckler, E. S. (2014). TASSEL-GBS: A high capacity genotyping by sequencing analysis pipeline. *PLoS One*, *9*(2), e90346. https://doi.org/10.1371/journal.pone.0090346

Goldman, I. L., & Navzio, J. P. (2003). History and breeding of table beet in the United States. In J. Janick (Ed.), *Plant breeding reviews* (Vol. 22, pp. 357–388). John Wiley & Sons. https://doi.org/10.1002/9780470650202.ch7

Goodstein, D. M., Shu, S., Howson, R., Neupane, R., Hayes, R. D., Fazo, J., Mitros, T., Dirks, W., Hellsten, U., Putnam, N., & Rokhsar, D. S. (2012). Phytozome: A comparative platform for a green plant genomics. *Nucleic Acids Research*, *40*, 1178–1186. https://doi.org/10.1093/nar/gkr944

Hanson, S. J., Dawson, J. C., & Goldman, I. L. (2021). *Beta vulgaris* ssp. chromosome 8 shows significant association with geosmin concentration in table beet. *G3: Genes|Genomes|Genetics*, *11*(12), jkab344. https://doi.org/10.1093/g3journal/jkab344

Hanson, S. J., & Goldman, I. L. (2019). Genotype is primarily responsible for variance in table beet geosmin concentration, but complex genotype × environment interactions influence variance in total dissolved solids. *Journal of the American Society for Horticultural Science*, *144*(6), 429–438. https://doi.org/10.21273/JASHS04758-19

Hesse, H., Sonnewald, U., & Willmitzer, L. (1995). Cloning and expression analysis of sucrose-phosphate synthase from sugar beet (*Beta vulgaris* L.). *Molecular Genetics and Genomics*, *247*, 515–520. https://doi.org/10.1007/BF00293155

Holland, J. B., Nyquist, W. E., & Cervantes-Martínez, C. T. (2002). Estimating and interpreting heritability for plant breeding: An update. *Plant Breeding Reviews*, *22*, 9–112. https://doi.org/10.1002/9780470650202.ch2

Huang, M., Liu, X., Zhou, Y., Summers, R. M., & Zhang, Z. (2019). BLINK: A package for the next level of genome-wide association studies with both individuals and markers in the millions. *GigaScience*, *8*(2), giy154. https://doi.org/10.1093/gigascience/giy154

Huber, S. C., & Huber, J. L. (1996). Role and regulation of sucrose-phosphate synthase in higher plants. *Annual Review of Plant Physiology and Plant Molecular Biology*, *47*, 431–444. https://doi.org/10.1146/annurev.arplant.47.1.431

Jiang, J., He, X., & Cane, D. E. (2007). Biosynthesis of the earthy odorant geosmin by a bifunctional *Streptomyces coelicolor* enzyme. *Nature Chemical Biology*, *3*(11), 711–715. https://doi.org/10.1038/nchembio.2007.29

Katuuramu, D. N., Levi, A., & Wechter, W. P. (2023). Genome-wide association study of soluble solids content, flesh color, and fruit shape in citron watermelon. *The Plant Genome*, *16*(4), e20391. https://doi.org/10.1002/tpg2.20391

Klee, H. J. (2010). Improving the flavor of fresh fruits: Genomics, biochemistry, and biotechnology. *New Phytologist*, *187*(1), 44–56. https://doi.org/10.1111/j.1469-8137.2010.03281.x

Korte, A., & Farlow, A. (2013). The advantages and limitations of trait analysis with GWAS: A review. *Plant Methods*, *9*, Article 29. https://doi.org/10.1186/1746-4811-9-29

Langmead, B., & Salzberg, S. L. (2012). Fast gapped-read alignment with Bowtie 2. *Nature Methods*, *9*, 357–359. https://doi.org/10.1038/nmeth.1923

Liato, V., & Aïder, M. (2017). Geosmin as a source of the earthy-musty smell in fruits, vegetables and water: Origins, impact on foods and water, and review of the removing techniques. *Chemosphere*, *181*, 9–18. https://doi.org/10.1016/j.chemoshpere.2017.04.039

Liu, X., Huang, M., Fan, B., Buckler, E. S., & Zhang, Z. (2016). Iterative usage of fixed and random effect models for powerful and efficient genome-wide association studies. *PLoS Genetics*, *12*(2), e1005957. https://doi.org/10.1371/journal.pgen.1005767

Lu, G., Edwards, C. G., Fellman, J. K., Mattinson, S. D., & Navazio, J. (2003). Biosynthetic origin of geosmin in red beets (*Beta vulgaris* L.). *Journal of Agricultural and Food Chemistry*, *51*, 1026–1029. https://doi.org/10.1021/jf020905r

Maher, L., & Goldman, I. L. (2018). Endogenous production of geosmin in table beet. *HortScience*, *53*(1), 67–72. https://doi.org/10.21273/HORTSCI12488-17

