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Identification of QTLs for Berry Acids and Sugar in a Vitis aestivalis-Derived 'Norton'-Based Population

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IDENTIFICATION OF QTLS FOR BERRY ACIDS AND SUGAR IN A *VITIS AESTIVALIS*-DERIVED 'NORTON'-BASED POPULATION

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Master of Science, Plant Science

By

Karlene L Negus

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ABSTRACT

Acidity and sugar content are among the grape berry quality traits that influence wine quality. Despite advantageous environmental tolerances of *Vitis aestivalis*-derived 'Norton', its acid and sugar concentrations often deviate from expectations set for *V. vinifera.* Identification of the genetic determinants of malic acid, tartaric acid, pH, and total soluble solids (TSS) can assist in the improvement of new hybrid cultivars. For this purpose, a 'Norton' and *V. vinifera* 'Cabernet Sauvignon' hybrid mapping population containing 223 individuals was used to construct a linkage map containing 384 simple sequence repeat (SSR) and 2084 genotyping-by-sequencing (GBS)-derived single nucleotide polymorphism (SNP) markers. The resulting map was 1441.9 cM in length with an average inter-marker distance of 0.75 cM and spanned 19 linkage groups (LGs). Quantitative trait loci (QTL) were detected for malic acid, tartaric acid, and pH. QTLs for malic acid (LG 8) and pH (LG 6) were observed across multiple years and explained approximately 17.7% and 18.5% of the phenotypic variation, respectively. Additionally, QTLs for tartaric acid were identified on linkage groups 1, 6, 7, 9, and 17 in single-year data. The QTLs for tartaric acid explained between 8.8-14.3% of the phenotypic variation. The markers linked to these QTLs can be used to improve hybrid cultivar breeding through marker-assisted selection.

KEYWORDS: *Vitis aestivalis*, 'Norton', simple sequence repeats, single nucleotide polymorphism, quantitative trait loci, malic acid, tartaric acid, total soluble solids, pH

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INTRODUCTION

Grapevine Cultivation & Breeding

In the time since the grape cultivation began, approximately 8,000 years ago, grapevines have grown to become one of the most important horticultural crops (McGovern et al. 2003). Today, nearly 7 million ha of vineyards worldwide are cultivated. In 2019 those vineyards produced an estimated 77.1 million metric tons of grapes which were sold as fresh table grapes, raisins, juice, or wine (FAO.org/faostat). While grapevines are cultivated throughout most regions of the world, production is primarily centered between 30°N and 50°N and within about 30°S and 40°S (Reisch et al. 2012). Even within the primary production regions, a broad range of climatic conditions and disease pressures exist. To accommodate these conditions, a diverse set of cultivated grapes exist and originate from parent species from Europe (*Vitis vinifera),* North America (*V. aestivalis, V. rupestris, V. riparia* - among others), and elsewhere. However, most cultivars of economic importance belong to *V. vinifera*.

European grapes originated from a wild progenitor species, *V. vinifera* subsp. *sylvestris* with the first evidence of cultivation found in the modern country of Georgia (McGovern et al. 2017). Few wild *sylvestris* grapes exist today, but the greater genetic diversity of *sylvestris* grapes in the Transcaucasia area (Georgia and neighboring regions) supports this region being the theorized origin of cultivated grapes. The wild *sylvestris* grapes were eventually domesticated and spread across the eastern hemisphere yielding the subspecies of cultivated grapevines *V. vinifera* subsp. *sativa.* (Zhou et al. 2019). Grape breeding in a controlled manner began roughly 200 years ago and was propelled forward with the introduction of North American diseases and insects into Europe shortly thereafter (Paul 1996). European vineyards experienced

much devastation in the mid-19th century due to imported pests and diseases like phylloxera (*Daktulosphaira vitifoliae Fitch*), powdery mildew *(Uncinula necator Burr*), and downy mildew (*Plasmopara viticola Berl.*) (Reisch et al. 2012). While North American grapevines had long grown under the pressures of these diseases, the *V. vinifera* cultivars found in Europe were highly susceptible. Much initial success was found using wild North American vines as rootstocks to confer resistance to phylloxera. Rootstock breeding programs soon began to include species like *V. aestivalis, V. rupestris,* and *V. berlandieri* (Cousins 2005). Scion breeding programs producing French-American hybrid (*V. vinifiera* × wild American *Vitis* spp.) grapes did have some early adaptation through the late 19th and first half of the 20th century in major grape growing regions such as France, however, few hybrids are grown there today (Reynolds 2015).

In less temperate regions of North America (where *V. vinifera* is ill-suited), breeders have found it necessary to utilize wild-species and hybrids which are better adapted to their regions. Breeding programs in North America also began around the early to mid-19th century and often included native species. The *V. labrusca* hybrid 'Concord' is an example of a hybrid cultivar which has gained widespread success and some name recognition. 'Concord', however, is more popularly used for juice, concentrate, jam, and jelly production rather than wine production. Overall, the table grape and raisin markets are those which most readily adopt cultivars resulting from modern breeding programs (Reisch et al. 2012). Despite the advantages in disease resistances and climate tolerances possessed by hybrid cultivars, there remains considerable bias toward hybrids for premium wine-production. Consumers often make purchasing decisions based on cultivar name recognition and, historically, American species-derived cultivars have not generated sales at the same level as *V. vinifera* (Lockshin and Corsi 2012). In recent years, as

the interest in organic and more sustainable viticultural practices has grown, a focus on breeding hybrid cultivars has returned (Zucca et al. 2009).

Cultivar Suitability

Vitis aestivalis-derived 'Norton', a North American species-based cultivar, is grown in the midwestern and eastern United States, regions with high disease pressure and cold winter temperatures for which *V. vinifera* is not adapted. (Viana et al. 2013; Sapkota et al. 2019). 'Norton' has strong resistance to powdery mildew and also exhibits less susceptibility to downy mildew, anthracnose, black rot, and phylloxera when compared to other *V. vinifera* and other hybrid cultivars (Ambers 2013). Because of these additional resistances, 'Norton' vineyards often require less inputs than *V. vinifera* cultivars in areas outside of the primary grape growing regions. However, *vinifera* cultivars continue to be planted in these environments which necessitates intense management. This is due to the dismissal of North American species-derived cultivars as being diminished in quality compared to *vinifera* cultivars (Ambers 2013). However, the "best red wine of all the nations," is how Dan Mouer, an eastern United States winemaker and writer, describes some 'Norton' wines produced from old Missouri vines, but he was not the first to state it. That distinction was awarded by judges of the 1873 World Fair in Vienna (Vizetelly 1873; Mouer 2019). 'Norton' lacks the intense "foxy" aromas associated with *V. labruscana* cultivars (e.g. 'Concord') (Ambers and Ambers 2004, Stover et al. 2009). Despite 'Norton' being recognized as a high quality North American species-derived cultivar, the chemical composition of 'Norton' berries still presents several challenges for premium wine production. Commonly reported challenges are high organic acids, and high pH (Main and Morris 2004; Liu et al. 2006, Jogaiah et al. 2013, Doerr 2014).

Berry Quality

The major organic acids in grapes are tartaric acid and malic acid which together constitute up to 90% of the total berry acids (Kliewer 1966). High concentrations of malic acid result in excessive sourness, which is associated with diminished wine quality (Rice 1974, Volschenk et al. 2006). Excessive tartaric acid is of less consequence than malic acid because the solubility of tartaric acid in wine is limited. Both tartaric and malic acid accumulate in the berry pre-veraison, but malic acid is the sole organic acid metabolized in grapes (Kliewer 1965; Ruffner 1982; Sweetman et al. 2009). Thus, malic acid content on a per-berry basis will decrease post-veraison, while tartaric acid remains stable. However, on a concentration basis, both organic acids will undergo dilution due to berry expansion during ripening (Iland and Coombe 1988). In 'Norton' malic acid and tartaric acid concentrations typically range from 3.2 to 7.8 g/L and 6.0 to 10.1 g/L, respectively, which are high compared to other red *V. vinifera* cultivars. *V. vinifera* values are often approximately 1.7-4.2 g/L for malic acid and 1.7-7.9 g/L for tartaric acid (Kliewer et al. 1967, Liu et al. 2007, Haggerty 2013).

Excessive wine pH facilitates oxidative and microbial spoilage (Conde et al. 2007). The relationship between pH and total acid is inverse but imperfectly correlated and influenced by variation in both organic acid compositions and the partial exchange of titratable protons for minerals, especially potassium (Hale 1977; Boulton 1980; Conde et al. 2007; Duchêne et al. 2014). Recently published ranges for pH in 'Norton' are approximately 3.4 to 3.9 (Main and Morris 2004, Jogaiah et al. 2013). These ranges are comparable to *V. vinifera* red's pH (3.2-4.2) (Kliewer et al. 1967, Liu et al. 2007, Haggerty 2013). A moderate pH for 'Norton' despite high organic acids is likely a consequence of its higher potassium content (up to 6 g/L) as compared to *V. vinifera* (Main and Morris 2004).

Sugar concentrations are generally evaluated through total soluble solids (TSS) measurements. Glucose and fructose, the dominant sugars in mature grapes, represent more than 90% of the total solids in grape juice (Kliewer 1966). Sugar accumulation in the vacuole is one of the main features of veraison and co-occurs with berry softening, acid reduction, and color change (Coombe 1992). Grape berries are non-climacteric and do not continue to ripen when removed from the vine so TSS is an important indicator of maturity for the timing of harvest especially when evaluated in conjunction with organic acid concentrations (Du Plessis 1984). For winemaking, glucose and fructose are critical components of fermentation as yeast transforms the sugars into alcohol. In *V. vinifera* cultivars TSS are generally 18-25 º Brix (another unit for the measure of total solids) (Cheynier et al. 1989; Waterhouse et al. 2016). 'Norton' has shown to have similar total soluble solids ranging from 21.2 to 23.7 º Brix (Main and Morris 2004, Jogaiah et al. 2013)

Cultivar Improvement

While cultivar-type and growing region are substantial determinants of organic acid composition, limited adjustment can be achieved with manipulation of the environment and cultural practices (Bobeica et al. 2015, Mirás-Avalos et al. 2019). It can instead be more effective to manipulate organic acids during vinification using practices such as potassium bitartrate precipitation, carbonate salt addition, and malolactic fermentation (Comuzzo and Battistutta 2019). However, these practices cannot fully address high acid musts – especially those with high malic acid – and may result in unwanted sensory changes (Beelman and Gallander 1979). Mature grapes with low sugar concentrations can yield wines with low alcohol content. Chaptalization, or the addition of sugar to grape must prior to fermentation, can be used

to increase the final alcohol content, but the practice is limited in many winemaking regions (Ribéreau-Gayon et al. 2005). Breeding selection informed by the underlying genetic determinants of important quality traits may be a more functional and sustainable method of control. The inheritance of berry traits with enological importance, such as acidity and sugar, has begun to be investigated in grapevine. Chen et al. (2015) reported six QTLs on two linkage groups for malic acid with each accounting for a small percentage of variation. Other studies have reported QTLs for malic acid, tartaric acid, and their ratio (Houel et al. 2015; Yang et al. 2016; Bayo-Canha et al. 2019; Duchêne et al. 2020). Several of these studies and others also included sugar traits, including TSS, ºBrix, fructose, glucose, and total sugar when investigating important agronomic traits (Viana et al. 2013; Chen et al. 2015; Houel et al. 2015; Bayo-Canha et al. 2019). However, the observed QTLs vary across studies and thus may be unique for a *V. aestivalis*-derived cultivar. Identifying the DNA markers linked to fruit quality traits will be important for the preservation of wine quality while maintaining pest and/or disease resistances possessed by the cultivar.

