Classifying Cider Apple Germplasm Using Genetic Markers for Fruit Acidity

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ABSTRACT. The organic acid concentration in apple (*Malus × domestica*) juice is a major component of hard cider flavor. The goal of this study was to determine if the malic acid markers, *Mal* and *Q8*, could classify the titratable acidity concentration in cider apple accessions from the United States Department of Agriculture *Malus* germplasm collection into descriptive classifications. Our results indicate that for diploid genotypes, the *Mal* marker alone and the *Mal* and *Q8* markers analyzed together could be used to predict cider apple acidity ($P < 0.0001$). Alone, the *Mal* marker categorized acidity into low ($<2.4 \text{ g} \cdot \text{L}^{-1}$), medium ($2.4\text{--}5.8 \text{ g} \cdot \text{L}^{-1}$), and high ($>5.8 \text{ g} \cdot \text{L}^{-1}$) groups. The combination of *Mal* and *Q8* markers provided more specificity, which would be useful for plant breeding applications. This work also identified a significant difference ($P = 0.0132$) in acidity associated with ploidy. On average, the triploids accessions had $0.33 \text{ g} \cdot \text{L}^{-1}$ higher titratable acidity than the diploid accessions. Based on the results of this work, we propose a genetics-based classification system for cider apples with the acidity component defined by the *Mal* and *Q8* markers.

Although fresh market apples comprise the majority of global apple (*Malus × domestica*) production, cider apples have been of particular interest recently because their higher acidity and tannin content (a group of polyphenols with bitterness and astringency) can potentially produce a hard cider with a depth of flavor similar to that of wine. Cider-specific apples can have five-times to 10-times more tannins than dessert apples and a wide range of organic acid concentrations (perceived as sourness and often described as sharpness) (Thompson-Witrick et al., 2014). In the United States, there is currently more demand than supply for

cider-specific apple cultivars (Pashow, 2018). In response to this supply chain imbalance, cider-specific cultivars, mostly of European origin, are being planted throughout the country (Miles et al., 2020). However, there is limited information regarding the juice quality characteristics of these apple cultivars.

The emerging U.S. cider industry has adopted a method of classifying cider apples that was originally developed at the Long Ashton Research Station (LARS) near Bristol, UK, over a century ago (Barker and Ertle, 1910). The LARS system classifies apple cultivars into one of four categories, sweet, bitter-sweet, sharp, or bittersharp, based on tannin and titratable acidity (TA) concentrations. Tannins were originally measured using the Löwenthal permanganate titration method (Snyder, 1893). A tannic acid concentration of $2 \text{ g} \cdot \text{L}^{-1}$ is used to separate nonbitter from bitter apples. Acidity was, and still is, measured using an acid–base titration at a pH endpoint of 8.1. Apples with a malic acid equivalent concentration less than $4.5 \text{ g} \cdot \text{L}^{-1}$ are classified as sweet, and those with a malic acid equivalent concentration greater than $4.5 \text{ g} \cdot \text{L}^{-1}$ are classified as sharp. Although plant genetics and sensory science have progressed greatly since the early 20th century, the LARS system has remained unchanged.

A French classification system is used to divide cider apples into the following six categories (English translation in parenthesis): amère (bitter); douce amère (bittersweet); douce (sweet); acidulée (subacid); aigre (sharp); and aigre amère (bittersharp) (Institut Français Des Productions Cidricoles, 2009). The acidity component of the French cider apple classification method has three categories, douce, acidulée, and aigre. The douce category is defined as having less than $4.5 \text{ g} \cdot \text{L}^{-1}$ TA, which is the same threshold used by the LARS classification system. The acidulée category is defined as TA values between

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4.5 and 6.75 g·L⁻¹ TA. The aigre category includes apples with greater than 6.75 g·L⁻¹ TA.

A Spanish cider classification system has undergone changes more recently than the LARS classification system and now includes the following six technical groups: sweet (<1.45 g·L⁻¹ tannic acid and <4.85 g·L⁻¹ TA); bittersweet (>1.45 g·L⁻¹ tannic acid and <4.85 g·L⁻¹ TA); semi-acid (<1.45 g·L⁻¹ tannic acid and 4.85–6.56 g·L⁻¹ TA); semi-acid-bitter (>1.45 g·L⁻¹ tannic acid and 4.85–6.56 g·L⁻¹ TA); acid (<1.45 g·L⁻¹ tannic acid and 6.56 g·L⁻¹ TA); and acid-bitter (>1.45 g·L⁻¹ tannic acid and >6.56 g·L⁻¹ TA) (Ministerio De Agricultura, Pesca y Alimentación, 2003).

The major organic acid in mature apple fruit is malic (≈90% to 95%, 3–5 g·L⁻¹), followed by quinic (≈4%; 0.2–0.5 mg·L⁻¹), citric (≈1.5%; 0.05–0.07 mg·L⁻¹), and trace amounts (<0.05 mg·L⁻¹) of ascorbic, shikimic, succinic formic, maleic, and tartaric acid (Wu et al., 2007; Zhang et al., 2010). The genetic underpinnings of apple acidity were first described in 1959, and subsequent studies have led to the identification and characterization of the malic acid (*Ma*) locus on linkage group 16 (Maliapaard et al., 1998; Nybom, 1959; Visser and Verhaegh, 1978; Xu et al., 2012; Yao et al., 2008). This locus has been reported to control 17% to 42.3% of the variation in acid concentration in apple fruit (Xu et al., 2012). The gene underlying *Ma*, named *Mal1*, has been identified to encode an aluminum-activated malate transporter-like protein (Bai et al., 2012; Khan et al., 2013). A single nucleotide mutation from the guanine (G) to adenine (A) at position 1455 in the coding sequence of *Mal1* results in a premature stop codon that truncates 84 amino acids at the C-terminus, causing low acidity (Bai et al., 2012; Li et al., 2020). Therefore, the *Mal1* allele, with “G” at position 1455, is associated with high acid (*Ma*), and the *Mal1* allele with “A” at position 1455 is associated with low acid (*ma*). This distinction defines the difference between the dominant *Ma* and recessive *ma* alleles. However, the dominance of the *Mal1* allele is incomplete, which was indicated by the wide range of TA values for the heterozygous *Mama* allele, suggesting that both additive and dominant effects of the *Mal1* allele exist (Verma et al., 2019; Xu et al., 2012). Recently, it was found that in response to excess nitrate accumulation, the *MdBT2* protein modulated and down-regulated the expression of *MdCibHLH1* and *MdMYB73*, which regulate malate-related genes, thus reducing acidity in apples (Zhang et al., 2020).

Linkage group 8 also contains an important quantitative trait locus (QTL), named *Ma3*, that regulates apple acidity (Kumar et al., 2013; Liebhard et al., 2003; Ma et al., 2015; Sun et al., 2015; Verma et al., 2019). Recently, the *Ma3* locus has been shown to have an incomplete dominance effect on apple acidity (Rymenants et al., 2020). Jia et al. (2018) identified two natural variations in hierarchical epistatic genes, *MdSAUR37* and *MdPP2CH*, that affect fruit acidity in the *Ma3* region. To genotype the *Ma3* locus, a sequence tagged site (STS) marker, named *Q8*, was developed, which is physically located at 11.1 Mb between genes *MdPP2CH* (8.7 Mb) and *MdSAUR37* (11.6 Mb) on chromosome 8 in the GDDH13 apple reference genome (Daccord et al., 2017; Jia et al., 2018). Additionally, three more QTLs, *Ma4*, *Ma5*, and *Ma6* located on chromosomes 6, 1, and 4, respectively, were found to be relevant for fruit acidity levels (Ban and Xu, 2020; Rymenants et al., 2020). These QTLs appeared to explain more variation in the background of *MaMa*

and *Mama* with relatively high acidity levels (>10 g·L⁻¹) (Ban and Xu, 2020).

Because of the major effects of *Mal1* and *Ma3* loci on fruit acidity levels less than 10 g·L⁻¹, we hypothesized that they could be used to predict acidity in apples, thereby allowing for classification into acidity ranges that would aid cider apple breeding, cultivar selection, and cider production. The goal of this study was to develop a genetic system for classifying *M. ×domestica* cider apple acidity using the *Mal1* and *Q8* markers.

Materials and Methods

STUDY LOCATION AND ACCESSION SELECTION. Apples were harvested in 2017, 2018, and/or 2019 from the *Malus* germplasm collection maintained by the U.S. Department of Agriculture (USDA) National Plant Germplasm System in Geneva, NY (lat. 42°53'40.3"N, long. 77°00'23.8"W). We compiled a list of 330 *M. ×domestica* cultivars within the *Malus* germplasm collection that are mentioned in historic European and American texts and/or are currently being used for hard cider (Supplemental Fig. 1). Additional *M. ×domestica* accessions were identified by searching the USDA Germplasm Resources Information Network (GRIN) Global database (USDA, 2020) for accessions with as-triangular, aromatic, and/or acidic fruit that were greater than 50 g at harvest. Trees that appeared unhealthy or had insufficient fruit for analyses were excluded. Ploidy data were obtained from a 20,000 single-nucleotide polymorphism (SNP) array as part of an ongoing collaborative apple pedigree reconstruction project (Denancé et al., 2020; Howard et al., 2018; Muranty et al., 2020). Accessions without associated ploidy data were removed from the dataset. These steps led to the selection of 217 *M. ×domestica* accessions (Supplemental Table 1).

DNA EXTRACTION AND ACCESSION GENOTYPING. Young leaves of the selected accessions were collected between 2017 and 2019. Leaf tissue (15–20 mg) was ground for 1 min using a tissue lyser (TissueLyserII; Qiagen, Venio, the Netherlands). Samples were incubated for 1 h in a hexadecyltrimethylammonium bromide (CTAB) extraction buffer containing polyvinylpyrrolidone (catalogue number BP431-500; Thermo Fisher Scientific, Waltham, MA) and β-mercaptoethanol (catalogue number BP176-100; Thermo Fisher Scientific) (Doyle and Doyle, 1987). A spectrophotometer (NanoDrop 1000; Thermo Fisher Scientific) was used for DNA quantification.