McGrath, J. M., Funk, A., Galewski, P., Ou, S., Townsend, B., Davenport, K., Daligault, H., Johnson, S., Lee, J., Hastie, A., Darracq, A., Willems, G., Barnes, S., Liachko, I., Sullivan, S., Koren, S., Phillippy, A., Wang, J., Liu, T., … Dorn, K. (2023). A contiguous *de novo* genome assembly of sugar beet EL10 (*Beta vulgaris* L.). *DNA Research*, *30*(1), dsac033. https://doi.org/10.1093/dnares/dsac033

McGrath, J. M., & Panella, L. (2018). Sugar beet breeding. In I. L. Goldman (Ed.), *Plant breeding reviews* (pp. 167–218). John Wiley & Sons, Inc. https://doi.org/10.1002/9781119521358

Merk, H. L., Yarnes, S. C., Deynze, A. V., Tong, N., Menda, N., Mueller, L. A., Mutschler, M. A., Loewen, S. A., Myers, J. R., & Francis, D. M. (2012). Trait diversity and potential for selection indices based on variation among regionally adapted processing tomato germplasm. *Journal of the American Society for Horticultural Science*, *137*(6), 427–437. https://doi.org/10.21273/JASHS.137.6.427

National Weather Service. (2022–2023). *NOWData Milwaukee/Sullivan, WI*. https://www.weather.gov/wrh/climate?wfo=mkx

Noonan, S. C., & Savage, G. P. (1999). Oxalate content of foods and its effect on humans. *Asia Pacific Journal of Clinical Nutrition*, *8*(1), 64–74. https://doi.org/10.1046/j.1440-6047.1999.00038.x

Price, A. L., Patterson, N. J., Plenge, R. M., Weinblatt, M. E., Shadick, N. A., & Reich, D. (2006). Principal components analysis corrects for stratification in genome-wide association studies. *Nature Genetics*, *38*, 904–909. https://doi.org/10.1038/ng1847

Reif, J. C., Liu, W., Gowda, M., Maurer, H. P., Möhring, J., Fischer, S., Schechert, A., & Würschum, T. (2010). Genetic basis of agronomically important traits in sugar beet (*Beta vulgaris* L.) investigated with joint linkage association mapping. *Theoretical and Applied Genetics*, *121*, 1489–1499. https://doi.org/10.1007/s00122-010-1405-7

Rosyara, U. R., De Jong, W. S., Douches, D. S., & Endelman, J. B. (2016). Software for genome-wide association studies in autopolyploids and its application to potato. *The Plant Genome*, *9*(2), plantgenome2015.08.0073. https://doi.org/10.3835/plantgenome2015.08.0073

Sánchez, F. B., Ribeiro, L. P., Cotrim, M. F., da Junior, C. A., Bhering, L. L., & Teodoro, P. E. (2020). Genetic gains with selection for yield and soluble solids content in cherry tomato hybrids. *HortScience*, *55*(4), 400–402. https://doi.org/10.21273/HORTSCI14415-19

Sandell, F. L., Pavese-Stralis, N., McGrath, J. M., Schulz, B., Himmelbauer, H., & Dohm, J. C. (2022). Genomic distances reveal relationships of wild and cultivated beets. *Nature Communications*, *13*, Article 2021. https://doi.org/10.1038/s41467-022-29676-9

Schneider, K., Schäfer-Pregl, R., Borchardt, C., & Salamini, F. (2002). Mapping QTL for sucrose content, yield, and quality in a sugar beet population fingerprinted by EST-related markers. *Theoretical and Applied Genetics*, *104*(6–7), 1107–1113. https://doi.org/10.1007/s00122-002-0890-8

Schumate, A., & Salzberg, S. L. (2021). Liftoff: Accurate mapping of genome annotations. *Bioinformatics*, *37*(12), 1639–1643. https://doi.org/10.1093/bioinformatics/btaa1016

Schwegler, D. D., Gowda, M., Schulz, B., Miedaner, T., Liu, W., & Reif, J. C. (2014). Genotypic correlations and QTL correspondence between line per se and testcross performance in sugar beet (*Beta vulgaris* L.) for the three agronomic traits beet yield, potassium content, and sodium content. *Molecular Breeding*, *34*, 205–215. https://doi.org/10.1007/s11032-014-0030-6

Shahwar, D., Khan, Z., & Park, Y. (2023). Molecular marker-assisted mapping, candidate gene identification, and breeding in melon (*Cucumis melo* L.): A review. *International Journal of Molecular Sciences*, *24*(20), 15490. https://doi.org/10.3390/ijms242015490

Thorvaldsdóttir, H., Robinson, J. T., & Mesirov, J. P. (2013). Integrated genomics viewer (IGV): High-performance genomics data visualization and exploration. *Briefings in Bioinformatics*, *14*(2), 178–192. https://doi.org/10.1093/bib/bbs017