Modern marker-assisted selection strategies involve the development of mapping populations and the construction of linkage maps. Linkage maps are useful for indicating the chromosomal position of DNA markers, particularly those which are associated with a trait of interest. A mapping population with segregating loci and segregating traits is critical for the identification of marker-trait associations through QTL mapping (Collard et al. 2005). The single nucleotide polymorphism (SNP) markers have become widely used for this purpose and successfully implemented in *Vitis* spp. in the last 10 years (Myles et al. 2011; Barba et al. 2014; Yang et al. 2016; Laucou et al. 2018). Specifically, genotyping-by-sequencing (GBS)-derived SNPs have proved to be a successful, fast, and low-cost marker system (Barba et al. 2014; Yang

et al. 2016; Sapkota et al. 2019), despite concerns regarding the high number of SNP calling errors in heterozygous species which results from low read depths associated with the method (Elshire et al. 2011; Swarts et al. 2014). Using SNPs in linkage map construction can overcome the low marker density of maps generated exclusively from simple sequence repeat (SSR) markers (Chen et al. 2015; Yang et al. 2016). However, SNP markers have limited transferability between *Vitis* species (Vezzulli et al. 2008). Given the widespread use of interspecific hybrids for developing improved cultivars, the high transferability of SSR markers remains an important advantage.

Study Focus

Previously, a mapping population of 183 genotypes from 'Norton' × 'Cabernet Sauvignon' was used to construct a linkage map via 411 SSR markers (Hammers et al. 2017). This map was then improved by integrating 1665 GBS-derived SNP markers to develop a high-density linkage map for further horticultural studies (Sapkota et al. 2019). The use of both SSR and SNP markers for linkage map construction provides the opportunity to improve both marker density and transferability.

The present study investigated the genetic determinants of tartaric acid, TSS, pH, and malic acid using an expanded 'Norton' × 'Cabernet Sauvignon' mapping population. The population was genotyped using GBS-derived SNP and SSR markers and phenotyped over three years. An improved, high-density linkage map was developed with additional markers and used to identify QTLs for all investigated traits.

METHODS

Plant Materials

The mapping population was developed from a crossing between *V. aestivalis*-based 'Norton' and *V. vinifera* 'Cabernet Sauvignon'. The population is located at the Missouri State Fruit Experiment Station (MSFES) in Mountain Grove, MO, USA. An initial crossing in 2005 yielded 79 progeny, and an additional planting in 2011 brought the population total to 279 individuals. Crosses were initiated by emasculation of the 'Norton' prior to anthesis. Next, pollen was collected from 'Cabernet Sauvignon' and dried overnight using a 60W light. Pollen was stored at room temperature until its use the morning following emasculation. Pollination was accomplished by brushing the dried pollen on the stigmas of the emasculated clusters. Paper bags were used to cover the inflorescences to prevent contamination following both emasculation and pollination. Seeds were extracted post-harvest and stratified for 3 months at 4°C prior to planting. Seeds germinated within 3 weeks of planting in seedling trays located in a greenhouse. Hybrid identification of the seedlings was completed using 6 SSR markers (Adhikari et al. 2014). True hybrid vines were planted in the field with spacing of 10-feet (3.0 m) between rows and 8-feet (2.4 m) within rows. A bilateral cordon system was used for vine training.

Genomic DNA was extracted from young leaves using the DNeasy Plant mini Kit (Qiagen, Valencia, CA) and the manufacturer's protocol. A NanoDrop spectrophotometer was used to quantify DNA concentration and purity (Adhikari et al. 2014). DNA was stored at -20°C prior to use in SSR genotyping. Additional leaf samples were collected and packaged using protocol recommended by the USDA-NIFA Specialty Crops Research Initiative *Vitis*Gen project (www.vitis gen.org) as previously described by Sapkota et al. (2019). Single leaf samples

approximately 10mm in diameter were partitioned using the wells of a Costar 96-well cluster tube collection plate (Corning Life Sciences, Tewksbury, MA, USA) for shipment to the *Vitis*Gen (www.vitisgen.org) genotyping center for the development of GBS-derived SNPs (Sapkota et al. 2019).

Genotyping

Genotypes for 413 polymorphic SSR markers were previously identified in the mapping population by Hammers et al. (2017). Briefly, SSR marker alleles were amplified via PCR using an M13-tailed forward primer (Oetting et al. 1995), a reverse primer, and a WellRED (Sigma–Aldrich, St. Louis, MO) labeled M13 sequence in each reaction (Adhikari et al. 2014). Primers were designed using previously published literature sourced from NCBI database uniSTS (http://www.ncbi.nlm.nih.gov), Vitis-EST database (http://cgf.ucdavis.edu), and Grape Genome Browser-Genoscope (http://www.genoscope.cns.fr/externe/GenomeBrowser/Vitis/). Primer design is further detailed by Hammers et al. (2017). PCR reactants included 1 µL of 15 ng/µL template DNA, 1 µM of 2 µM WellRed M13 primer, 1.8 µL of 0.1 µM of forward primer and 2 µM of reverse primer, 5 µL AmpliTaq GoldR 360 Master Mix buffer (Life Technologies, Grand Island, NY) and 0.2 µL of 25 mM $MgCl_2$. The touchdown PCR protocol was used for amplification (Adhikari et al. 2014). SSR allele sizes were evaluated using a GenomeLab GeXP capillary sequencer and software (Beckman Coulter, Brea, CA). To account for the M13 sequence, 18 base pair was removed from the allele size yielded from the capillary sequencer when recording SSR marker genotypes.

A subset of the population (159 individuals) was previously genotyped using GBS (Sapkota et al. 2019). An additional 64 individuals from the mapping population were

subsequently genotyped using an identical procedure. Briefly, GBS was carried out according to the protocol of Elshire et al. (2011) using ApeKI restriction digestion and Illumina Highseq 2000 platform for sequencing which was performed at the Cornell University Biotechnology Resource Center (BRC, Ithaca, NY, USA). Individuals in the F_1 mapping population were all sequenced once and aligned to the 12x v2 *V. vinifera* 'PN40024'. Parental DNA was sequenced in three separate replicates. Burrows-Wheeler alignment tool (BWA) with default settings was used for alignment (Li and Durbin 2009; Barba et al. 2014). The resulting SNP data, contained within a VCF file, were filtered in TASSEL 5.2.04 (Bradbury et al. 2007). Markers possessing both parental genotypes, genotypes for at least 90% of the population, a minor allele frequency (MAF) of at least 0.10, and a heterozygous proportion of at least 0.10 were retained. The remaining markers were evenly thinned based on their physical positions to total 142 to 216 markers per chromosome.

Linkage Map Construction

Genetic map construction was accomplished in Lep-MAP3 v. 0.2 (Rastas 2017). SNP markers were encoded into the format used in JoinMap 4 (Van Ooijen 2006) (lm×ll, nn×np, hk×hk) using the Next Generation Sequencing Eclipse Plugin (NGSEP) (Perea et al. 2016). SSR markers were concatenated with the SNP markers. Linkage phases for all markers were extracted from JoinMap 4. The marker file was converted using an awk command provided by Lep-MAP3 and used, with the pedigree, in the *ParentCall2* module. Markers were separated into linkage groups using distortion-aware LOD scores using *SeparateChromosomes2*. LOD limits for grouping ranged from 6-10, as needed to achieve 19 linkage groups (LGs). Remaining single, ungrouped markers were iteratively joined to existing groups via *JoinSingles2All*. Finally, using

the *OrderMarkers2* module, markers were ordered into a sex-averaged map based on the maximized order likelihood of 100 mapping iterations. Mapping distances and marker orders were extracted from the output of *OrderMarkers2* and formatted for use with MapQTL6 (Van Ooijen 2009).

Phenotyping

Berries were sampled from the population at post-veraison maturity in the years 2017, 2018, and 2019. Harvest dates varied from year to year in accordance with a visual assessment of grape maturity (stage 38-40 on modified E-L system) (Coombe 1995). All samples were collected during the same harvest period within each year. Berry samples contained 200-300 g from each genotype. Two sampling replicates of each genotype, as fruit availability allowed, were collected and stored at -20°C. One replicate was kept at Missouri State University and used for this project, and the second was overnight shipped with dry ice to the *Vitis*Gen Fruit Quality Phenotyping Center for additional berry quality phenotyping (not described here) at Cornell University (Ithaca, NY, USA).

For acid, TSS, and pH evaluation, berries (100 g) were destemmed and thawed at room temperature for approximately 2 h before being crushed in a Stomacher 80 Biomaster (Seward, Worthing West Sussex, UK) for 1 min at 'normal' speed. Samples were transferred to 50 mL plastic centrifuge tubes and centrifuged at 12,000x g for 15 min, and supernatant transferred into 20 mL plastic centrifuge tubes.

The concentrations of malic and tartaric acids were evaluated using an Agilent 1100 Series HPLC system (Agilent Technologies, Santa Clara, CA, USA) with a Synergi Hydro-RP column (Phenomenex, Torrance, CA, USA) and an Agilent 1100 Diode Array Detector. The

column was held at 22°C. Samples were eluted with a 20 mM KH_2PO_4 solution at a pH of 2.9, a flow rate of 0.40 mL per min, and at a UV absorbance of 220 nm. The injection volume was 5.0 µL of undiluted juice. Duplicate analysis was completed on each sample. Malic and tartaric acid concentrations were calculated using standard curves of (L-(-)-malic and L-(-)-tartaric acid (Sigma-Aldrich, St. Louis, MO, USA) and expressed in g/L. An Orion Star A211 pH meter (Thermo Fisher Scientific, Waltham, MA, USA) was used to evaluate the pH of extracted juice samples. TSS was measured using a Pocket Brix-Acidity Meter for grape & wine (PAL-BX|ACID2) (Atago, Bellevue, WA, USA) and expressed in ºBrix.

Statistical Evaluations

Normality, transformations, heritability, correlations, and best linear unbiased predictor (BLUP) estimates were performed in the statistical software R v3.6.3 (R Core Team 2020). The normality of trait distributions was evaluated using the Shapiro-Wilk's test. All phenotypes were Box-Cox transformed using MASS package v7.3-51.6 (Venables and Ripley 2002) to improve normality of the traits for QTL analysis.

The full model used for BLUPs and broad sense heritability estimates was $P_{ij}=\mu+G_i+E_j+r_{ij,}$ where P_{ij} was the trait phenotype for genotype i in year j, μ the overall mean, G_i the random effect of genotype i, E_j the random effect of year j, and r_{ij} the residual term. BLUPs were calculated for traits which were phenotyped in at least three years and individuals for which at least two sets of data were available. Broad sense heritability was estimated for each trait as the proportion of phenotypic variance which is explained by the genotypic variance using the following equation: $\sigma_G^2 \,/\, (\sigma_G^2 + \sigma_E^2)$ where σ_G^2 is the variance due to genotype and σ_E^2 is the variance due to environment. The linear model and variances were calculated using the lme4

package v. 1.1-21 (Bates et al. 2015). Correlations between years of data were determined using the Pearson test in Hmisc v. 4.4-0 (Harrell Jr and Dupont 2020). Partial correlations of traits were evaluated using ppcor v. 1.1 with default methods (Kim 2015).