A cleaved amplified polymorphic sequence marker (CAPS₁₄₅₅) targeting base 1455 in the open reading frame of the *Mal1* gene was used to distinguish the SNP between the *Mal1* alleles, as previously described (Bai et al., 2012). Briefly, polymerase chain reaction (PCR)-amplified products were digested overnight with BspHI (New England Bio Laboratories, Ipswich, MA) in a 37 °C water bath overnight. Digested products were visualized on a 1.5% agarose gel, and *Mal1* genotypes were determined based on band patterning. The PCR program included 2 min at 98 °C, 35 cycles of 10 s at 98 °C, 15 s at 55 °C, and 90 s at 72 °C, and then a final 5 min at 72 °C. The reactions were conducted in 20-μL volumes containing 1 × PrimeSTAR MAX DNA Polymerase (R045A; Takara/Clontech, Mountain View, CA), 0.5 mM of each primer, and 30 ng of genomic DNA in a gradient thermal cycler (Mastercycler[®] EP; Eppendorf, Hamburg, Germany). Restriction digestion was performed for 12 h at 37 °C in 20-μL reactions that contained 10 μL PCR products, 2 units of BspHI restriction enzyme (New England Biolabs,

Ipswich, MA), and 1 × NEBuffer 4 (New England Biolabs). After sample incubation, 7 µL of sample and 3 µL of loading dye were injected into each well of a 1.5% (w/v) agarose gel. The samples were suspended in a 1-N Tris/Acetate/EDTA (TAE) buffer solution. After 1 h of electrophoresis, the gels were stained with ethidium bromide at a concentration of 2 µL to 100 mg of gel. Then, the banding patterns in the gel were illuminated with a 110-V ultraviolet light transilluminator (Thermo Fisher Scientific). The banding patterns in the gel images were visually scored and the *Mal* alleles for each accession were recorded.

The *Q8* marker primer sequences were 5'-AAAAATTGAACTTGTGGATCGTT-3' (forward primer) and 5'-AAATCAAAAGCATACCACCACA-3' (reverse primer). The marker was PCR-amplified using 1× OneTaqDNA Polymerase (New England BioLabs) with the following conditions: 2 min at 98 °C, 35 cycles of 30 s at 94 °C, 30 s at 54 °C, and 45 s at 68 °C. The PCR products were visualized on 1.5% agarose gel and scored for *Q8* genotypes.

FRUIT SAMPLING AND PROCESSING PROCEDURES. Of the 217 *M. × domestica* accessions, 32 accessions were collected for 3 years, 114 accessions were collected for 2 years, and 71 accessions were collected for 1 year (Supplemental Table 1). The biennial bearing habit of many of these accessions made fruit unavailable during every year. Fruit was sampled from mid-August to mid-November. Before harvest, two fruit per accession that were near the reported harvest date in GRIN-Global were field-tested in situ for maturity using the cortex starch pattern index (SPI) (Blanpied and Silsby, 1992). An iodine solution (0.22% w/v iodine, 0.88% w/v potassium iodine) (EMD Millipore Corp., Billerica, MA) was applied to the stem-side of an equatorial cross-section of the apple. A visual rating of the stained cortex flesh (hypanthium and mesocarp) was conducted and recorded using a scale ranging from 1 to 8, where 1 = 100% staining (no starch degradation) and 8 = 0% staining (complete starch degradation). As much as possible, fruit was harvested when they were rated to have a SPI of 6 or more.

Fifteen fruit were randomly harvested from different regions of the tree canopy while avoiding selecting two or more fruit from the same branch. The unique identifying plant introduction (PI) number given to each accession in the USDA-PGRU collection was recorded and used to track the fruit throughout the phenotyping process. After harvest, the 15 fruit were randomly divided into three groups of five apples to allow for three subsamples of five fruit per sampled accession (Evans et al., 2012). The fruit were stored at 4 °C in a commercial storage room under ambient atmospheric gases for 1 to 4 weeks before fruit processing analysis at the Cornell University Agricultural Experiment Station Research Orchards in Ithaca, NY.

The SPI was determined for all sample apples as described. The calyx half of each pooled subsample of five apples was milled and pressed in a juicer (model 280; Norwalk Juicers, Bentonville, AR). Juice from each subsample replication was stirred and aliquoted into 50-mL tubes. All juice-extracting equipment was rinsed with water between samples to minimize cross-contamination. Juice samples were stored at −80 °C until titrations were performed.

JUICE TITRATABLE ACIDITY AND pH. Samples were thawed to room temperature and then vortexed for 10 s. Juice TA and pH were measured with an automatic titrator (Unitrode pH meter, 778 sample processor, and 800 Dosino dosing device; Metrohm, Herisau, Switzerland). Juice acidity was measured by titrating a

5-mL juice aliquot against a standardized 0.1 N NaOH solution to an endpoint of pH 8.1 and expressed in grams per liter of malic acid equivalents.

STATISTICAL ANALYSIS. Linear models were developed using *Mal*, *Q8*, or both markers as predictor variables and TA as the response variable with RStudio (version 1.1.442; RStudio, Boston, MA). The linear models were used to predict 95% confidence intervals (CIs) with the Estimated Marginal Means data analysis package. The data were not transformed before analysis. $P \leq 0.05$ was considered statistically significant.

Results

FRUIT MATURITY, TITRATABLE ACIDITY, AND pH. The SPI for the 217 evaluated accessions ranged from 5 to 8 (mean, 6.96; SE ± 0.06) (Supplemental Table 1). More than 85% of the accessions had an average SPI more than 6. The TA ranged from 1.09 to 11.53 g·L^{−1} (mean, 4.48 ± 0.16 g·L^{−1}) (Fig. 1A). The pH ranged from 2.3 to 5.1 (mean, 3.84 ± 0.03) (Fig. 1B). The sample population had 113 (52%) accessions with TA less than the mean and 104 (48%) accessions with TA greater than the mean. There were two peaks in the TA distribution, one in the lower range representing most of the accessions with very low acidity with a peak at 1.5 g·L^{−1}, and the other between 4.6 and 6.6 g·L^{−1}, suggesting a bimodal distribution for fruit TA within our sample population.

ALLELIC DEMOGRAPHICS. Of the 217 genotyped accessions, 181 (83%) were diploid accessions and 36 (17%) were triploid accessions (Supplemental Table 1). For the *Mal* marker, 17 (9%) of the diploid accessions were homozygous dominant (*MaMa*), 107 (59%) were heterozygous (*Mama*), and 57 (32%) were homozygous recessive (*mama*) (Table 1). For the *Q8* marker, which is a likely marker for the *Ma3* gene, 129 (71%) of the diploid accessions were homozygous dominant (*Q8Q8*), 48 (27%) were heterozygous (*Q8q8*), and 4 (2%) were homozygous recessive (*q8q8*). The most common genotype combinations were *Mama-Q8Q8* (66 accessions; 37%), *mama-Q8Q8* (49 accessions; 27%), and *Mama-Q8q8* (37 accessions; 20%) (Table 1). The other allelic combinations each represented less than 10% of the total sample population, including three *MaMa-Q8q8* accessions, four *Mama-q8q8* accessions, and eight *mama-Q8q8* accessions. There were no *MaMa-q8q8* or *mama-q8q8* combinations among the accessions.

Allele diversity was less robust for the triploid accessions. The *mamama-Q8Q8Q8* combination comprised 39% of our triploid samples, *Mama-Q8Q8Q8* comprised 28% of our triploid samples, and *Mama-Q8q8* comprised 33% of our triploid samples (Table 1). This was partly because of a smaller sample size ($N = 36$) and the inability to identify the third allele in triploid heterozygous accessions.

RELATIONSHIPS AMONG THE GENOTYPES AND PHENOTYPES. Ploidy significantly influenced the TA concentration ($P = 0.0132$), with diploid accessions having 0.33 g·L^{−1} lower TA than triploids (4.43 and 4.76 g·L^{−1}, respectively) (Table 2). The pH was not significantly affected by the ploidy level. The interactions between *Mal* and *Q8* and ploidy were not significant, indicating that ploidy is independent of the *Mal* and *Q8* allele composition. Because of ploidy being a significant factor for TA, diploid and triploid accessions were analyzed separately for each *Mal* and *Q8* allelic combination using estimated marginal means analyses and CIs (Tables 3 and 4).

Fig. 1. The distribution of (A) titratable acidity and (B) pH of cider apple accessions (N = 217) harvested between 2017 and 2019, from the U.S. Department of Agriculture *Malus* germplasm collection in Geneva, NY.

Table 1. The number of cider apple accessions for each allelic combination of the *Mal* and *Q8* markers. Leaves from the sample population (N = 217) were collected from the U.S. Department of Agriculture *Malus* germplasm collection in Geneva, NY.

Allele	Diploid accessions			Triploid accessions	
	<i>MaMa</i> (no.)	<i>Mama</i> (no.)	<i>Mama</i> (no.)	Allele ^z	(no.)
<i>Q8Q8</i>	14	66	49	<i>mamama_Q8Q8Q8</i>	14
<i>Q8q8</i>	3	37	8	<i>Mama_Q8Q8Q8</i>	10
<i>q8q8</i>	0	4	0	<i>Mama_Q8q8-</i>	12

^z“—” = A missing allele in the heterozygous triploid accessions.

Table 2. Fixed effects from a simple linear model of the *Mal* and *Q8* markers and ploidy in a study of cider apple accessions (N = 217) harvested between 2017 and 2019 from the U.S. Department of Agriculture *Malus* germplasm collection in Geneva, NY.