University of Wisconsin-Madison. (2022–2023). *Wisconsin's environmental mesonet yearbook, 2022–2023*. https://wisconet.wisc.edu

VanRaden, P. M. (2008). Efficient methods to compute genomic predictions. *Journal of Dairy Science*, *91*(11), 4414–4423. https://doi.org/10.3168/jds.2007-0980

Vega, A., & Goldman, I. L. (2023). Planting density does not affect root shape traits associated with market class in carrot. *HortScience*, *58*(9), 996–1004. https://doi.org/10.21273/HORTSCI17232-23

Vos, P. G., Paulo, M. J., Voorrips, R. E., Visser, R. G., van Eck, H. J., & van Eeuwijk, F. A. (2017). Evaluation of LD decay and various LD-decay estimators in simulated and SNP-array data of tetraploid potato. *Theoretical and Applied Genetics*, *130*(1), 123–135. https://doi.org/10.1007/s00122-016-2798-8

Wang, J., & Zhang, Z. (2021). GAPIT version 3: Boosting power and accuracy for genomic association and prediction. *Genomics, Proteomics & Bioinformatics*, *19*(4), 629–640. https://doi.org/10.1016/j.gpb.2021.08.005

Wang, M., Xu, Y., Wang, W., Wu, Z., Xing, W., & Zhang, H. (2019). Quantitative trait locus (QTL) mapping of sugar yield-related traits in sugar beet (*Beta vulgaris* L.). *Sugar Tech*, *21*(1), 125–144. https://doi.org/10.1007/s12355-018-0632-0

Wang, Y. H., Liu, P. Z., Liu, H., Zhang, R. R., Liang, Y., Xu, Z. S., Li, X. J., Luo, Q., Tan, G. F., Wang, G. L., & Xiong, A. S. (2023). Telomere-to-telomere carrot (*Daucus carota)* genome assembly reveals carotenoid characteristics. *Horticulture Research*, *10*(7), uhad103. https://doi.org/10.1093/hr/uhad103

Weber, W. E., Borchardt, D. C., & Koch, G. (1999). Combined linkage maps and QTLs in sugar beet (*Beta vulgaris* L.) from different populations. *Plant Breeding*, *118*(3), 193–204. https://doi.org/10.1111/j.1439-0523.1999.tb01513.x

Weber, W. E., Borchardt, D. C., & Koch, G. (2000). Marker analysis for quantitative traits in sugar beet. *Plant Breeding*, *119*(2), 97–106. https://doi.org/10.1046/j.1439-0523.2000.00482.x

Wigg, K. S., Brainard, S. H., Metz, N., Dorn, K. M., & Goldman, I. L. (2023). Novel QTL associated with *Rhizoctonia solani* Kühn resistance identified in two table beet × sugar beet $F_{2:3}$ populations using a new table beet reference genome. *Crop Science*, *63*(2), 535–555. https://doi.org/10.1002/csc2.20865

Wolyn, D. J., & Gabelman, W. H. (1990). Selection for betalain pigment concentrations and total dissolved solids in red table beets. *Journal of the American Society for Horticultural Science*, *115*(1), 165–169.

Würschum, T., Maurer, H. P., Kraft, T., Janssen, G., Nilsson, C., & Reif, J. C. (2011). Genome-wide association mapping of agronomic traits in sugar beet. *Theoretical and Applied Genetics*, *123*(7), 1121–1131. https://doi.org/10.1007/s00122-011-1653-1

Yadav, S. K., & Sehgal, S. (2003). Effect of domestic processing and cooking on selected antinutrient contents of some green leafy vegetables. *Plant Foods for Human Nutrition*, *58*, 1–11. https://doi.org/10.1023/B:QUAL.0000040359.40043.4f

Yang, J., Zaitlen, N. A., Goddard, M. E., Visscher, P. M., & Price, A. L. (2014). Advantages and pitfalls in the application of mixed-model

association methods. *Nature Genetics*, *46*, 100–106. https://doi.org/10.1038/ng.2876

Yau, Y., Santos, K., & Simon, P. (2005). Molecular tagging and selection for sugar type in carrot roots using co-dominant, PCR-based markers. *Molecular Breeding*, *16*, 1–10. https://doi.org/10.1007/s11032-004-6669-7

Yu, J., Pressoir, G., Briggs, W. H., Bi, I. V., Yamasaki, M., Doebley, J. F., McMullen, M. D., Gaut, B. S., Neilsen, D. M., Holland, J. B., Kresovich, S., & Buckler, E. S. (2006). A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. *Nature Genetics*, *38*(2), 203–208. https://doi.org/10.1038/ng1702

## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**How to cite this article:** Pelikan, A., & Goldman, I. L. (2025). Variation for QTL alleles associated with total dissolved solids among crop types in a GWAS of a *Beta vulgaris* diversity panel. *The Plant Genome*, *18*, e70014. https://doi.org/10.1002/tpg2.70014