QTL Analysis

QTL analysis was performed in MapQTL6 (Van Ooijen 2009). The restricted multiple QTL model (rMQM) mapping method was used with cofactor selection for final analysis of all phenotypes. Cofactor selection was accomplished using the "automatic cofactor selection" tool. The percentage of variance explained (PVE) by the QTL and QTL position was extracted from the final rMQM result. Confidence intervals for the QTL were defined by a 1.5-LOD support interval. Significance thresholds were estimated using 1000 permutations of each phenotype. The LOD threshold equivalent to α=0.05 at the genome-wide (GW) level was considered significant. QTLs observed in at least two years were considered stable. QTLs detected in a single year were reported but considered unstable.

An analysis of variance (ANOVA) evaluating differences in trait means between genotypes at a given locus was used for single year effect sizes. BLUP values were used in place of single year phenotypes for loci located at stable QTL. Default settings for 'Anova' function from the car package (v. 3.0-6) were used (Fox and Weisberg 2019). All markers within the QTL regions and the closest flanking markers were tested. Alleles of the greatest effect size for the QTL and flanking marker alleles effect sizes were reported for single-year QTL. Multi-year QTL effect sizes were reported for all markers within the support interval. Tukey's honest significant difference (HSD) test ($p < 0.05$) was conducted to evaluate the differences in each haplotype combination's average effect for QTL flanking markers and one interior maker. P-values were

also extracted from the analysis of variance model. All effect sizes are reported in units corresponding to the trait values and are not standardized.

RESULTS

Genotyping & Genetic Maps

A total of 63488 SNPs were generated from GBS. Using TASSEL to filter to minimums of 90% population coverage, 0.10 MAF, and 0.10 heterozygous proportion, 9663 markers remained. After thinning by physical position, the GBS marker number was 3366. All SNPs were successfully converted to JoinMap format using NGSEP. Markers which sorted into incorrect groups based on the reference genome were discarded after an initial mapping. In total, 2502 SNP and 402 SSR were used at the outset of the final map construction. The final consensus map (Appendix) included 2468 markers (384 SSR and 2084 SNP) which covered 1441.9 cM and represented 19 LGs (Table 1). Linkage groups varied in size and marker number from 63.6 cM (LG 15) to 95.7 cM (LG 7) and 81 markers (LG 15) to 171 markers (LG 14). Marker density averaged 0.75 cM between markers.

Of the 2468 markers used, the dominant segregation patterns were informative for one of two parents. Those which varied in only 'Norton' (lm×ll) included 1400 (56.7%) SNPs and 120 (4.9%) SSRs while markers segregating exclusively in 'Cabernet Sauvignon' (nn×np) numbered 552 (22.4%) SNPs and 29 (1.2%) SSRs. Markers that segregated with two identical alleles in both parents (hk×hk) numbered 132 (5.3%) SNPs and 3 (0.12%) SSR. The remaining 232 (9.4%) markers segregated with more than 2 alleles. SSRs which were fully informative for both parents (ab×cd) totaled 147 (6.0%) and tri-allelic SSRs (ef×eg) counted 85 (3.4%). Distorted markers ($\alpha < 0.05$) were seen randomly distributed on every LG with regions of clustering also present. These markers were included in the final genetic linkage map unless they hindered linkage map construction.

Table 1 Marker distributions of the 'Norton' × 'Cabernet Sauvignon' consensus genetic map

LG	No. of Markers			Inter-marker distances (cM)		Length (cM)
	Total	SSR	SNP	Average	Maximum	
1	149	29	120	0.72	3.4	80.3
2	109	15	94	0.78	3.4	69.1
3	89	13	76	0.86	6.8	63.7
4	154	29	125	0.72	4.5	79.0
5	121	21	100	0.71	4.3	70.4
6	164	17	147	0.67	3.8	78.7
7	170	27	143	0.69	3.6	95.8
8	167	24	143	0.66	3.8	84.8
9	99	14	85	0.85	3.4	70.7
10	99	14	85	0.75	3.6	64.2
11	110	21	89	0.79	5.9	71.4
12	122	14	108	0.72	3.8	66.0
13	145	13	132	0.70	3.1	87.7
14	171	45	126	0.62	3.8	87.0
15	81	8	73	0.96	8.4	63.6
16	114	17	97	0.82	4.3	74.3
17	125	13	112	0.72	2.7	75.1
18	167	36	131	0.72	5.4	94.5
19	112	14	98	0.76	3.4	65.9
Total	2468	384	2084	0.75	8.4	1441.9

Phenotypic Evaluations

All traits varied continuously (Figure 1). Each year, traits were evaluated in 138 to 186 individuals (Table 2). In 2019, pH and malic acid mean values were lower than other years while tartaric acid was greater in 2019 than 2018. The smallest range for each trait was reported in 2018. Tartaric acid in 2019 and TSS in 2019 were more broadly distributed across the trait range than seen in the other reported year(s). The traits which were normally distributed included pH in all years and TSS in 2019, according to a Shapiro-Wilk's test ($P \geq 0.05$). The normality of all traits was improved through the Box-Cox transformation.

Malic acid reported here (0.50-12.3 g/L) exceeded the range reported in *V. vinifera* populations (approx. 1.1-7.6 g/L) (Liu et al. 2007; Duchêne et al. 2014) but was within the range of other *Vitis* spp. populations (approx. 0.80-21.3 g/L) (Chen et al. 2015; Yang et al. 2016). Tartaric acid values (1.7—19.2 g/L) exceeded those reported for *V. vinifera* (1.4-7.9 g/L) (Liu et al. 2007; Duchêne et al. 2014) and in 2019 slightly exceeded the range seen in some other interspecific hybrid populations (1.5-17.2 g/L) (Chen et al. 2015). The pH range (2.2-4.3) was similar to other *V. vinifera* populations (3.0-4.3) (Viana et al. 2013, Duchêne et al. 2014). TSS means in the population (20.4-22.4) were near observed values for 'Norton' (21.6-22.6), *V. vinifera* (20.8) and other *V. spp* (20.6-21.0) (Sun et al. 2011; Jogaiah et al. 2013). Overall, malic and tartaric acid values exceeded those seen in *V. vinifera* populations while pH and TSS were near previously observed values.

Broad sense heritability was 0.24 for tartaric acid, 0.65 for TSS, 0.32 for pH, and 0.70 for malic acid (Table 2). Malic acid was significantly correlated ($P<0.05$; Pearson test) between the year combinations (Table 3). Tartaric acid was significantly correlated ($P<0.05$; Pearson test) between the years 2018 and 2019 (0.23) (Table 4). Significant correlations also existed for pH

and TSS between 2017 and 2018 (0.57, 0.52), 2017 and 2019 (0.50, 0.57, 0.33), and 2018 and 2019 (0.66, 0.65, 0.38) (Tables 5 and 6). Due to the logarithmic nature of the pH scale, pH was converted to hydrogen-ion concentration ($[H^+]$) for evaluation of linear partial correlation. Results of the partial correlations, malic acid and pH (as $[H^+]$) were correlated in 2019 and 2018 ($P \leq 0.05$) with coefficients of 0.19 and 0.20, respectively (Tables 7 and 8). TSS was negatively correlated (-0.21) with malic acid in 2018. TSS was also significantly correlated with tartaric acid in 2018 (0.18) and 2019 (0.24). TSS and pH were also correlated in 2017 (0.26) (Table 9) and 2019 (-0.19). The remaining traits were not significantly correlated.

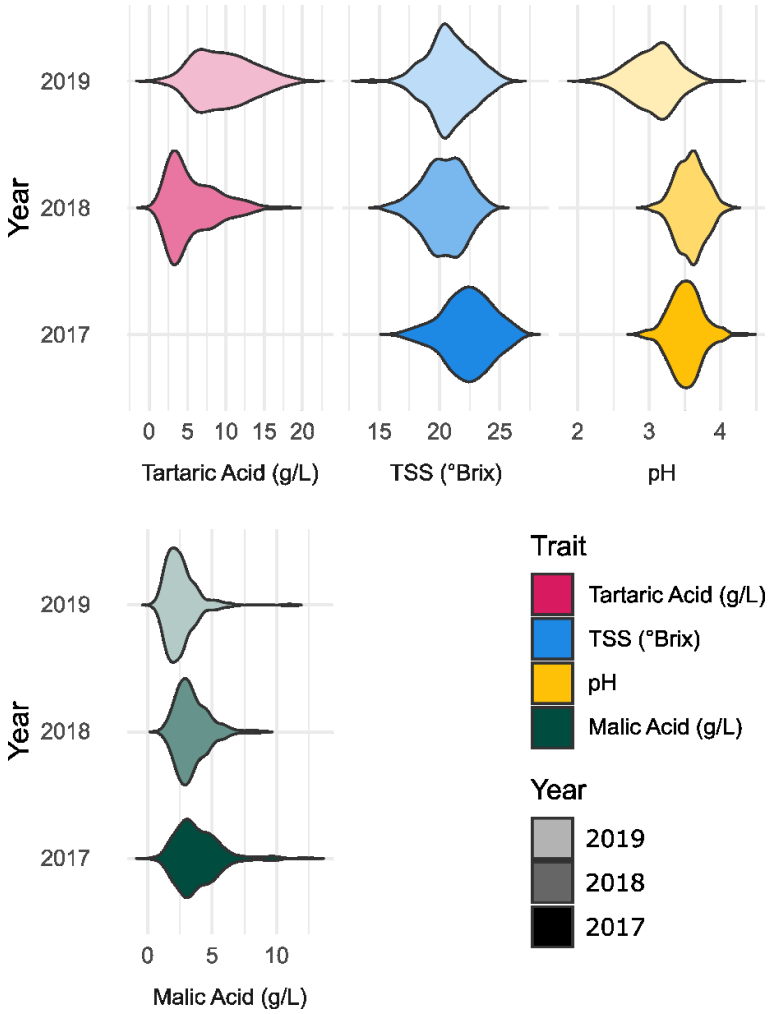

Fig. 1 Frequency distribution (violin plot) of berry quality traits by year

Table 2 Summary statistics and broad-sense heritability values for evaluated traits

Trait	Year	N	Mean	H^2	Minimum	Median	Maximum
Tartaric acid (g/L)	18	138	5.5	0.24	1.7	4.4	16.6
	19	183	9.7		1.9	9.3	19.2
TSS (ºBrix)	17	179	22.4		17.0	22.4	26.4
	18	143	20.4	0.65	15.9	20.5	24.0
	19	185	20.9		14.4	20.7	25.5
pH	17	179	3.5		2.9	3.5	4.3
	18	143	3.6	0.32	3.0	3.6	4.1
	19	186	3.1		2.2	3.1	4.0
Malic acid (g/L)	17	178	3.9		0.52	3.5	12.3
	18	138	3.4	0.70	1.3	3.2	8.5
	19	185	2.5		0.50	2.3	11.0

Table 3 Malic acid correlations between years

Year	2018	2017
2019	0.66 ***[a]	0.5 ***
2018		0.66 ***

[a] *** :P<0.001

Table 4 Tartaric acid correlations between years

Year	2018	2017
2019	0.23 *[a]	NA
2018		NA

[a] *: P<0.05; NA: data not available

Table 5 pH correlations between years

Year	2018		2017	
2019	0.65	***[a]	0.57	***
2018			0.57	***

[a] *** :P<0.001

Table 6 TSS correlations between years

Years	2018		2017	
2019	0.38	***[a]	0.33	***
2018			0.52	***

[a] ***: P<0.001

Table 7 Partial correlations between traits in 2019

Trait	Tartaric acid		pH		TSS	
Malic acid	-0.02	ns[a]	0.19	*	-0.08	ns
Tartaric acid			0.14	-	0.24	**
pH					-0.19	**

[a] ns: not significant; - :P<0.1; * :P<0.05; ** :P<0.01

Table 8 Partial correlations between traits in 2018

Trait	Tartaric acid		pH		TSS	
Malic acid	0.09	ns[a]	0.28	**	-0.21	*
Tartaric acid			0.15	-	0.18	*
pH					-0.15	-

[a] ns: not significant; - :P<0.1; * :P<0.05; ** :P<0.01

Table 9 Partial correlations between traits in 2017

Trait	Tartaric acid	pH		TSS	
Malic acid	NA[a]	0.01	ns	-0.12	ns
Tartaric Acid		NA		NA	
pH				0.26	***

[a] ns: not significant; *** :P<0.001; NA: data not available

QTL Detection

Phenotypes (tartaric acid, pH, TSS, and malic acid) were analyzed in 3 data sets which were divided by year. At the genome-wide (GW) level of significance (P<0.05), 11 QTLs were identified and distributed across 9 linkage groups (Table 10). Tartaric acid, pH, and malic acid showed 5, 3, and 3 QTLs, respectively. The GW significance thresholds varied in each trait and were relatively high, ranging from a LOD score of 5.1 (pH 2017, malic acid 2019) to 5.9 (tartaric acid 2018), so individual thresholds were retained for each trait. The maximum LOD scores for the reported QTLs varied between 5.1 (pH LG 6) and 9.0 (pH LG 4, malic acid LG 8) and the PVE by each ranged from 8.8 (tartaric acid LG 6) to 21.6 (pH LG 4). Significant QTLs were not identified for TSS.