	Titratable acidity	pH
Fixed effects	<i>P</i> value	
<i>Mal</i>	<0.0001	<0.0001
<i>Q8</i>	0.5032	0.2361
<i>Mal</i> + <i>Q8</i>	0.0001	<0.0003
Ploidy	0.0132	0.1375
<i>Mal</i> + <i>Q8</i> × ploidy	0.2275	0.6368

For the diploids, the *Mal* marker had a significant effect on TA ($P < 0.0001$) and pH ($P < 0.0001$), but the *Q8* marker did not show a significant effect for either TA or pH (Table 2). However, a detailed analysis of each *Ma* genotype group indicated that there was a significant difference between *Q8Q8* and *q8q8* within the *Mama* genotype, where all the *q8q8* accessions were found. This suggests that the insignificant effect of *Q8* on acidity of the 181 diploid accessions may have been related to the limited number of accessions (N = 4) that possessed the recessive *q8q8* in our sample population (Tables 1 and 3, Fig. 2A). The analysis also revealed that for *Mama* and *MaMa*, the *Q8Q8*

genotype exhibited higher TA than *Q8q8*, although that difference was not significant (Fig. 2A, Table 3). In the *mama* group, the difference in acidity between the *Q8Q8* and *Q8q8* genotypes were negligible (Fig. 2A, Table 3). Consequently, when the *Mal* and *Q8* markers were considered in combination, both TA ($P < 0.0001$) and pH ($P < 0.0001$) were correlated with the genotype (Table 2).

CLASSIFYING CIDER APPLES BY PHENOTYPE, GENOTYPE, AND REGION OF ORIGIN. The 217 cider apple accessions genotyped during this study were classified under the different existing regional classification systems based on acidity (Fig. 3). Using the 4.5 g·L⁻¹ threshold from the LARS classification system, there was an approximately equal distribution of sweet (52%) and sharp (48%) cider apples. The French and Spanish classification systems showed a very similar spread of cider apple accessions, with 52% and 57% in the sweet category, 30% and 24% in the semi-acid category, and 18% and 19% in the acidic category, respectively (Fig. 3).

The 95% CI range (lower to upper CI) from the estimated marginal means test for each *Mal* allele alone and *Mal* and *Q8* allele combinations were used to develop classification thresholds for the diploid and triploid accessions separately (Table 3, Fig. 3). The *Mal* marker-based classification system discretely categorized the cider apple accessions with minimal overlap among the genotypes (Fig. 3). For the diploid accessions, the *Mal* marker could be classified into low [*mama* (<2.4 g·L⁻¹)], medium [*Mama* (2.4 to 5.8 g·L⁻¹)], and high [*MaMa* (>5.8 g·L⁻¹)] acidity levels. There were only two accessions that overlapped between the upper threshold for diploid *Mama* (5.78 g·L⁻¹) and the lower threshold for *MaMa* (5.60 g·L⁻¹). The diploid *Mal*

and *Q8* allelic combinations categorized cider apples with multiple category overlaps; however, they provided more specificity. There was a significant difference between the *Mama_q8q8* (1.62–4.81 g·L⁻¹) and *Mama_Q8Q8* (5.5–6.8 g·L⁻¹) alleles.

The triploid accessions were classified into two categories solely using the *Mal* allele (*mamama* and *Mama-*). Homozygous dominant triploid *Mal* alleles were absent in the sample population. Using both the *Mal* and *Q8* alleles for the triploid accessions, there were three identified categories of *Mal* and *Q8* alleles (*mamama_Q8Q8Q8*, *Mama-Q8q8-*, and *Mama-Q8Q8Q8*). The triploid homozygous recessive *mal* alleles and homozygous

Table 3. Estimated marginal means and the lower confidence intervals (LCIs) and upper confidence intervals (UCIs) for juice titratable acidity and pH for the *Mal* alleles alone and *Mal* + *Q8* allelic combinations among diploid (N = 181) cider apple accessions harvested between 2017 and 2019 from the U.S. Department of Agriculture *Malus* germplasm collection in Geneva, NY. Some of the *Mal* and *Q8* allele combinations were omitted because of nonavailability of accessions with those genotypes.

Allelic combinations	Titratable acidity [mean $\pm$ SE (g·L ⁻¹)]	LCI (g·L ⁻¹)	UCI (g·L ⁻¹)	pH (mean $\pm$ SE)	LCI	UCI	Accessions (no.)
<i>Mal</i> allele alone							
<i>Mama</i>	1.92 $\pm$ 0.226 c ^z	1.47	2.38	4.41 $\pm$ 0.038 a	4.34	4.49	57
<i>Mama</i>	5.46 $\pm$ 0.165 b	5.13	5.78	3.59 $\pm$ 0.027 b	3.53	3.64	107
<i>MaMa</i>	6.42 $\pm$ 0.415 a	5.60	7.24	3.53 $\pm$ 0.069 c	3.39	3.67	17
<i>Mal</i> + <i>Q8</i> allele							
<i>mama_Q8q8</i>	1.64 $\pm$ 0.571 d	0.51	2.77	4.53 $\pm$ 0.102 a	4.32	4.73	8
<i>mama_Q8Q8</i>	1.96 $\pm$ 0.231 d	1.51	2.42	4.39 $\pm$ 0.041 a	4.31	4.48	49
<i>Mama_q8q8</i>	3.21 $\pm$ 0.808 cd	1.62	4.81	3.90 $\pm$ 0.145 b	3.61	4.19	4
<i>Mama_Q8q8</i>	4.93 $\pm$ 0.262 bc	4.42	5.45	3.64 $\pm$ 0.047 b	3.54	3.73	37
<i>MaMa_Q8q8</i>	5.24 $\pm$ 0.933 abc	3.4	7.08	3.60 $\pm$ 0.168 b	3.27	3.93	3
<i>Mama_Q8Q8</i>	5.89 $\pm$ 0.199 ab	5.5	6.28	3.54 $\pm$ 0.035 b	3.47	3.61	66
<i>MaMa_Q8Q8</i>	6.67 $\pm$ 0.432 a	5.82	7.52	3.51 $\pm$ 0.077 b	3.36	3.67	14

^zMeans within columns followed by the same lowercase letter are not significantly different based on Tukey's means comparison at $\alpha = 0.05$ and were analyzed separately for *Mal* alone and *Mal* + *Q8* allele combinations.

Table 4. The estimated marginal means and the lower confidence intervals (LCIs) and upper confidence intervals (UCIs) for juice titratable acidity and pH within each of the *Mal* allelic combinations among triploid cider apple accessions (N = 36) harvested between 2017 and 2019 from the United States Department of Agriculture *Malus* germplasm collection in Geneva, NY. Some of the allelic combinations were omitted because of very low or no availability of accessions with those genotypes. The third allele in heterozygous triploid accessions was not identified; therefore, “—” represents an unknown allele.

Allelic combinations	Titratable acidity [mean $\pm$ SE (g·L ⁻¹)]	LCI (g·L ⁻¹)	UCI (g·L ⁻¹)	pH (mean $\pm$ SE)	LCI	UCI	Accessions (no.)
<i>Mal</i> allele alone							
<i>Mamama</i>	2.13 $\pm$ 0.508 b ^z	1.10	3.17	4.34 $\pm$ 0.066 a	4.20	4.47	14
<i>Mama—</i>	6.51 $\pm$ 0.415 a	5.66	7.35	3.50 $\pm$ 0.054 b	3.39	3.62	22
<i>Mal</i> + <i>Q8</i> alleles							
<i>mamama_Q8Q8Q8</i>	2.13 $\pm$ 0.480 b	1.16	3.11	4.34 $\pm$ 0.062 a	4.21	4.46	14
<i>Mama—_Q8q8—</i>	5.76 $\pm$ 0.549 a	4.64	6.88	3.61 $\pm$ 0.071 b	3.46	3.75	12
<i>Mama—_Q8Q8Q8</i>	7.33 $\pm$ 0.568 a	6.17	8.48	3.39 $\pm$ 0.073 b	3.24	3.54	10

^zMeans within columns followed by the same lowercase letter are not significantly different based on Tukey's means comparison at $\alpha = 0.05$ and were analyzed separately for *Mal* alone and *Mal* + *Q8* allele combinations.

Fig. 2. The variation in titratable acidity for the *Mal* and *Q8* alleles among (A) diploid accessions (N = 181) and (B) triploid accessions (N = 36) between 2017 and 2019, from the U.S. Department of Agriculture *Malus* germplasm collection in Geneva, NY. The blue dots represent the estimated marginal means for each accession. The red plus signs represent outliers, as defined by the estimated marginal means test.

dominant *Q8* alleles (*mamama_Q8Q8Q8*) had an upper 95% CI value of 3.11 g·L⁻¹, which was 0.69 g·L⁻¹ higher than their diploid counterpart (*mama_Q8Q8*). There were six accessions that

overlapped in TA between the upper CI of the *Mama—_Q8q8—* (6.88 g·L⁻¹) and lower CI of *MaMa—_Q8Q8Q8* (6.17 g·L⁻¹) allele combinations (Table 4, Supplemental Table 1).

Fig. 3. Comparison of cider apple acidity classification systems for cider apple accessions (N = 217) harvested between 2017 and 2019, from the U.S. Department of Agriculture *Malus* germplasm collection in Geneva, NY. For the Long Ashton Research Station (LARS), French, and Spanish classification systems, the percentages of accessions were calculated by titratable acidity. For the marker-based classification systems, the percentages of accessions were calculated according to the *Ma1* and *Q8* allelic combinations for diploid (N = 181) and triploid (N = 36) accessions. The third allele in heterozygous triploid accessions was not identified; therefore, “—” represents an unknown allele.

Fig. 4. The variations in titratable acidity and pH among cider apple accessions (N = 205) harvested between 2017 and 2019, from the U.S. Department of Agriculture *Malus* germplasm collection in Geneva, NY, sorted according to their country or region of origin.