Malic acid and pH had QTLs stable across three years. The LOD maximums for the stable malic acid QTL on LG 8 were positioned within a 2.3 cM range in all three years. The 1.5 LOD confidence intervals converged on the interval between 30.6 and 31.2 cM and was associated with the physical interval from 9992297-10385236 bp. Within this region, alleles of the greatest effect all were informative for 'Norton' (Table 11). For marker VVS4, individuals possessing the allele size of 183 bp (progeny genotypes of 183/169 bp and 183/176 bp) had an average malic acid concentration 0.74 g/L greater than individuals which inherited the 169 bp allele from 'Norton' (progeny genotypes 169/169 bp and 169/176 bp) (Figure 2). Both SSR and

SNP marker effects reported for this QTL were significant (P<0.05). Additional single year (2019) QTLs were detected for malic acid on LGs 14 and 18. On LG 14, phenotype averages differed between 'Cabernet Sauvignon' alleles (Figure 3) while on LG 18 significant effects were seen from 'Norton' alleles (Figure 4). Effect sizes from LGs 14 and 18 were smaller (0.60 and 0.54 g/L) than those on LG 8 (0.75 g/L).

A stable QTL associated with pH was located on LG 6 at a common interval of 43.9 to 46.0 cM on the linkage map and 11607560 and 15724160 bp in physical position. An increased average pH corresponded with allele size 128 (Cabernet Sauvignon) of marker VVS5 (Figure 5) The effect size at this marker was a 0.10 difference between alleles (Table 11). Single year QTLs for pH were also detected on LGs 4 and 8. The effects of alleles on LGs 4, 6, and 8 associated with pH were all attributed to Cabernet Sauvignon. Effects on LG 4 (0.20-0.25) were the greatest of those reported for pH (Figure 6). The pH QTL on LG8, which appeared to co-locate with the stable malic acid QTL, was also attributed to 'Cabernet Sauvignon' but only nearly significant (P<0.10) allele effects were seen (Figure 7).

The remaining trait – tartaric acid – demonstrated only single year QTLs. QTLs associated with tartaric acid were located on LG 1, 6, 7, 9, and 17. 'Norton'-associated allele effects were found on LG 1, 9, and 17, (Figures 8, 9, and 10) while 'Cabernet Sauvignon' alleles explained variation on LGs 6 and 7 (Figures 11 and 12). Only two markers were reported on LG 9 as 9_240934 was a marker interior to the reported physical region but also the most distal informative marker.

Table 10 Summary of QTLs for acid-related traits in 'Norton' × 'Cabernet Sauvignon' F_1 population

Trait	LG	Year	Max LOD	GW LOD Threshold	PVE	Peak (cM)	1.5-LOD Interval (cM)	Physical Interval (bp)
Tartaric	1	19	7	5.2	12.3	43	41.5-44.6	8335044-9738524
acid	6	19	5.2	5.2	8.8	39.1	30.6-41.5	6679935-7615750
	7	19	6.9	5.2	12.1	36.7	35.8-37.3	8215450-9475359
	9	18	7	5.9	14.1	5.2	5.0-6.1	198079-460535
	17	18	7.1	5.9	14.3	56.8	56.4-56.8	9295617-10144592
pH	4	19	9	5.3	21.6	53.4	51.8-56.4	16450987-18940320
	6	17	5.1	5.1	16.2	2.8	2.8-43.9	141387-11607560
		18	7	5.4	19.2	46	44.0-46.0	14849083-15724160
		19	6.6	5.3	20.2	49.4	43.4-54.0	8139288-16573962
	8	18	5.9	5.4	13	32.8	32.0-33.2	8553958-10498296
Malic	8	17	9	5.2	19.6	30.5	30.2-36.0	9992297-11975066
acid		18	7.3	5.3	20.1	32.8	14.0-39.6	2400696-11975066
		19	7.6	5.1	13.4	31.2	30.6-31.2	9992297-10385236
	14	19	6.8	5.1	13.7	7.9	6.4-8.2	1426689-1955725
	18	19	5.9	5.1	9.5	21.2	21.2-21.8	4020114-4997180

Table 11 Allele effects and parental genotypes of markers associated with berry acid QTLs

Trait	LG	Marker	Allele	Effect Size		Parental Genotype N	Parental Genotype CS	Physical Position (bp)
Tartaric	1	1_8118509	T	1.5	*[a]	AT	AA	8118509
Acid		VVIP60	332	-2.4	***	310/332	307/315	8803413
		VMC7g5	167	2.3	***	157/167	171/186	9804330
	6	VMC2G2	131	-1.3	*	127/127	127/131	5819478
		6_6679935	A	-1.6	**	GG	AG	6679935
		VMC2F10	105	1.5	*	95/114	95/105	7615750
	7	ctg9481	403	-0.40	ns	394/397	394/403	7446186
		VMC1A2	149	0.52	ns	108/217	108/149	8230151
		7_16971155	A	-1.2	-	GG	AG	16971155
	9	9_240934	T	-0.30	ns	GT	GG	240934
		FAM26	301	1.0	-	295/301	295/303	461325
	17	17_9295617	C	0.62	ns	CT	TT	9295617
		17_9884306	C	1.7	**	AC	AA	9884306
		17_10144592	C	-1.1	-	AC	AA	10144592
pH	4	VVIN75	194	0.20	***	196/212	188/194	14823485
		VMC2E10	59	0.25	***	53/55	57/59	17456655
		VVIP77	191	0.21	***	180/186	186/191	19317923
	6[b]	6_9366152	A	0.09	**	GG	AG	9366152
		6_12192456	T	-0.11	***	CC	CT	12192456
		VVS5	128	0.10	***	113/NULL	104/128	12956677
		6_13625084	C	0.14	***	GG	CG	13625084
		6_14347587	T	0.12	***	CC	CT	14347587
		VMC4G6	130	0.12	***	124/140	124/130	15179752

[a] ns: P>0.10, -: P<0.10, *: P < 0.05, **: P < 0.01, ***: P < 0.001

[b] QTL effects were calculated using BLUP values in place of single year data

Table 11 Continued Allele effects and parental genotypes of markers associated with berry acid QTLs

Trait	LG	Marker	Allele	Effect Size		Parental Genotype		Physical Position (bp)
						N	CS	
pH	8	8_7718022	T	0.03	ns	CC	CT	7718022
		VMC7H2	135	0.07	-	125/132	122/135	10149243
		8_10699999	T	-0.03	ns	CC	CT	10699999
Malic	8[b]	VVS4	183	0.74	***	169/183	169/176	9992297
Acid		VMC7H2	125	0.68	***	125/132	122/135	10149243
		8_10159975	T	0.69	***	CT	CC	10159975
		8_10222803	G	0.53	**	AG	AA	10222803
		FAM16	326	0.67	***	326/329	329/329	10384476
	14	VVC62	204	0.65	**	184/188	184/204	1426689
		14_1714481	A	0.60	**	CC	AC	1714481
		14_2083206	A	0.51	*	GG	AG	2083206
	18	18_4020114	T	0.42	*	AT	AA	4020114
		18_4607745	G	0.54	*	CG	CC	4607745
		VMCNg1b9	151	0.22	ns	151/160	160/160	5645610

[a] ns: P>0.10, -: P<0.10, *: P < 0.05, **: P < 0.01, ***: P < 0.001

[b] QTL effects were calculated using BLUP values in place of single year data

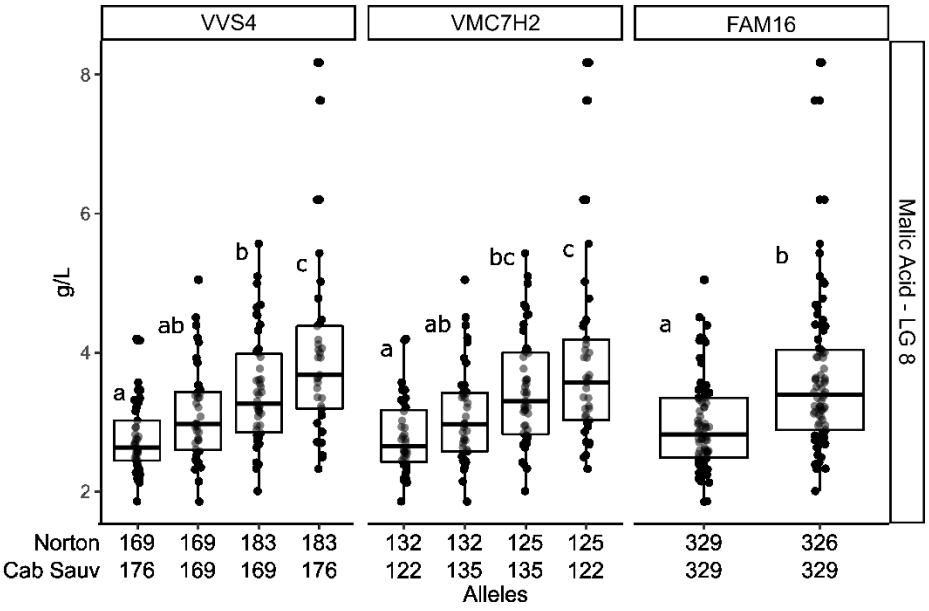

Fig. 2 Malic acid phenotypes grouped according to genotype using markers associated with QTL on LG 8. Phenotypes represent BLUP values of data from 2017-2019. Statistically significant differences are designated by letter ($p < 0.05$, Tukey's HSD test).

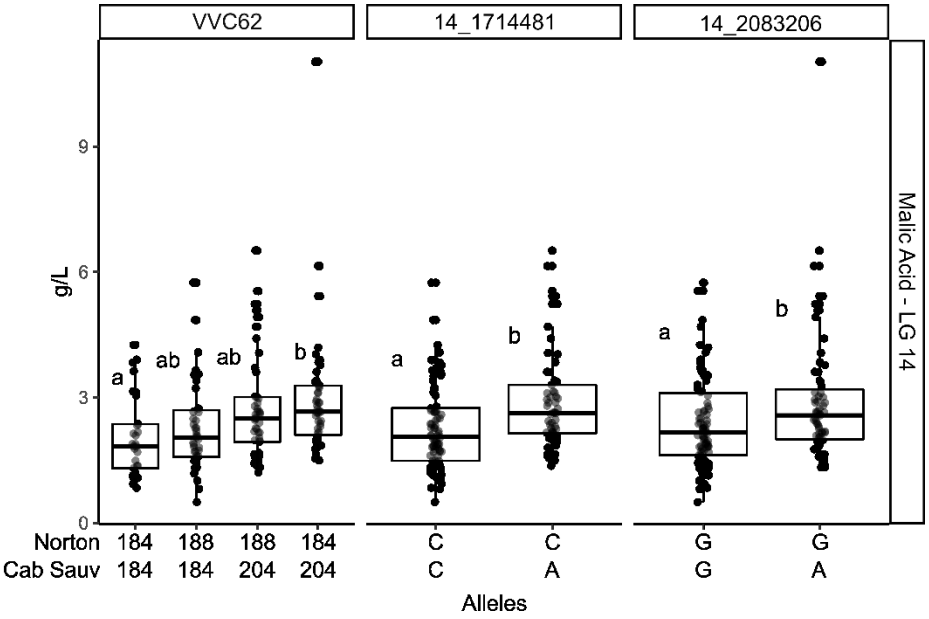

Fig. 3 Malic acid phenotypes grouped according to genotype using markers associated with QTL on LG 14. Phenotypes represent data from 2019. Statistically significant differences are designated by letter ($p < 0.05$, Tukey's HSD test).

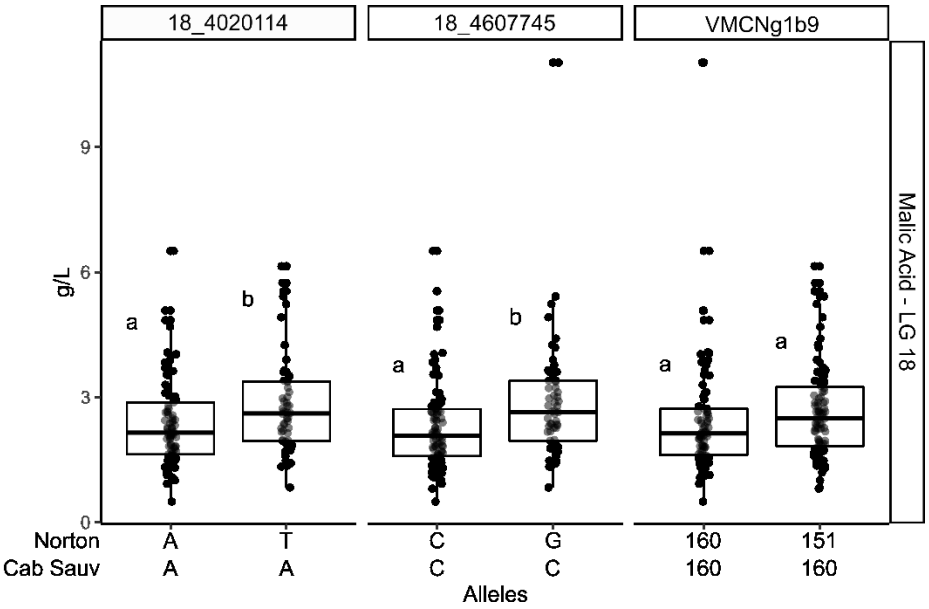

Fig. 4 Malic acid phenotypes grouped according to genotype using markers associated with QTL on LG 18. Phenotypes represent data from 2019. Statistically significant differences are designated by letter ($p < 0.05$, Tukey's HSD test).

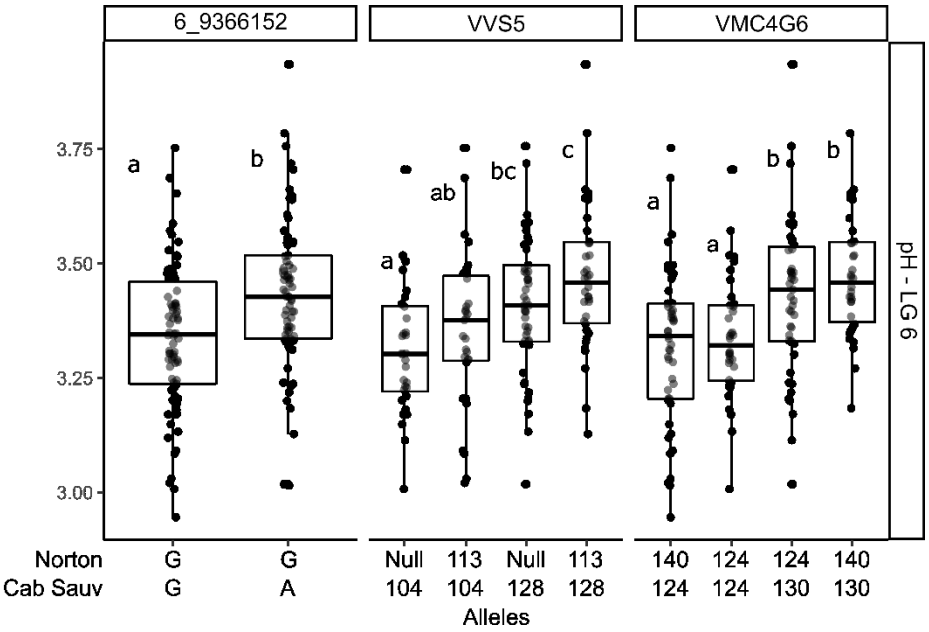

Fig. 5 pH phenotypes grouped according to genotype using markers associated with QTL on LG 6. Phenotypes represent BLUP values of data from 2017-2019. Statistically significant differences are designated by letter ($p < 0.05$, Tukey's HSD test).

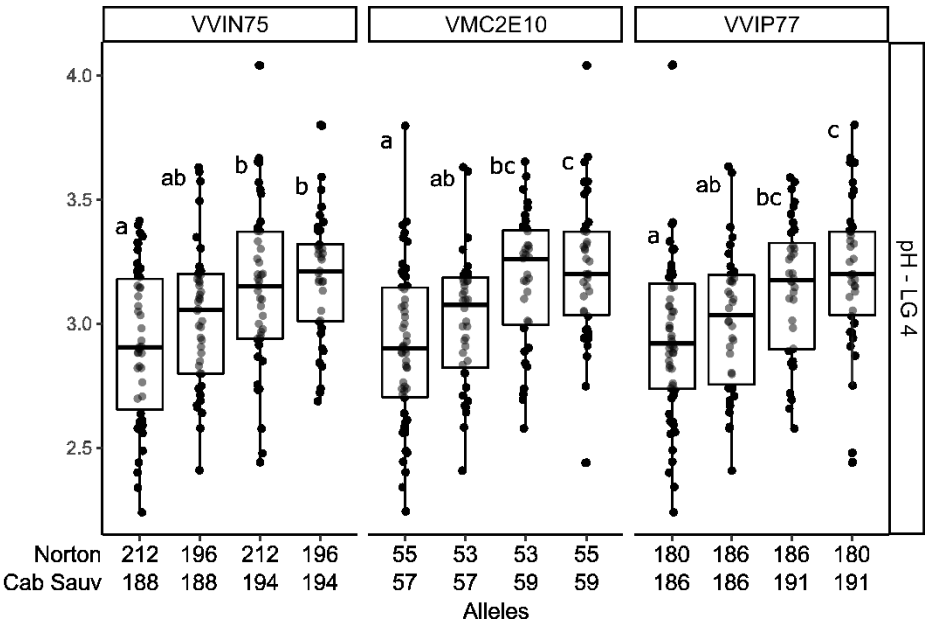

Fig. 6 pH phenotypes grouped according to genotype using markers associated with QTL on LG 4. Phenotypes represent data from 2019. Statistically significant differences are designated by letter ($p < 0.05$, Tukey's HSD test).

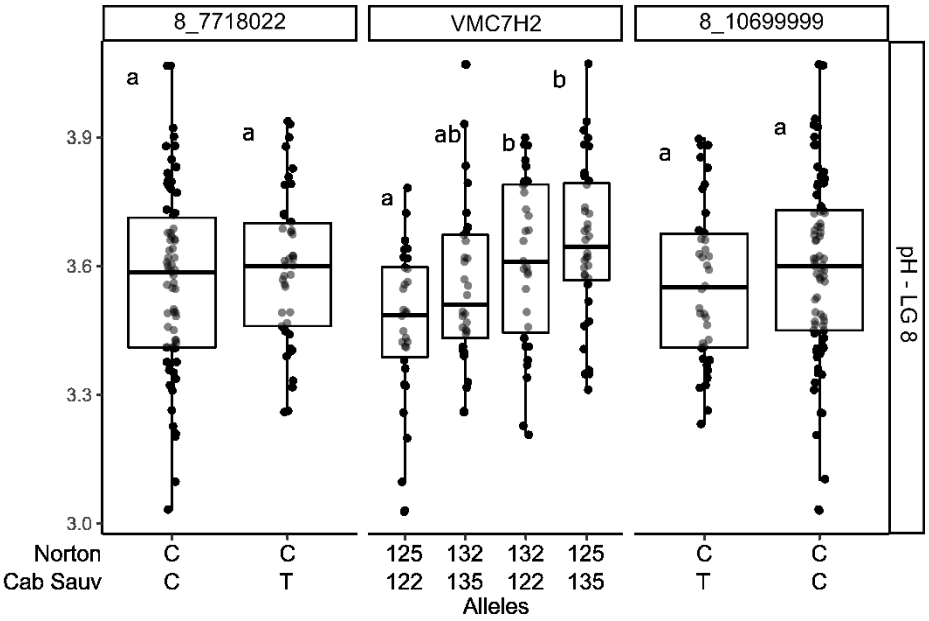

Fig. 7 pH phenotypes grouped according to genotype using markers associated with QTL on LG 8. Phenotypes represent data from 2018. Statistically significant differences are designated by letter ($p < 0.05$, Tukey's HSD test).

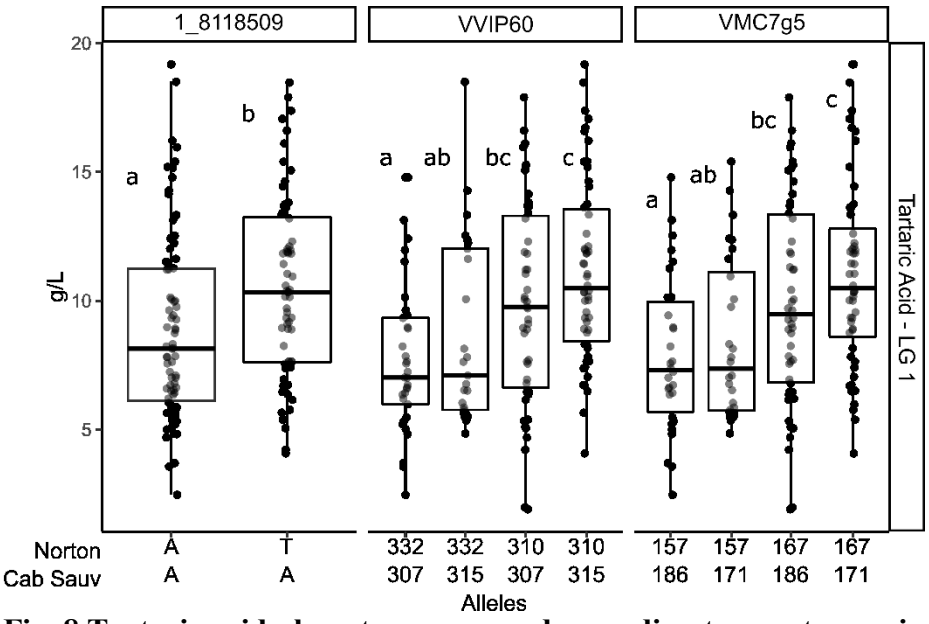

Fig. 8 Tartaric acid phenotypes grouped according to genotype using markers associated with QTL on LG 1. Phenotypes represent data from 2019. Statistically significant differences are designated by letter ($p < 0.05$, Tukey's HSD test).