When the accessions were separated into broad geographic regions of origin, they exhibited a similar distribution, with accessions representing a TA range between 1.09 and 11.53 g·L⁻¹ and pH between 2.3 and 5.1 (Fig. 4). Five accessions were removed because of unknown regions of origin and seven accessions were removed due to low representation (≤ 3 accessions). Triploid accessions were represented in all major regions of origin. Among the 80 French accessions, there was a noticeable skew toward low to medium acid accessions, with 55 accessions with a LARS acidity threshold less than 4.5 g·L⁻¹ and only 25 accessions with a LARS acidity threshold more than 4.5 g·L⁻¹. Among the 55 accessions with low acid, 41 had the *mama_Q8Q8* genotype. Within accessions that originated in England (N = 48), approximately one-quarter (N = 13) also possessed the *mama_Q8Q8* genotype. Twenty-two accessions originated in Central Europe; 19 of which possessed the heterozygous *Mama* alleles, indicating a strong skew toward medium acid accessions. All 14 Spanish accessions in the dataset had the homozygous dominant *Q8Q8* allele and possessed all three types of the *Ma1* alleles (*MaMa*, *Mama*, and *mama*). Three of the four accessions that possessed the homozygous recessive *q8q8* genotype originated in North America.

Discussion

MARKER-BASED SYSTEM FOR CATEGORIZING CIDER APPLES. This study lays the groundwork for using the *Ma1* and *Q8* (*Ma3*) acidity markers to categorize cider apple germplasm. Both the diploid and triploid *Malus* accessions had statistically significant correlations between the TA concentration and the *Ma1* marker alone or *Ma1* + *Q8* markers analyzed together, but not the *Q8* marker alone. Based on the *Ma1* marker and the estimated marginal mean model with a 95% CI, the diploid accessions could be grouped into ranges of low

[*mama* (<2.4 g·L⁻¹), medium [*Mama* (2.4 to 5.8 g·L⁻¹), and high [*MaMa* (>5.8 g·L⁻¹)] acidity. These three categories had negligible overlap in TA concentrations among accessions in our sample population. However, using only the *Mal* marker resulted in less precision than using both the *Mal* and *Q8* markers because of the larger range for each acidity category. Using both the *Mal* and *Q8* markers resulted in more specificity (smaller ranges), but also extensive overlap among the seven allelic combinations. For example, there were 16 accessions that overlapped between the upper CI of the heterozygous *Mama* allele and the lower CI of the homozygous dominant *MaMa* when the homozygous dominant *Q8* allele was constant between both *Mal* allele types. Therefore, the utility of using one or both markers would depend on the end-user's goals. Using both the *Mal* and *Q8* markers would be very beneficial to plant breeders in terms of precision breeding and making marker-assisted selections, whereas using the *Mal* marker alone would be useful for establishing a broad marker-based cider apple acidity classification system. The *Mal* marker is presently used in SNP arrays by breeders; however, there are no SNPs that are currently associated with the *Q8* marker.

Even though we analyzed a robust list of cider apple accessions, there were only four diploid and no triploid homozygous recessive *q8q8* genotypes. The lack of some allelic groups limited our ability to make conclusions about this allelic combination for identifying fruit acidity. It is unclear why there are very few cider apples with the homozygous recessive *q8q8* alleles; however, the reason may be related to human selection of apples with higher acid for cider production.

There were 128 accessions with a TA concentration for the *Mama* genotype group that spanned both sides of the LARS 4.5 g·L⁻¹ threshold. Therefore, the *Mal* marker cannot be used as a predictor of the acidity component of the LARS cider apple classification system. With three acid categories, the French and Spanish systems were somewhat more closely matched to the *Mal* acidity markers. For example, the *Mal* marker categorized accessions with less than 2.4 g·L⁻¹ as low acidity, and the French and Spanish systems classified apples with less than 4.5 and 4.85 g·L⁻¹ as *douce* and *sweet*, respectively. Medium acidity ranged from 2.4 to 5.8 g·L⁻¹ for the marker-based system, whereas the medium ranges were 4.5 to 6.75 g·L⁻¹ and 4.85 to 6.56 g·L⁻¹ for the French and Spanish systems, respectively. In general, there was a 1- to 2-g·L⁻¹ difference among the French, Spanish, and *Mal* marker-based classification systems. Although apple classification systems are widely used by apple growers and cider producers, we have not found documentation that explains how the specific TA thresholds were chosen. The variations among the English, French, Spanish, and our proposed marker-based systems could be attributable to the perception of other flavor components in the apples, such as astringency, bitterness, and sweetness, which affect the way that acidity is perceived (Hampson et al., 2000). More sensory studies must be conducted to analyze the interdependency and perception thresholds among these important flavor components.

Similar to other studies that investigated apple fruit acidity genetics, our analyses indicate that the *Mal* gene is a reliable predictor ($P < 0.0001$) of TA (Bai et al., 2012; Khan et al., 2013). Therefore, our study adds to the body of literature indicating that the *Mal* gene largely determines apple acidity (Bai et al., 2015; Brown and Harvey, 1971; Kouassi et al., 2009; Nybom, 1959; Visser and Verhaegh, 1978; Xu et al., 2012). Although apple fruit acidity is affected by geographic, horticultural,

and seasonal variations, the controlling genes are static and can assist breeders in making selections and/or crosses and apple growers in choosing cultivars to plant.

For the *mama* genotype, Xu et al. (2012) reported a mean TA concentration of 2.06 g·L⁻¹, which is similar to the concentration of 1.92 g·L⁻¹ obtained during our study. However, for the *Mama* and *MaMa* genotypes, Xu et al. (2012) reported mean TA values of 8.46 and 10.38 g·L⁻¹, respectively, whereas our data indicate mean TA concentrations of 5.45 and 6.42 g·L⁻¹, respectively. The discrepancies between these studies may be attributed to the sample populations and differences in maturity at harvest. The study by Xu et al. (2012) used two mapping populations containing a maternal 'Royal Gala' and two paternal *M. sieversii* parents, whereas the sample population in our dataset consisted almost entirely of *M. ×domestica* cider cultivars. Duan et al. (2017) proposed that cultivated apples *M. ×domestica* possess two distinct genetic regions of substantially reduced genetic diversity near the *Mal* gene in comparison with progenitor species *M. sieversii*. Therefore, increased genetic diversity in *M. sieversii* could have resulted in the higher TA for the *Mama* genotype in the Xu et al. (2012) study.

The *Mal* and *Q8* markers were the focus of our study because of their large genetic effect on fruit acidity that has been commonly detected in *M. ×domestica* accessions with acidity levels lower than 10 g·L⁻¹ TA. We propose that the *Ma4*, *Ma5*, and *Ma6* markers should be used in future studies to further delineate cider apples into more precise classes, particularly those with acidity levels higher than 10 g·L⁻¹ (Ban and Xu, 2020; Rymerants et al., 2020).

Because organic acids are metabolized during the ripening process, pH usually decreases (Ma et al., 2015). It should be noted that pH is in a logarithmic scale, and small differences can result in sensorially detectable perceptions of the overall flavor and taste of the fruit (Hampson et al., 2000). The low-acid *mama* genotypes had a pH range between 4 and 5, which is greater than the ideal pH range (3.2–3.8) for microbial control during cider fermentation (Kosseva et al., 2016). Higher pH (>3.8) could result in microbial contamination and the development of off flavors during cider fermentation. The pH values of diploid and triploid accessions are presented in Supplemental Fig. 2A and B.

WITHIN AND AMONG YEAR VARIABILITY. Apple fruit acidity can be affected by within year (for example, harvest maturity and fruit location within the canopy) and year-to-year variations (for example, environmental conditions, crop load, and other biotic factors such as disease pressure) (Brown and Harvey, 1971). The variation in TA has been found to be dependent on cultivar and year more so than horticultural practices in the orchard (Bourvellec et al., 2015). Multiple years of acidity data collection for many of the accessions in our study minimized those effects. Additionally, fruit from different parts of the tree were mixed to obtain a representative sample. Furthermore, to reduce variability attributable to the ripening stage, all accessions in this study were harvested at a similar maturity, as measured using the SPI.

Previous studies examining the relationship between the *Mal* gene and TA evaluated the fruit at a mean SPI between 4 and 6 (Bai et al., 2012, 2015; Xu et al., 2012). However, the current study was focused on apple accessions for the hard cider industry; therefore, the goal was to test the fruit when the SPI was between 6 and 8, a later stage in the maturity process. This late-stage timing was unique to this study and helped to gain a cider-specific focus on developing a genetic marker-based

classification system for cider apples. Because TA fluctuates because of biotic and abiotic factors, a marker-based system provides a general range of how much acidity to expect; however, exact concentrations vary based on the aforementioned factors.

DIPLOID AND TRIPLOID ACCESSIONS. This study is one of the first to elucidate a significant difference in acidity concentrations in cider apples attributable to ploidy. On average, the diploid accessions had a TA concentration $0.33 \text{ g} \cdot \text{L}^{-1}$ less than that of triploid accessions, despite the lack of the dominant *MaMaMa* triploid genotype in our sample population. Therefore, the mean triploid acidity values were skewed toward the heterozygous and homozygous recessive *Mal* genotypes. This study corroborates the evidence from other fruit crops, such as citrus (*Citrus* sp.) and blueberry (*Vaccinium* sp.), that ploidy increases acidity levels (Ahmed et al., 2020; Mengist et al., 2020). The effect of ploidy on increasing acidity levels in cider apples could be attributable to the quantitative and additive natures of acid biosynthesis controlled by the major *Mal* gene (Brown and Harvey, 1971). Therefore, more copies of the dominant *Mal* allele would increase acidity concentrations.