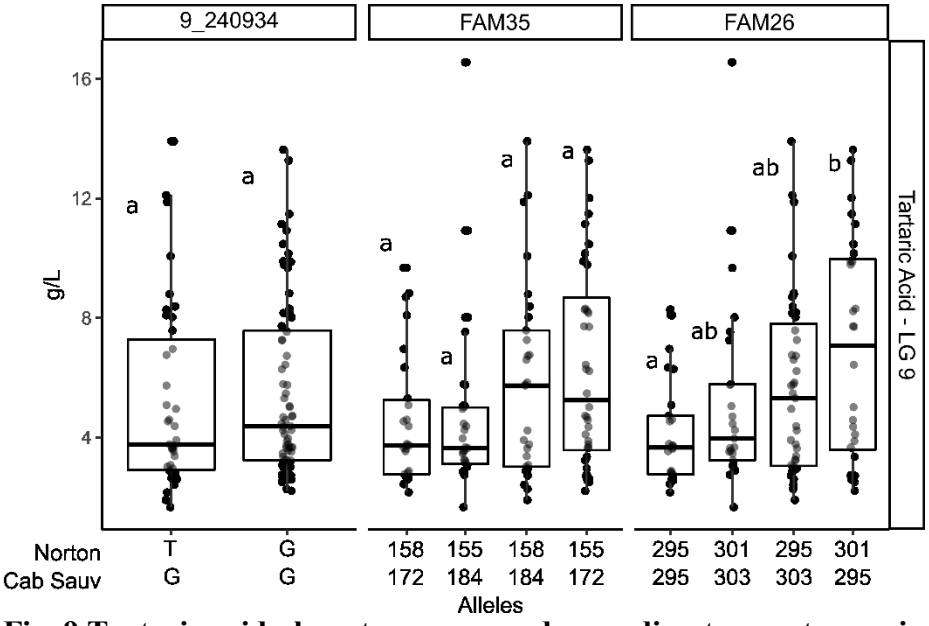

Fig. 9 Tartaric acid phenotypes grouped according to genotype using markers associated with QTL on LG 9. Phenotypes represent data from 2018. Statistically significant differences are designated by letter ($p < 0.05$, Tukey's HSD test).

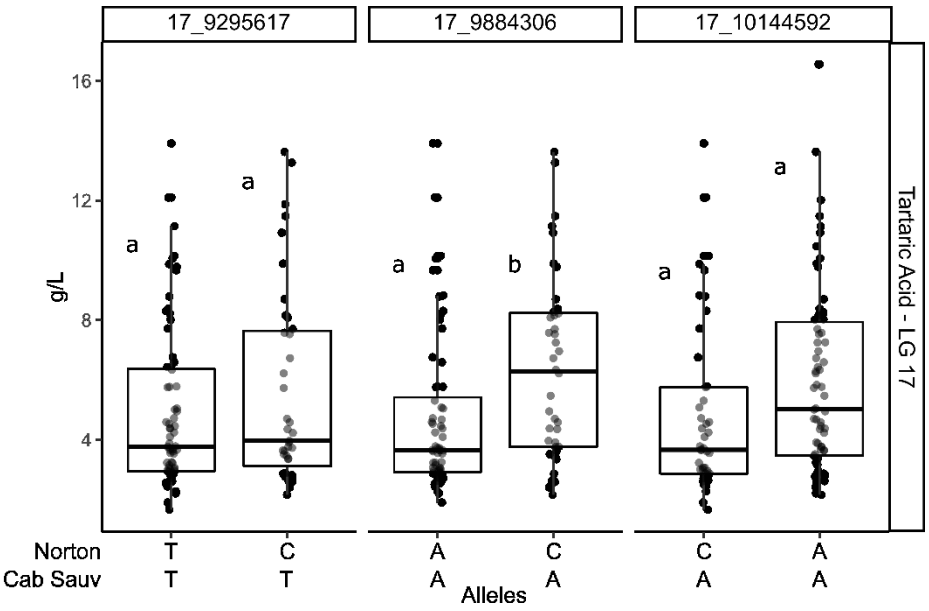

Fig. 10 Tartaric acid phenotypes grouped according to genotype using markers associated with QTL on LG 17. Phenotypes represent data from 2018. Statistically significant differences are designated by letter ($p < 0.05$, Tukey's HSD test).

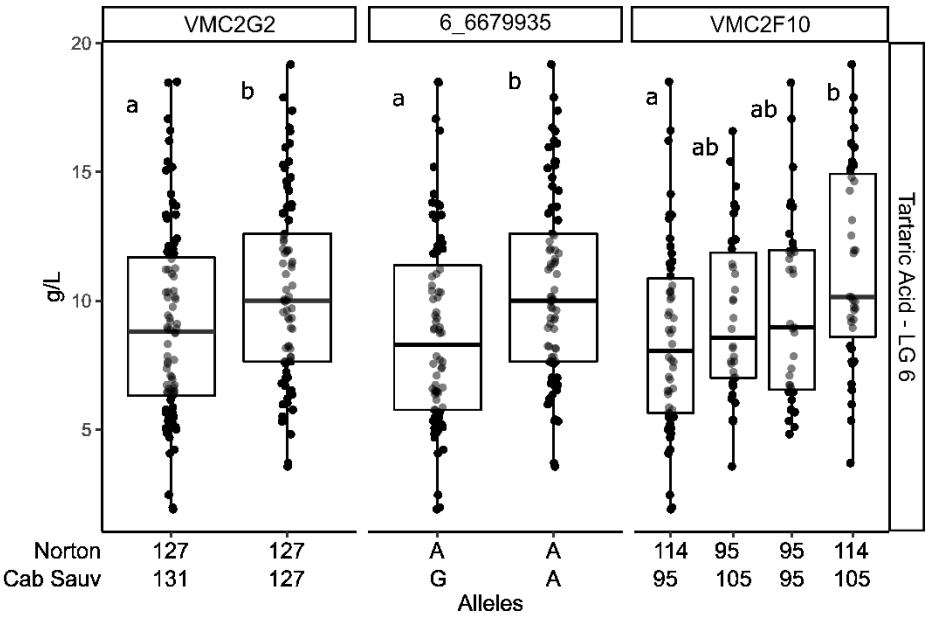

Fig. 11 Tartaric acid phenotypes grouped according to genotype using markers associated with QTL on LG 6. Phenotypes represent data from 2019. Statistically significant differences are designated by letter ($p < 0.05$, Tukey's HSD test).

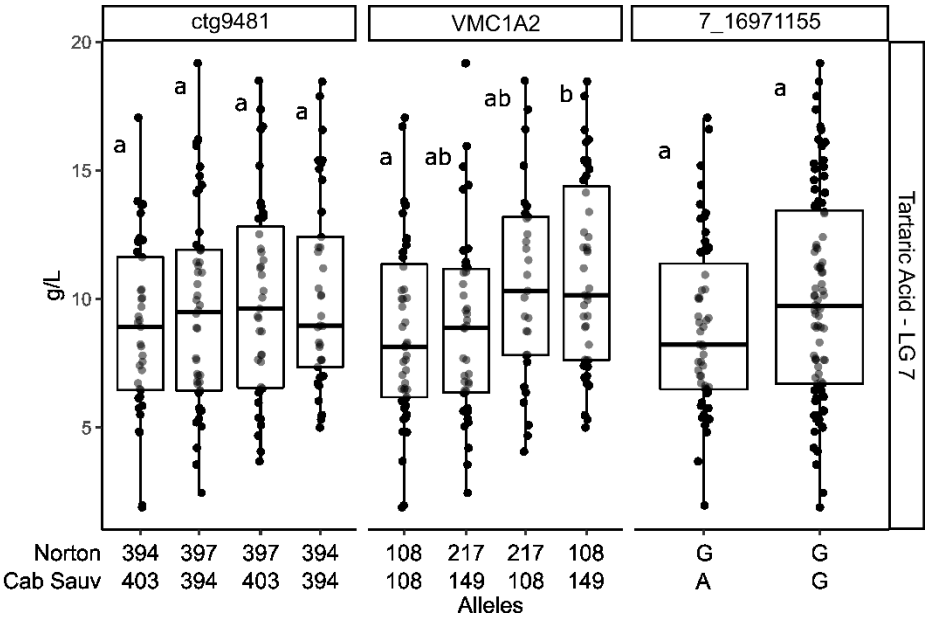

Fig. 12 Tartaric acid phenotypes grouped according to genotype using markers associated with QTL on LG 7. Phenotypes represent data from 2019. Statistically significant differences are designated by letter ($p < 0.05$, Tukey's HSD test).

DISCUSSION

V. aestivalis-derived 'Norton' has achieved regional acceptance in states such as Missouri, Arkansas, and Virginia (Ambers and Ambers 2004). Its popularity with growers may be attributed to high levels of resistance to many destructive diseases, pests and winter temperatures. However, the flavor chemistry of 'Norton' (such as high malic acid) diminishes its acceptability as compared to international red *V. vinifera* cultivars, such as 'Cabernet Sauvignon'. Prior to this investigation of berry quality traits, a 'Norton' × 'Cabernet Sauvignon' mapping population was developed (Adhikari et al. 2014) and two linkage maps were constructed for 'Norton' (Hammers et al. 2017; Sapkota et al. 2019). Here we report on the increase of the mapping population, improvement upon previous maps, and identification of QTLs related to berry traits. These advancements may be useful in the marker-assisted selection of progeny possessing the complex agronomical traits of 'Norton'.

Genotyping & Genetic Maps

QTL analysis results that approach fine-mapping to the greatest degree possible are desirable for accurate marker-assisted selection. Increasing mapping resolution requires capturing more recombination events within a region of interest. This can be accomplished through larger population sizes and increased marker numbers (Collard et al. 2005). The integrated SSR and SNP-based map for this population was improved by both the addition of new F_1 genotypes and a greater number of markers compared to the previous SSR (Hammers et al. 2017) and integrated SSR and SNP (Sapkota et al. 2019) maps (Table 12). In comparison to the 2019 map, this map was constructed using 64 more F_1 individuals in the mapping population

and 396 more markers. The map size was reduced by 236.7 cM (1678.6 cM) from the 2017 map and 761.6 cM from the 2019 map (2203.5 cM). The average interval between markers was decreased by 3.4 cM and 0.35 cM from 2017 and 2019, respectively. In addition, there are no gaps in length greater than 10.0 cM which is an improvement over the previous maps which contained 26 (2017) and 4 (2019) such gaps (Hammer et al. 2017; Sapkota et al. 2019). The reduction in map size likely results from improved genotype error handling and minimized missing data (Cartwright et al. 2007; Ball et al. 2010). Lep-MAP3 uses genotype-likelihoods rather than observed genotypes for all calculations which makes map ordering more robust against potential genotyping errors (Rastas 2017). Additionally, the SNP marker diversity is more reflective of increased heterogeneity expected from a hybrid *Vitis* spp. cultivar -'Norton' in comparison to a *V. vinifera* cultivar -'Cabernet Sauvignon'. Of the markers segregating in a single parent (2102 SSRs and SNPs) nearly three-fourths (72.3%) were polymorphic for 'Norton'. Further improvements included the percentage of missing data allowed in the initial marker set being decreased to less than 10%. The map size of 1441.9 cM was maintained within the range of other published maps (1301.0-1967.4 cM) as was the average marker spacing of 0.75 cM (0.41-2.5 cM) (Barba et al. 2014; Chen et al. 2015; Teh et al. 2017; Su et al. 2020). Segregation distorted loci were included during map construction to minimize potential marker-gaps, as distorted markers are often clustered which was also observed in the final map (Zuo et al. 2019). The longest gap of 8.4 cM was located on LG 15 (Table 1). Linkage group 15 contains the fewest markers, so the increased marker spacing compared to other LGs should be expected.

Table 12 Comparison between the previously published and current genetic map constructed for the 'Norton' × 'Cabernet Sauvignon' F_1 population

Year	Marker Type	Population size	Number of markers	Map size (cM)	Average distance between loci (cM)	Number of gaps >10 cM
2017	SSR	183	413	1678.6	4.1	26
2019	SSR, SNP	159	2072	2203.5	1.1	4
2020	SSR, SNP	223	2468	1441.9	0.75	0

Phenotyping

Variation in traits among years indicated the influence of environment. Substantial defoliation, which has been shown to impact berry quality, did occur during all years because of considerable Japanese beetle (*Popillia japonica*) populations during and after veraison. Increased sun exposure and leaf removal has been shown to decrease malic acid in 'Norton' (Jogaiah et al. 2012, 2013). The malic acid levels from Jogaiah et al. (2012) decreased from 5.8 g/L with full shading to 3.2 g/L with full sun exposure. Jogaiah et al. (2013) similarly showed a reduction in malic acid from 7.8 g/L to 3.9 g/L with leaf removal at fruit set. If similar reductions in malic acid occurred in our 'Norton' × 'Cabernet Sauvignon' population in 2017- 2019 due to leaf damage, a difference of approximately 3.0 g/L malic acid as has been previously seen in 'Norton', would be enough to shift the population means (3.9, 3.4 and 2.5 g/L) from the low end of the expected value range to the mid-upper section of the range. In 2019, slightly warmer temperatures from veraison until harvest than in previous years in combination with the defoliation likely contributed to the higher tartaric acid and lower malic acid means seen during that year. An additional possible factor contributing to the variability of tartaric acid and pH includes potassium bitartrate precipitation as sample preparation did not include heating which would dissolve any precipitates which occurred from freezing and thawing of the berries. A

narrower range was observed for all trait values in 2018 than in other years. This attribute may be generally due to the reduced genotypes available in 2018 for tartaric acid, malic acid, TSS, and pH as this trend was not also seen in the ranges of the 95% confidence intervals for the means (data not shown).

The broad sense heritability value of malic acid (0.70) (Table 2) was intermediate to the ranges reported by Liu et al. (2007) of 0.79-0.91 and Bayo-Canha et al. (2019) of 0.51-0.69. The broad sense heritability of tartaric acid (0.24) was lower than previously seen by Liu et al (2007) (0.59-0.84) and Bayo-Canha et al (2019) (0.49-0.56) but greater than the reports of Houel et al. (2015) (0.13). The heritability of pH was lower (0.32) than those of Duchêne et al. (2014) (0.71-0.93). The heritability of TSS (0.65) was higher than the heritability of 0.19 for total sugars reported by Houel et al. (2015), but within the range of values (0.54 – 0.84) given by Bayo-Canha et al. (2019). Overall, the broad sense heritability scores indicated the traits are considerably impacted by genetic factors. The between year correlations seen for malic acid (0.50-0.66), tartaric acid (0.23), pH (0.52-0.67), and TSS (0.57-0.65) demonstrate the stability of these trait across years (Tables 3, 4, 5 and 6). Correlation between pH (on a $[H^+]$ basis) and malic acid in 2018 (0.28) and 2019 (0.19) but not for 2017 or tartaric acid in 2018 or 2019 demonstrates the variable relationship between pH and organic acids. Significant and nonsignificant correlations between pH and organic acids have also been seen elsewhere (Pavlqušek and Kumšta 2011, Bayo-Canha et al. 2012). TSS and pH had differences in correlation directionality by year. In 2017 a positive correlation existed while in 2019 and 2018 the relationship was negative. The difference was not related to the absence of tartaric acid from the partial correlation calculations in 2017 as the negative correlation remained if tartaric acid was omitted from the 2018 and 2019 calculations (data not shown). Rather it may be related to

the absence of a correlation between malic acid and pH in 2017 as malic acid had a positive relationship with pH and inverse relationship with TSS.

QTLs

QTLs related to malic acid have been reported on LGs 5, 6, 8, 15, and 18 (Chen et al. 2015; Yang et al. 2016; Bayo-Canha et al. 2019; Duchêne et al. 2020). QTLs for malic acid on LG 8 have been reported in *V. vinifera* populations, but not previously in an interspecific hybrid population (Bayo-Canha et al. 2019; Duchêne et al. 2020). Duchêne et al. (2020) investigated the region from 8669988 to 13093399 on chromosome 8 for both malic and tartaric candidate genes, but the malic acid QTL may be attributed to a more defined region based on their SSR-based map (Duchêne et al. 2012). That confidence interval was flanked by markers at physical positions of 10702981 and 14039528. Bayo-Canha et al. (2019) presented a QTL flanked by nearest markers at positions of 5145874-14039528 bp. The confidence interval for this QTL spans approximately 8.9 Mbps making it difficult to validate against the position of the QTL on LG 8 reported here. However, the cofactors associated with the QTL of Bayo-Canha et al. (2019) may indicate a more concise location of the responsible region as they are all located within the interval of 5145874-8533120 bp. The ambiguity of QTL reported on low-density maps or with relatively large confidence intervals can make it difficult to validate the uniqueness of new findings. The QTL on LG 8 reported here (9992297-10385236 bp) appears to be unique to 'Norton' as shown by the attributed alleles in Table 11. Two single year QTL for malic acid in 2019 were also identified on LGs 14 and 18. A QTL on LG 18 has previously been reported in an interspecific hybrid population (Chen et al. 2015). The QTL on LG 14 has been found for pH (Duchêne et al. 2020) but it has not been shown to be connected to malic acid.

QTLs for pH have been published on LGs 10, 11, 13, and 14 (Duchêne et al. 2020). However, a stable QTL for pH on LG 6 identified here has not previously been reported. Additional pH QTLs on LGs 4 and 8 were seen in single years. On LG 8, the pH QTL is located at the same position we reported for malic acid. However, differing parental alleles were shown to be responsible with pH allelic effects originating from 'Cabernet Sauvignon' rather than 'Norton' as seen for malic acid. Tartaric acid QTLs have previously only been reported on LGs 2, 4, 6, 7, 8, and 13 (Houel et al. 2015; Duchêne et al. 2020). Tartaric acid QTLs reported here on LGs 6 and 7 coincide with those reported by Houel et al. (2015) and Duchêne et al. (2020), both of which used *V. vinifera*-based populations. Allele effects by the *V. vinifera* parent in this study further supports the QTL being identical to those previously reported. Duchêne et al. (2020) also showed a QTL for the malic-tartaric acid ratio located on LG 1 which coincided with the location of the tartaric acid QTL from this study. QTL for tartaric acid on LGs 9 and 17 have not previously been reported.

The study presented here confirms that GBS-derived SNP and SSR markers can be combined to generate a high-density genetic map for study of an interspecific F_1 population. Significant QTLs for berry quality traits with enological importance were identified, some of which have not previously been seen. QTL variability within and across populations demonstrates the importance of data replication across environments and high-resolution genetic mapping for accurate QTL identification. QTLs for malic acid, tartaric acid, and pH were identified for the first time in a *V. aestivalis*-based population. Stable QTLs across at least two years for malic acid and pH can help guide the selection of new hybrid cultivars for improved berry quality.