EVALUATED GERMLASM. The USDA *Malus* germplasm collection contains the world's largest and most diverse catalog of *Malus* accessions, including a large collection of European and historic American cider apple cultivars, which was ideal for our analyses (Volk and Henk, 2016). The European cider cultivars in our study also included recently acquired traditional Spanish cider cultivars and traditional English and French bittersweet and bittersharp cultivars (Supplemental Table 1). A limitation to our study was that the USDA *Malus* germplasm collection consists of a single tree per accession and, thus, no field replication. Nonetheless, the sheer diversity of the collection has often been used to study the genetics of other important crop traits, such as dihydrochalcone content, volatile profiles, and anthocyanin production (Gutierrez et al., 2018; Sugimoto et al., 2015). Additionally, the geographically and genetically diverse accessions used for our study captured wide ranges of juice TA and pH that allowed us to identify potential genetic signatures to categorize cider apple genotypes. This diversity was further exploited to analyze accessions according to their country or region of origin.

Every country or region of origin had representative samples of low, medium, and high TA. There were some interesting trends observed during the analyses of acidity and region of origin. For example, 41 of the 80 analyzed French accessions possessed the homozygous recessive *mama* alleles and homozygous dominant *Q8Q8* alleles, indicating a strong selection preference for low-acid accessions. The dominant *Q8Q8* allele was observed in all the Spanish accessions, indicating that this genotype could be fixed in most Spanish accessions. Future pedigree and genetic linkage studies will uncover the history, movement, and introgression of progenitor species and lineages among the cider apple cultivars.

FUTURE CIDER APPLE CLASSIFICATION RECOMMENDATIONS. This is the first study to use a marker-based classification system to identify cider apples. Although we concluded that the *Mal* and *Q8* markers could usefully segregate accessions in our sample pool, more germplasm must be evaluated to encompass a wider range of allelic variability, particularly for triploids and accessions with the recessive *q8* alleles. Identification of additional acidity genes in apples would also help to account for more variability. Furthermore, sensory analyses would help to understand the thresholds for the human perception of acidity at different TA concentrations, as well as elucidate how other factors, such as sugar and polyphenol content, could affect the perception of acid in apples and cider (Hampson

et al., 2000; Rymenants et al., 2020). Finally, adding genetic markers for sugar and polyphenol content would create a robust suite of markers for plant breeders, horticulturists, and commercial cider producers to use to rapidly identify potential cider apple cultivars in germplasm collections and breeding populations.

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Supplemental Fig. 1. Flowchart illustrating the selection process for the cider apple accessions (N = 217) from the U.S. Department of Agriculture *Malus* germplasm collection in Geneva, NY that were used in this study.

Supplemental Fig. 2. The variation in pH for each of the combinations of *Ma1* and *Q8* alleles among (A) diploid accessions (N = 181) and (B) triploid accessions (N = 36) between 2017 and 2019 from the U.S. Department of Agriculture *Malus* germplasm collection in Geneva, NY. There were no *mama-q8q8* or *MaMa-q8q8* gene combinations. The blue dots represent the estimated marginal means for each accession. The red plus signs represent outliers as defined by the estimated marginal means test.

Supplemental Table 1. The cider apple accessions (N = 217) from the U.S. Department of Agriculture *Malus* germplasm collection in Geneva, NY used in this study. The Plant Introduction (PI) number is a unique inventory number assigned by the U.S. Department of Agriculture. The *Malus* UniQue genotype (MUNQ) code is based on single sequence repeat (SSR) data (Denancé et al., 2020). The accessions were genotyped for the *Mal* and *Q8* alleles and phenotyped for titratable acidity (TA) and pH. The *Mal* and *Q8* markers are unable to distinguish the third allele in heterozygous triploid accessions, thus “-” represents a missing allele. Titratable acidity, pH, and the starch pattern index (SPI) values are a mean of three biological replicates for each year the accession was harvested. The table is sorted by ploidy, followed by ascending TA concentration.

Accession name	PI (no.)	MUNQ (no.) ^z	Ploidy	<i>Mal</i>	<i>Q8</i>	TA (g·L ⁻¹)	pH	Date(s) harvested	SPI	Region of origin
Boutteville	162723	n/a	2	<i>mama</i>	<i>Q8Q8</i>	1.09	5	27 Sept. 2018	8	France
Sweet Coppin	589688	2574	2	<i>mama</i>	<i>Q8Q8</i>	1.13	4.4	06 Oct. 2017, 05 Oct. 2016	7.1	England
Stembridge Jersey	589693	1455	2	<i>Mama</i>	<i>Q8q8</i>	1.15	4.7	15 Sept. 2017, 07 Sept. 2018, 20 Sept. 2019	7.5	England
Taylor's	589663	2572	2	<i>mama</i>	<i>Q8q8</i>	1.19	5.1	21 Sept. 2017, 17 Sept. 2018	6.7	England
Harry Masters Jersey	589653	2589	2	<i>mama</i>	<i>Q8q8</i>	1.21	4.7	07 Sept. 2018	6.4	England
Perthre	589674	127	2	<i>mama</i>	<i>Q8Q8</i>	1.21	4.6	15 Sept. 2017, 07 Sept. 2018	8	England
Dabinett	589073	802	2	<i>mama</i>	<i>Q8Q8</i>	1.24	4.6	27 Sept. 2018	8	England
Reinette De Cuzy	590136	1060	2	<i>mama</i>	<i>Q8Q8</i>	1.33	4.4	15 Sept. 2017, 16 Sept. 2018	5.1	France
Le Bret	589690	n/a	2	<i>mama</i>	<i>Q8Q8</i>	1.34	4.6	19 Oct. 2017, 19 Oct. 2018	7.4	England
Holaart Doux	589585	2108	2	<i>mama</i>	<i>Q8Q8</i>	1.35	4.8	06 Oct. 2017, 25 Oct. 2018, 30 Oct. 2019	7.7	Northern Europe
Gala	392303	739	2	<i>Mama</i>	<i>Q8q8</i>	1.36	4.2	27 Sept. 2018	7.8	New Zealand
Ellis Bitter	589650	2593	2	<i>mama</i>	<i>Q8Q8</i>	1.4	4.6	01 Sept. 2017, 24 Aug. 2018, 04 Sept. 2019	7.8	England
PRI 1744-1	589789	n/a	2	<i>mama</i>	<i>Q8Q8</i>	1.4	4.6	15 Sept. 2017, 15 Aug. 2018	7.2	North America
Binet Blanc	122598	2358	2	<i>mama</i>	<i>Q8Q8</i>	1.41	4.6	29 Sept. 2017, 24 Oct. 2019	7.5	France
Sweet Alford	589081	n/a	2	<i>mama</i>	<i>Q8Q8</i>	1.41	4.4	06 Oct. 2017, 05 Oct. 2018	6	England
Bedan des Parts	123733	2377	2	<i>mama</i>	<i>Q8Q8</i>	1.43	4.7	19 Oct. 2018	5.3	France
Coat Jersey	589175	2598	2	<i>mama</i>	<i>Q8Q8</i>	1.45	4.6	15 Sept. 2017, 18 Sept. 2019	8	England
Colozette	162712	2357	2	<i>mama</i>	<i>Q8q8</i>	1.48	4.1	12 Oct. 2018	4.7	France
Frequin	162503	6564	2	<i>mama</i>	<i>Q8Q8</i>	1.48	4.9	21 Sept. 2017, 24 Sept. 2019	7.7	France
Binet Rouge	158730	2360	2	<i>mama</i>	<i>Q8Q8</i>	1.54	4.6	26 Oct. 2017, 12 Oct. 2018	5.6	France
Frequin Audievre	161838	n/a	2	<i>MaMa</i>	<i>Q8Q8</i>	1.61	4.8	05 Oct. 2018	7.7	France
Amer Gauthier	136243	6548.1	2	<i>mama</i>	<i>Q8Q8</i>	1.64	4.6	19 Oct. 2017, 30 Oct. 2019	8	France
American Forestier	173978	4779	2	<i>mama</i>	<i>Q8Q8</i>	1.64	4.5	06 Oct. 2017, 12 Oct. 2018	7.6	France
Dekkers Glorie	188515	2497	2	<i>Mama</i>	<i>q8q8</i>	1.65	4.3	21 Sept. 2017, 17 Sept. 2018	7.3	Northern Europe
Repinaldo do Liebana	105528	n/a	2	<i>mama</i>	<i>Q8Q8</i>	1.66	4.5	29 Sept. 2017, 09 Oct. 2019	7.5	Spain
Dunkerton Late Sweet	589666	2595	2	<i>mama</i>	<i>Q8q8</i>	1.67	4.7	19 Oct. 2017, 19 Oct. 2018	6.5	England
Pepa	680620	n/a	2	<i>mama</i>	<i>Q8Q8</i>	1.68	4.4	05 Oct. 2018, 30 Oct. 2019	7.5	Spain
Pommier Llorca	240817	n/a	2	<i>mama</i>	<i>Q8Q8</i>	1.72	4.8	01 Sept. 2017, 07 Sept. 2018	7.4	Northern Africa
Damelot	162722	4696	2	<i>mama</i>	<i>Q8q8</i>	1.73	4.6	19 Oct. 2017, 19 Oct. 2018, 24 Oct. 2019	7.4	France
Fuero Rous	187352	n/a	2	<i>mama</i>	<i>Q8Q8</i>	1.81	4.6	06 Oct. 2017, 19 Oct. 2018, 30 Oct. 2019	6	France
Marin Onfroy	173982	6559	2	<i>mama</i>	<i>Q8Q8</i>	1.81	4.5	15 Sept. 2017, 20 Sept. 2019	7.8	France
Doux-AMR	122616	535	2	<i>mama</i>	<i>Q8q8</i>	1.85	4.5	29 Sept. 2017, 09 Oct. 2019	7.8	France
Jouveaux	162731	326	2	<i>Mama</i>	<i>Q8Q8</i>	1.89	4.3	21 Sept. 2017, 12 Oct. 2018, 24 Oct. 2019	7.7	France
Coloradona	681633	n/a	2	<i>Mama</i>	<i>Q8Q8</i>	1.9	4.2	21 Sept. 2017, 27 Sept. 2018, 20 Sept. 2019	6.6	Spain
Manch Rouge	162716	n/a	2	<i>Mama</i>	<i>Q8Q8</i>	1.9	4.2	05 Oct. 2018, 15 Oct. 2019	7	France
Muscadet de Lense	173985	4625	2	<i>Mama</i>	<i>Q8Q8</i>	1.9	4.2	15 Aug. 2018, 20 Sept. 2019	7.6	France
Doux Normandie	589667	2597	2	<i>Mama</i>	<i>Q8q8</i>	1.94	4.2	29 Sept. 2017, 12 Oct. 2018	7.6	France
Muscadet de Dieppe	589493	2213	2	<i>Mama</i>	<i>Q8Q8</i>	1.95	4.6	16 Sept. 2018, 24 Sept. 2019	6.7	France
Amere de Bertheocourt	127311	1624	2	<i>Mama</i>	<i>Q8Q8</i>	1.98	4.4	29 Sept. 2017, 27 Sept. 2018, 24 Sept. 2019	7.9	France

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Accession name	PI (no.)	MUNQ (no.) ^z	Ploidy	MaI	Q8	TA (g·L ⁻¹)	pH	Date(s) harvested	SPI	Region of origin
Royal Jersey	175545	2549	2	Mama	Q8Q8	1.99	4.4	06 Oct. 2017	7.9	England
Major	150649	6559	2	Mama	Q8Q8	2.03	4.5	06 Oct. 2017, 27 Sept. 2018	7.9	England
Michelin	589670	535	2	mama	Q8q8	2.05	4.3	15 Sept. 2017, 27 Sept. 2018	7.8	France
C'Huero Biz Bras	187297	n/a	2	mama	Q8Q8	2.06	4.6	21 Sept. 2017, 20 Sept. 2019	6.9	France
Frequin Tardive de la Sarthe	589689	367	2	mama	Q8Q8	2.07	4.4	26 Oct. 2017, 24 Oct. 2019	7.6	France
Pomme Thoury	162548	4184	2	mama	Q8Q8	2.08	4.2	21 Sept. 2017, 16 Sept. 2018, 24 Oct. 2019	7.6	France
Tale Sweet	589691	2573	2	mama	Q8Q8	2.08	4.3	12 Oct. 2018, 30 Oct. 2019	6.5	England
Twistbody Jersey	175551	2561	2	mama	Q8Q8	2.08	4.4	15 Sept. 2017, 18 Sept. 2019	7.8	England
Belle de Crollon	162544	4625	2	mama	Q8Q8	2.09	4.2	06 Oct. 2017, 05 Oct. 2018, 24 Oct. 2019	7.9	France
Fillbarrel	589679	2592	2	mama	Q8Q8	2.16	4.2	29 Sept. 2017, 30 Oct. 2019	7.9	England
Frequin Lacaille	247314	6644.1	2	mama	Q8Q8	2.16	4.4	21 Sept. 2017, 27 Sept. 2018, 09 Oct. 2019	8	France
Crow Egg	589196	4674	2	Mama	Q8Q8	2.18	4	19 Oct. 2017, 12 Oct. 2018	7.3	North America
White Jersey	175553	2568	2	mama	Q8Q8	2.21	4.5	21 Sept. 2017, 07 Sept. 2018, 09 Oct. 2019	7.1	England
Bramtot	158731	6557.1	2	mama	Q8Q8	2.22	4.3	06 Oct. 2017, 05 Oct. 2018	6.8	France
Muscadet Bernay	200780	4625	2	mama	Q8Q8	2.23	4.2	19 Oct. 2018, 30 Oct. 2019	7.7	France
Launette	162732	n/a	2	mama	Q8Q8	2.28	4.3	29 Sept. 2017, 27 Sept. 2018, 15 Oct. 2019	7.3	France
Tardive Forestier	175548	723	2	mama	Q8Q8	2.3	4.13	29 Sept. 2017, 17 Sept. 2018, 09 Oct. 2019	7.9	France
Pomme Framboise	131975	345	2	Mama	Q8q8	2.33	3.8	01 Sept. 2017, 15 Aug. 2018	8	Central Europe
La Paix	589598	488	2	Mama	Q8q8	2.42	3.9	15 Sept. 2017, 07 Sept. 2018	7.2	Central Europe
Medaille d'Or	594108	256	2	mama	Q8Q8	2.42	4.3	29 Sept. 2017, 27 Sept. 2018	7.5	France
Daux Belan	162062	4708	2	mama	Q8Q8	2.51	4.5	06 Oct. 2017, 05 Oct. 2018, 24 Oct. 2019	6.4	France
Geeveston Fanny	589123	4674	2	Mama	Q8Q8	2.51	4	26 Oct. 2017, 05 Oct. 2018	7.8	Australia
Djulabia	264689	n/a	2	Mama	Q8Q8	2.56	3.8	21 Sept. 2017, 17 Sept. 2018	n/a	Central Europe
Hubbardston Nonsuch	589202	941	2	Mama	Q8q8	2.66	3.8	27 Sept. 2018	5.5	North America
Rousse Latour	136604	n/a	2	mama	Q8Q8	2.69	4.1	01 Sept. 2017, 24 Oct. 2019	5.8	France
Noel Deschamps	173986	4728	2	mama	Q8Q8	2.72	4.2	19 Oct. 2017, 24 Oct. 2019	7.8	France
Reinette Jaune De Butzel	131978	n/a	2	Mama	Q8q8	3.01	3.9	27 Sept. 2018	7.3	Central Europe
Improved Lambrook Pippin	589682	1454	2	Mama	Q8Q8	3.1	3.8	15 Sept. 2017, 12 Sept. 2019	7	England
Empire	588842	656	2	MaMa	Q8q8	3.11	3.6	19 Oct. 2018	7.3	North America
Bella de Jardins	105498	96	2	Mama	Q8Q8	3.12	3.7	21 Sept. 2017, 27 Sept. 2018	7.8	France
Hudson's Golden Gem	590157	6528	2	Mama	q8q8	3.14	4	25 Oct. 2018	7.3	North America
Blahova Oranzova Renetor	341067	931	2	Mama	Q8q8	3.15	4	21 Sept. 2017, 05 Oct. 2018	7.9	Central Europe
Binet Blanc Dore	158729	n/a	2	mama	Q8Q8	3.16	4.1	26 Oct. 2017, 05 Oct. 2018, 30 Oct. 2019	7.3	France
Royal Gala	651008	739.2	2	Mama	Q8q8	3.24	3.8	25 Aug. 2018	1.3	New Zealand
Margil	264558	732	2	Mama	Q8q8	3.46	4	26 Oct. 2017, 05 Oct. 2018	7.2	n/a
Langworthy	161851	2505	2	Mama	Q8Q8	3.61	3.6	06 Oct. 2017	8	England
Ben Davis	588953	106	2	Mama	q8q8	3.64	3.7	19 Oct. 2017, 14 Nov. 2018	7	North America
Macoun	589895	1055.1	2	Mama	Q8Q8	3.72	3.6	17 Sept. 2018	5.7	North America
Redstreak	175543	n/a	2	MaMa	Q8q8	3.9	3.9	25 Oct. 2018	7	England
White Winter Pearmain	613887	50.1	2	Mama	Q8Q8	3.9	3.7	26 Oct. 2017, 02 Nov. 2018	7	North America
Pohorka	588745	2699	2	Mama	Q8q8	3.93	3.6	06 Oct. 2017	8	Former USSR
Gewurzluiken	132225	315	2	Mama	Q8q8	3.95	3.7	29 Sept. 2017	8	Central Europe

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Supplemental Table 1. Continued.