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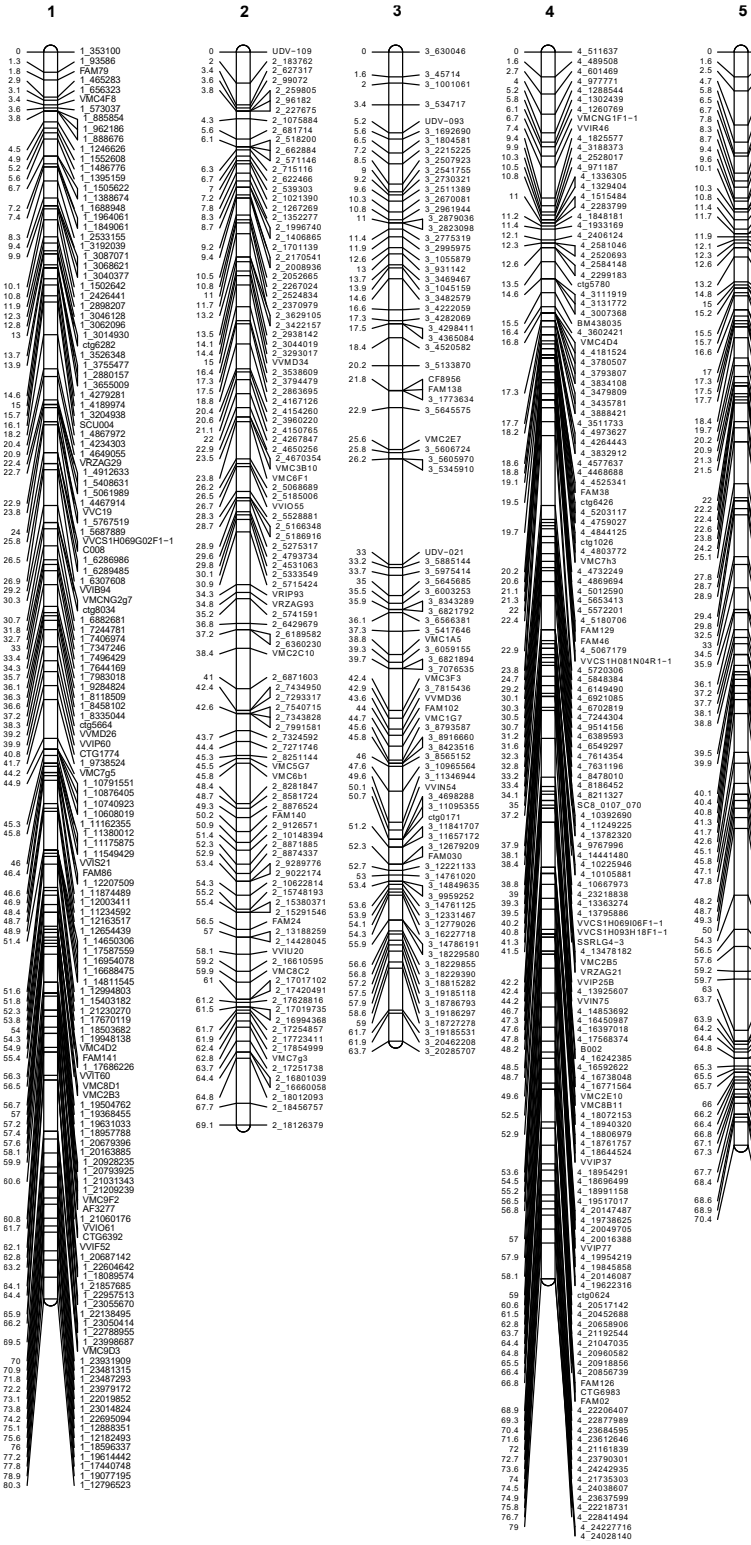
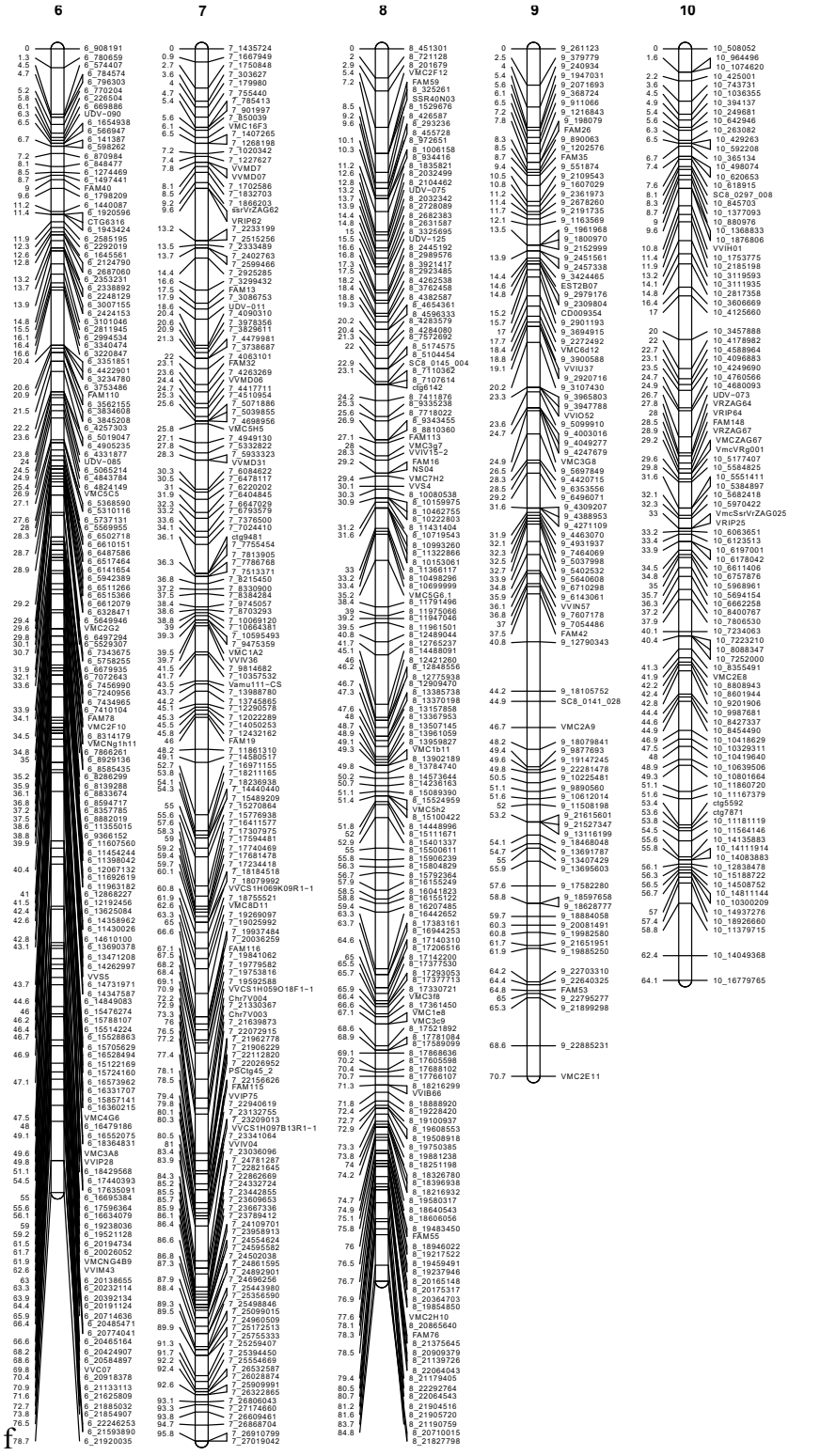
APPENDIX

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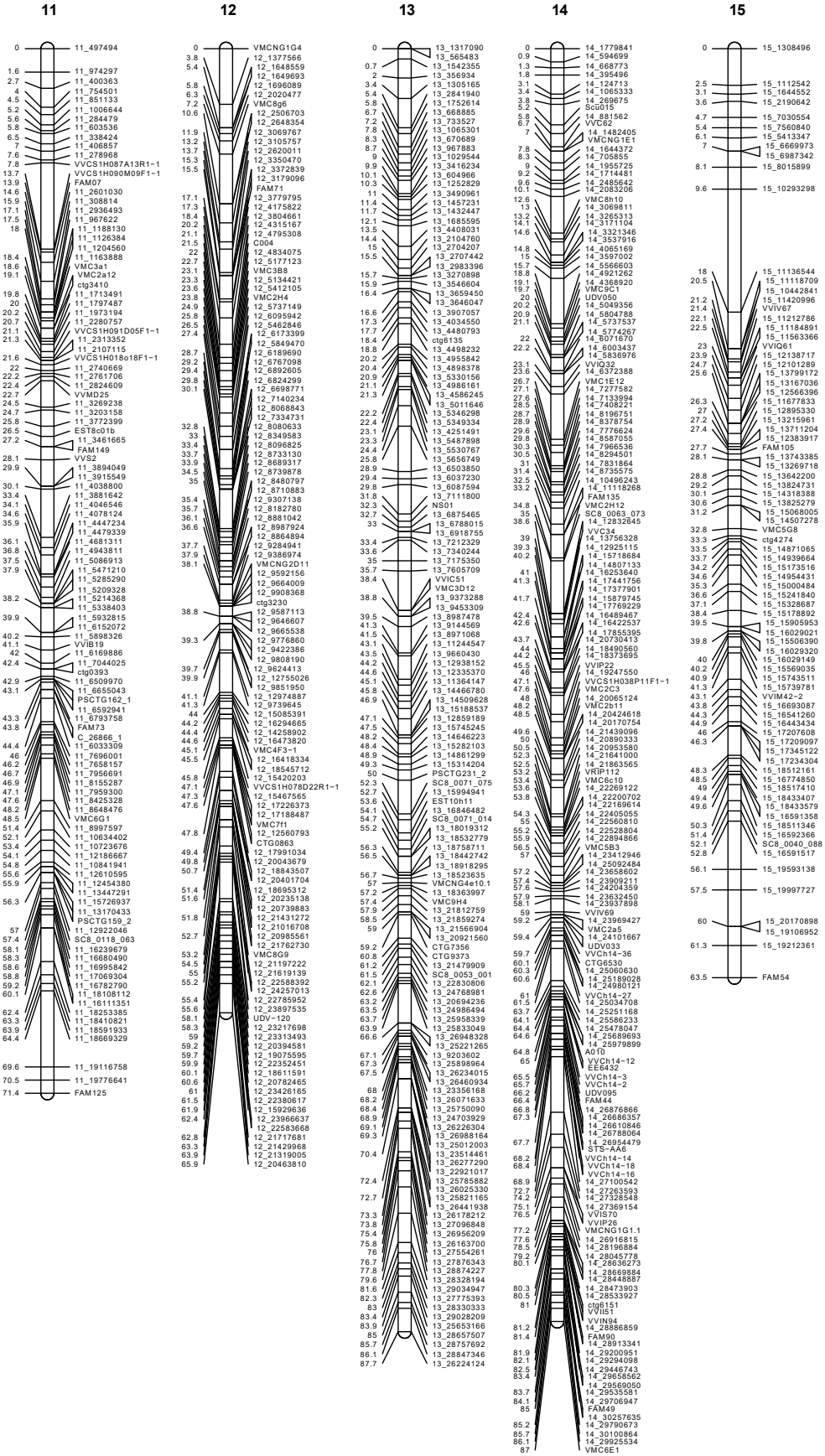
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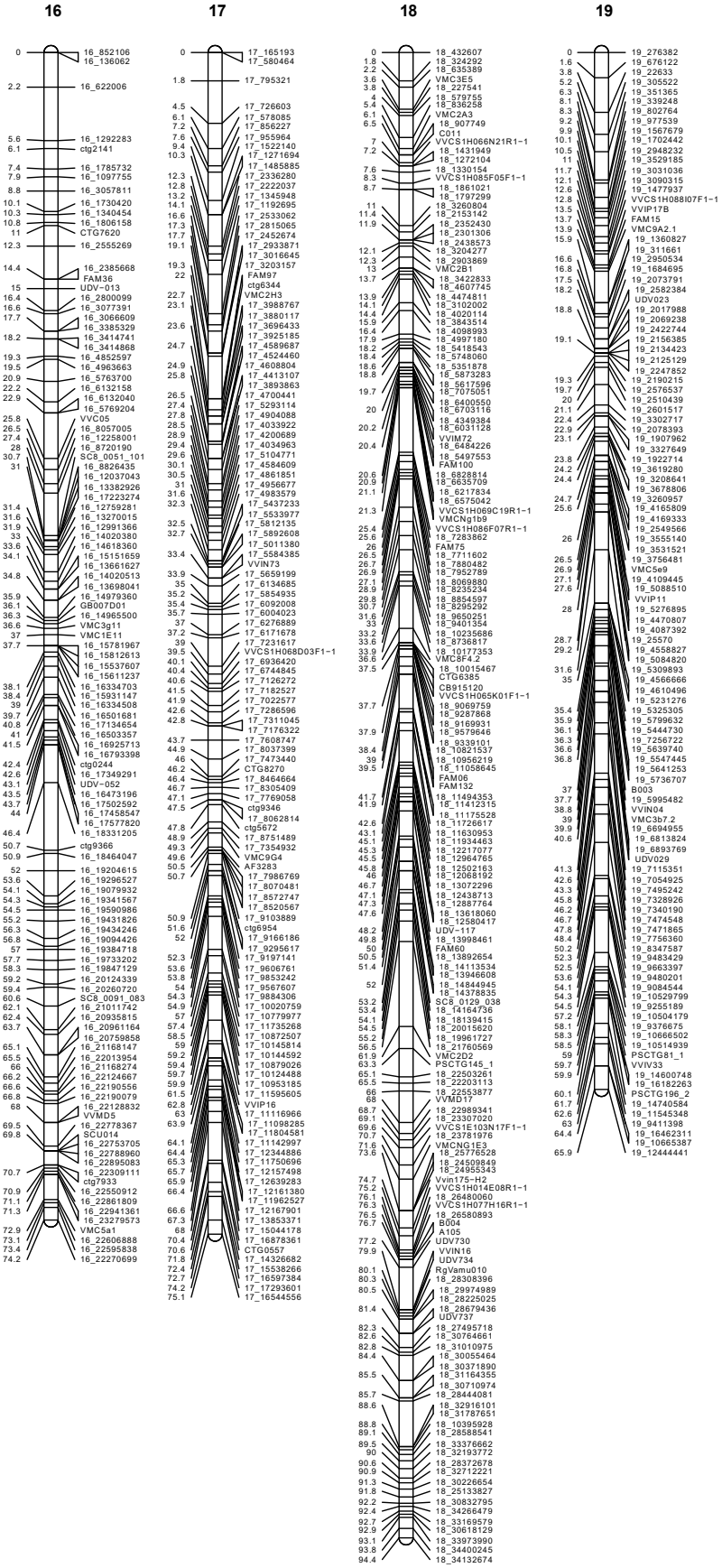
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10.3 16_1340454
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FAM36
15 UDV~013
16.4 16_2800099
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