Accession name	PI (no.)	MUNQ (no.) ^z	Ploidy	MaI	Q8	TA (g·L ⁻¹)	pH	Date(s) harvested	SPI	Region of origin
Kingston Black	589703	632	2	Mama	Q8Q8	3.97	3.8	21 Sept. 2017, 07 Sept. 2018, 09 Oct. 2019	6.7	England
Belle Sans Pepin	588951	2705	2	Mama	Q8Q8	3.98	3.8	26 Oct. 2017, 02 Nov. 2018	7.6	France
Jefferis	589185	2865	2	Mama	Q8Q8	3.98	3.7	21 Sept. 2017, 07 Sept. 2018	5.8	North America
Lande	162724	67.2	2	Mama	Q8Q8	4	3.7	21 Sept. 2017, 12 Oct. 2018	6.3	France
Herring's Pippin	136001	82	2	MaMa	Q8Q8	4.02	3.6	15 Aug. 2018	8	England
Cortland	588848	503	2	Mama	Q8Q8	4.1	3.6	12 Oct. 2018	5	North America
Northern Spy	588872	23	2	Mama	Q8q8	4.26	3.5	06 Oct. 2017	8	North America
Drap d'Or Guemene	131823	92	2	Mama	Q8q8	4.31	3.7	06 Oct. 2017, 05 Oct. 2018	7.6	France
Belle Fleur Rouge	245048	n/a	2	Mama	Q8Q8	4.36	3.5	05 Oct. 2018	6.7	France
American Summer Pearmain	589214	2986	2	Mama	q8q8	4.41	3.6	07 Sept. 2018	7.4	North America
Grimes Golden	588791	3190	2	Mama	Q8q8	4.41	3.6	02 Nov. 2018	5	North America
Reine des Reinettes x 82	279325	732	2	Mama	Q8q8	4.44	3.7	21 Sept. 2017, 27 Sept. 2018	7.4	France
Fenouillet de Ribours	590126	255	2	Mama	Q8Q8	4.49	3.6	29 Sept. 2017, 12 Oct. 2018, 09 Oct. 2019	5.3	France
Golden Delicious	590184	65	2	Mama	Q8q8	4.49	3.7	21 Sept. 2017, 12 Oct. 2018	6.8	North America
Pomme Raisin	134669	670	2	Mama	Q8Q8	4.52	3.5	15 Sept. 2017, 27 Sept. 2018	7.2	Central Europe
Cristalina	681639	n/a	2	Mama	Q8Q8	4.62	3.7	25 Oct. 2018, 30 Oct. 2019	6.2	Spain
Foxwhelp (misidentified)	589318	n/a	2	Mama	Q8Q8	4.62	3.5	21 Sept. 2017, 07 Sept. 2018, 09 Oct. 2019	7.6	England
Stoke Red	589697	2575	2	Mama	Q8q8	4.62	3.7	17 Sept. 2018, 09 Oct. 2019	7.7	England
Reine des Reinettes x 1700	279326	37	2	Mama	Q8Q8	4.64	3.7	06 Oct. 2017, 27 Sept. 2018	7.5	France
Vagnon Ascher	175552	273	2	Mama	Q8Q8	4.65	3.8	29 Sept. 2017, 12 Oct. 2018, 09 Oct. 2019	7.5	England
Golden Russet	589892	2862	2	Mama	Q8q8	4.72	3.6	25 Oct. 2018	5.7	n/a
Sunset	589694	164	2	Mama	Q8q8	4.82	3.4	17 Sept. 2018	6.5	England
Winesap	588799	447	2	Mama	Q8q8	4.9	3.6	26 Oct. 2017, 02 Nov. 2018	4.8	North America
Pigeonnet Rouge	132273	1509	2	Mama	Q8Q8	4.96	3.5	21 Sept. 2017, 27 Sept. 2018, 18 Sept. 2019	6.9	France
Reine des Pommes	132571	285	2	Mama	Q8Q8	5.05	3.7	19 Oct. 2017	8	France
Perico	680621	n/a	2	MaMa	Q8Q8	5.14	3.6	25 Oct. 2018, 24 Oct. 2019	4.9	Spain
Marshall McIntosh	588998	508	2	Mama	Q8Q8	5.16	3.5	21 Sept. 2017, 17 Sept. 2018	6.6	North America
Red Ralls	437047	546	2	Mama	Q8Q8	5.18	3.6	19 Oct. 2017, 19 Oct. 2018	7.5	Central Europe
Hubbards Pearmain	590130	2476	2	Mama	Q8Q8	5.24	3.6	29 Sept. 2017, 27 Sept. 2018	7.8	England
Stembridge Cluster	589692	1454	2	Mama	Q8q8	5.32	3.5	29 Sept. 2017, 27 Sept. 2018, 09 Oct. 2019	7	England
De La Riega	680619	n/a	2	MaMa	Q8Q8	5.33	3.5	12 Oct. 2018, 24 Oct. 2019	6.5	Spain
Esopus Spitzenburg	588785	522	2	Mama	Q8Q8	5.33	3.5	19 Oct. 2017, 19 Oct. 2018	7	North America
Thorgauer Weinapfel	506361	1333	2	Mama	Q8Q8	5.37	3.6	21 Sept. 2017, 12 Oct. 2018	6.8	Central Europe
Court Pendu Rose	589587	105	2	Mama	Q8Q8	5.39	3.7	19 Oct. 2017	7	France
McIntosh Summerland Red	588817	508	2	Mama	Q8Q8	5.4	3.4	01 Sept. 2017, 16 Sept. 2018	6.8	North America
Friandise	590127	378	2	Mama	Q8Q8	5.43	3.6	21 Sept. 2017, 25 Oct. 2018	5.1	Northern Europe
Bella di Pontoise	102537	87	2	Mama	Q8q8	5.5	3.5	19 Oct. 2018	6.3	France
Reinette Da Mana	322032	131	2	Mama	Q8Q8	5.52	3.6	25 Oct. 2018	6.5	France
Court Pendu Gris	589602	105	2	Mama	Q8Q8	5.56	3.5	06 Oct. 2017, 19 Oct. 2018	5.3	France
Landsberger Reinette	589565	61	2	MaMa	Q8Q8	5.56	3.5	15 Sept. 2017, 16 Sept. 2018	7.4	Central Europe
Renetta Dorata	104034	38.1	2	Mama	Q8Q8	5.63	3.5	05 Oct. 2018	5.4	Southern Europe
Liberty	588943	585	2	Mama	Q8Q8	5.72	3.4	12 Oct. 2018	6.6	North America

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Accession name	PI (no.)	MUNQ (no.) ^z	Ploidy	MaI	Q8	TA (g·L ⁻¹)	pH	Date(s) harvested	SPI	Region of origin
Blanquina	681637	n/a	2	MaMa	Q8Q8	5.79	3.5	05 Oct. 2018, 30 Oct. 2019	7	Spain
Edelroter	590125	173	2	Mama	Q8q8	5.79	3.7	06 Oct. 2017, 27 Sept. 2018	7.3	Central Europe
Pomme Grise	589242	n/a	2	Mama	Q8q8	5.79	3.5	25 Oct. 2018	6.6	North America
Arkansas Black	589117	44	2	Mama	Q8Q8	5.8	3.5	06 Oct. 2017, 02 Nov. 2018	6	North America
D'Arcy Spice	590122	1923	2	Mama	Q8q8	5.85	3.5	05 Oct. 2018	3.2	England
Clear Heart	206022	2733	2	Mama	Q8Q8	5.92	3.6	07 Sept. 2018	7.1	Northern Europe
Collaos	666188	n/a	2	MaMa	Q8Q8	5.95	3.6	05 Oct. 2018	5.3	Spain
Teign Harvey	175549	2545	2	Mama	Q8Q8	6	3.5	21 Sept. 2017, 17 Sept. 2018, 20 Sept. 2019	6.6	England
Golden Pippin	590129	1441	2	Mama	Q8Q8	6.01	3.4	21 Sept. 2017, 27 Sept. 2018	6.4	n/a
Yellow Bellflower	589195	77	2	Mama	Q8q8	6.07	3.6	19 Oct. 2017, 12 Oct. 2018	8	North America
Cap of Liberty	161830	n/a	2	Mama	Q8q8	6.13	3.5	06 Oct. 2017, 10 Oct. 2019	7.8	England
Weidners Goldreinette	590143	3040	2	Mama	Q8Q8	6.14	3.4	21 Sept. 2017, 27 Sept. 2018	6.7	Central Europe
Jonathan	590185	57	2	Mama	Q8q8	6.16	3.4	29 Sept. 2017, 05 Oct. 2018	7.7	North America
Skyrme's Kernel	161846	n/a	2	Mama	Q8q8	6.19	3.4	21 Sept. 2017, 17 Sept. 2018, 09 Oct. 2019	6.3	England
Reinette Franche	590137	278	2	Mama	Q8Q8	6.22	3.5	26 Oct. 2017, 12 Oct. 2018, 24 Oct. 2019	7.8	France
Grenadier	589684	93	2	Mama	Q8Q8	6.23	3.4	15 Sept. 2017, 07 Sept. 2018	7.5	England
Pigeonnet Blanc	132272	n/a	2	Mama	Q8q8	6.23	3.3	19 Oct. 2018	7	France
Blue Pearmain	590180	n/a	2	Mama	Q8q8	6.26	3.6	06 Oct. 2017, 27 Sept. 2018	6.2	North America
Belle de Nordhausen	589584	99	2	Mama	Q8Q8	6.3	3.3	27 Sept. 2018	7.7	Central Europe
Yellow Newtown	588773	787	2	Mama	Q8Q8	6.4	3.5	29 Sept. 2017, 25 Oct. 2018	6.7	North America
Cheddar Cross	589656	2696	2	Mama	Q8Q8	6.41	3.4	15 Aug. 2018	7.8	England
Court Pendu	589601	105	2	Mama	Q8Q8	6.42	3.4	05 Oct. 2018	6	France
Weisser Winter Taffetapfel	590144	234	2	Mama	Q8Q8	6.44	3.6	06 Oct. 2017, 05 Oct. 2018	7.3	Central Europe
Reinette do Chenee	131828	n/a	2	Mama	Q8Q8	6.48	3.6	06 Oct. 2017	7.8	Central Europe
Lambrook Pippin	161839	n/a	2	Mama	Q8Q8	6.5	3.4	12 Oct. 2018	7.7	England
Old Pearmain	590133	598	2	Mama	Q8q8	6.57	3.4	21 Sept. 2017, 17 Sept. 2018	6.5	England
Raxao	681640	n/a	2	Mama	Q8Q8	6.65	3.4	29 Sept. 2017, 07 Sept. 2018, 24 Oct. 2019	7.8	Spain
Redfield	589211	n/a	2	Mama	Q8q8	6.68	3.4	29 Sept. 2017, 25 Oct. 2018	7.8	North America
Nanot	161763	n/a	2	Mama	Q8q8	6.81	3.2	21 Sept. 2017, 09 Oct. 2019	8	France
Reinette Grise	589588	2114	2	Mama	Q8Q8	6.84	3.5	23 Aug. 2018	6.9	France
Duffin	175542	2554	2	Mama	Q8Q8	6.86	3.4	07 Sept. 2018	7.2	England
Reinette Clochard	589444	118	2	Mama	Q8Q8	6.9	3.5	19 Oct. 2017, 19 Oct. 2018	6.1	France
Bonne-Hotture	590120	124	2	Mama	Q8q8	7.07	3.5	06 Oct. 2017, 05 Oct. 2018	7	France
Grosse Mouche	162545	n/a	2	Mama	Q8Q8	7.23	3.4	06 Oct. 2017	8	France
Reineta do Caravia	105524	n/a	2	Mama	Q8Q8	7.24	3.4	06 Oct. 2017, 19 Oct. 2018	6.1	Spain
Finkenwerder Herbstprinz	651010	2889	2	MaMa	Q8Q8	7.27	3.4	12 Oct. 2018	6.1	Central Europe
Calville Blanc	589596	95	2	Mama	Q8q8	7.39	3.4	25 Oct. 2018	7	France
Reinette Thouin	590140	134	2	Mama	Q8Q8	7.41	3.5	06 Oct. 2017, 12 Oct. 2018	7.9	France
Inducoa No. II	613830	16	2	Mama	Q8Q8	7.49	3.5	27 Sept. 2018	7.8	Spain
Ross Nonpareil	590141	2100	2	Mama	Q8q8	7.58	3.6	06 Oct. 2017, 19 Oct. 2018, 09 Oct. 2019	6.3	n/a
Golden Harvey	590128	2841	2	Mama	Q8Q8	7.79	3.5	21 Sept. 2017, 19 Oct. 2018	7.2	England
Pomme Cloche	134668	85	2	MaMa	Q8Q8	7.89	3.2	29 Sept. 2017, 12 Oct. 2018	7.3	Central Europe

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Supplemental Table 1. Continued.

Accession name	PI (no.)	MUNQ (no.) ^z	Ploidy	MaI	Q8	TA (g·L ⁻¹)	pH	Date(s) harvested	SPI	Region of origin
Champagne Reinette	264688	91	2	Mama	Q8Q8	8.07	3.3	06 Oct. 2017, 19 Oct. 2018	7.5	France
Sturmer Pippin	307382	5249	2	MaMa	Q8Q8	8.34	3.3	26 Oct. 2017, 14 Nov. 2018	5.4	England
William Crump	589309	333	2	Mama	Q8Q8	8.34	3.3	15 Sept. 2017, 19 Oct. 2018	6.4	England
Edward VII	392312	74	2	MaMa	Q8Q8	8.36	3.2	19 Oct. 2017, 19 Oct. 2018	7.7	England
Lorna Doone	161840	2694	2	Mama	Q8q8	8.45	3.3	24 Aug. 2018	5.4	England
Lombart's Calville	589920	9	2	Mama	Q8Q8	8.61	3.4	05 Oct. 2018	6.3	Northern Europe
Wickson	613818	n/a	2	MaMa	Q8Q8	8.69	3.5	19 Oct. 2017, 25 Oct. 2015	7.2	North America
King David	589156	71	2	MaMa	Q8q8	8.71	3.3	12 Oct. 2018	6.6	North America
Xuanina	680623	n/a	2	MaMa	Q8Q8	8.71	3.3	12 Oct. 2018, 30 Oct. 2019	7.3	Spain
Vista Bella	588819	366	2	Mama	Q8Q8	8.75	3.4	21 Sept. 2017	8	North America
Court Pendu Plat	123960	105.2	2	Mama	Q8Q8	8.88	3.6	25 Oct. 2018	4.8	France
Toreno	245145	n/a	2	MaMa	Q8Q8	9	3.3	19 Oct. 2018	3.7	Spain
Pigeonnet	589597	2112	2	MaMa	Q8Q8	9.01	3.3	05 Oct. 2018	6.2	France
Tremlett's Bitter (misidentified)	175550	n/a	2	Mama	Q8Q8	9.07	3.3	21 Sept. 2017, 12 Oct. 2018	7.8	n/a
Boche	162549	n/a	2	Mama	Q8Q8	9.39	3.2	21 Sept. 2017	7.9	France
Zuccamaglio	589569	56	2	Mama	Q8Q8	10.89	3.4	05 Oct. 2018	5.8	Central Europe
Isle of Wight Pippin	590131	n/a	2	Mama	Q8Q8	11.36	3.4	19 Oct. 2019	6.8	England
Court Royal	589671	n/a	3	mamama	Q8Q8Q8	1.43	4.5	29 Sept. 2017	7.8	England
Miron Sacharanij	154164	n/a	3	mamama	Q8Q8Q8	1.61	4.3	15 Aug. 2018	8	Central Europe
Domaine	173979	n/a	3	mamama	Q8Q8Q8	1.66	4.6	12 Oct. 2018	7.4	France
Arkansas	588952	n/a	3	mamama	Q8Q8Q8	1.84	4.6	21 Sept. 2017, 27 Sept. 2018, 20 Sept. 2019	7.6	North America
Belle Fille	162709	n/a	3	Mamama	Q8Q8Q8	1.88	4.6	29 Sept. 2017, 16 Sept. 2018, 26 Sept. 2019	7.6	France
C'Huero Ru Bienn	187298	n/a	3	Mama-	Q8Q8Q8	1.9	4.5	26 Oct. 2017, 12 Oct. 2018	7.5	France
Bulmer Norman	588808	618	3	Mamama	Q8Q8Q8	1.96	4.4	21 Sept. 2017, 16 Sept. 2018, 20 Sept. 2019	7.2	France
Doucet Rouge	161760	n/a	3	mamama	Q8Q8Q8	2.03	4.2	15 Aug. 2018, 20 Sept. 2019	7.5	France
Nehou	175544	n/a	3	mamama	Q8Q8Q8	2.21	4.5	17 Sept. 2018	7.3	France
Gros Frequin	131105	n/a	3	mamama	Q8Q8Q8	2.26	4.2	27 Sept. 2018, 09 Oct. 2019	7	France
Surpasse Frequin	125566	n/a	3	mamama	Q8Q8Q8	2.58	4	18 Sept. 2019	7.9	France
Teint Fraise	127370	n/a	3	mamama	Q8Q8Q8	2.65	4.2	06 Oct. 2017, 19 Oct. 2018, 30 Oct. 2019	7.6	France
Doux Tardif	162715	n/a	3	mamama	Q8Q8Q8	2.69	4	12 Oct. 2018, 30 Oct. 2019	6.2	France
Peau De Vache	136489	n/a	3	mamama	Q8Q8Q8	3.17	4.1	05 Oct. 2018, 24 Oct. 2019	8	France
Double Bon Pommier	131104	n/a	3	Mama-	Q8q8-	3.31	4.4	21 Sept. 2017	8	France
Notaire	137094	n/a	3	Mama-	Q8q8-	3.88	4	21 Sept. 2017, 27 Sept. 2018	7.1	France
Stayman	588975	117.2	3	Mama-	Q8q8-	4.28	3.6	19 Oct. 2017, 02 Nov. 2018	5.9	North America
Rott Jarnpple	102148	n/a	3	Mama-	Q8q8-	4.37	3.5	21 Sept. 2017	7	Central Europe
Mutsu	223602	874.2	3	Mama-	Q8q8-	4.73	3.5	15 Sept. 2017, 19 Oct. 2018	6.4	Japan
Smokehouse	589903	n/a	3	Mama-	Q8q8-	4.79	3.5	05 Oct. 2018	6	North America
Ribston	588840	907.2	3	Mama-	Q8q8-	4.86	3.6	21 Sept. 2017, 7 Sept. 2018	7.3	England
Reinette Jamin	135645	n/a	3	Mama-	Q8Q8Q8	4.97	3.6	29 Sept. 2017, 27 Sept. 2018	7.9	France
Reinette van Ekenstein	188521	n/a	3	Mama-	Q8Q8Q8	5.06	3.5	21 Sept. 2017, 17 Sept. 2018	7.5	Northern Europe
Rhode Island Greening	589520	n/a	3	Mama-	Q8Q8Q8	5.59	3.5	26 Oct. 2017, 19 Oct. 2018	7.5	North America
Reinette Jaegers	131561	n/a	3	Mama-	Q8q8-	6.2	3.5	15 Sept. 2017, 12 Oct. 2018	7.9	Central Europe

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Accession name	PI (no.)	MUNQ (no.) ^z	Ploidy	MaI	Q8	TA (g·L ⁻¹)	pH	Date(s) harvested	SPI	Region of origin
Tom Putt	125271	n/a	3	Mama-	Q8Q8Q8	6.37	3.4	06 Oct. 2017	6.6	England
Vandevere	589060	n/a	3	Mama-	Q8Q8Q8	6.56	3.4	19 Oct. 2017, 12 Oct. 2018	7.3	North America
Ashmead's Kernel	589654	1546	3	Mama-	Q8q8-	6.7	3.4	19 Oct. 2017, 19 Oct. 2018	6	England
Roxbury Russet	588971	n/a	3	Mama-	Q8Q8Q8	6.76	3.4	19 Oct. 2017, 25 Oct. 2018	6.6	North America
Reinette Ontz	590139	n/a	3	Mama-	Q8Q8Q8	7.79	3.3	19 Oct. 2018	6.2	England
Canavial-14	183961	n/a	3	Mama-	Q8Q8Q8	7.93	3.4	26 Oct. 2017, 02 Nov. 2018	5.7	Central Europe
Rouge Belle De Boskoop	589143	886.2	3	Mama-	Q8q8-	9.07	3.4	07 Sept. 2018	7.7	France
Reinette Tres Tardive	162741	890	3	Mama-	Q8Q8Q8	10.73	3.2	19 Oct. 2017, 12 Oct. 2018, 09 Oct. 2019	5	France
Reinette D'Anjou	590135	n/a	3	Mama-	Q8q8-	11.17	3.3	12 Oct. 2018	6.7	France
Piel De Sapo	681634	913	3	Mama-	Q8Q8Q8	11.53	3.2	14 Nov. 2018	5	Spain

^zMUNQ value ending in "0.1", doubt regarding value due to small differences in SSR genotypes. MUNQ value ending in "0.2", value attributed only because of name. Value ending in "0.3", MUNQ attributed only because of name, but the pedigree is consistent between SSR and SNP data. Not available (n/a).