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Tree Genetics & Genomes (2022) 18:40
https://doi.org/10.1007/s11295-022-01571-w

ORIGINAL ARTICLE

Mapping of QTLs for citrus quality traits throughout the fruit maturation process on clementine (*Citrus reticulata* × *C. sinensis*) and mandarin (*C. reticulata* Blanco) genetic maps

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Received: 22 July 2022 / Revised: 7 October 2022 / Accepted: 16 October 2022

Abstract

Citrus fruit quality is defined as the combination of physical and chemical traits; some of which may change during the ripening phase, e.g., acidity and sugar content. A clear understanding of their genetic control would be very helpful for marker-assisted breeding programs especially with regard to the juvenile phase and some reproductive features that hamper the selection of improved hybrids. A genetic study was thus performed on the heredity of quality traits and QTL detection based on segregation in a progeny generated from a cross between clementine *cv* "Commun" (*Citrus* × *reticulata cv clementine*) and mandarin *cv* "Willow leaf" (*C. reticulata* Blanco). Parental and consensus genetic linkage maps were constructed using 645 SNP and SSR markers. These maps were represented by 10 linkage groups in clementine and 12 linkage groups in mandarin, representing 75% and 58% respectively of the previously published clementine reference map. A total of 16 traits, including fruit mass, equatorial diameter, juice percentage, total soluble solids, acidity, pH, glucose, fructose, sucrose, and citric and malic acid concentrations were evaluated at three maturation dates. High variations indicating transgressive segregation were found for all traits, with normal or close to normal distributions. QTL analysis performed using the multiple QTL model allowed the detection of 34 QTLs on the three maps. QTLs were distributed in different linkage groups and generally detected at only one date of the ripening phase. The percentage of total variation explained ranged from 12 to 37% per QTL. Major QTLs ($R^2 \geq 30\%$) were detected for equatorial diameter, glucose, and fructose (expressed in percentage dry matter) on linkage groups 8 and 9. Co-localization of QTLs controlling correlated and uncorrelated traits were mainly found on linkage groups 2, 4, 8, and 9, particularly between fruit mass and acidity.

Keywords Heredity · SNP · SSR · Acidity · Soluble Sugars · Fruit mass · Fruit ripening

Communicated by C. Chen

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Introduction

Modern environment-friendly citriculture requires the development of new varieties with higher yield and nutritional quality, as well as better tolerance to biotic and abiotic constraints (Gmitter et al. 2007). Fruit quality and its development during maturation are based on environmental factors and internal complex traits, with juice percentage, acid, and sugar contents being major determinants of internal fruit quality (Iglesias et al. 2007). Some of these traits show continuous variation during fruit ripening (Spiegel-Roy and Goldschmidt 1996). During the maturation of orange and mandarin-like varieties, fruit acidity, mainly due to citric acid, decreases while fruit sweetness increases (Bain 1958). In addition to the ratio between total sugar content (evaluated with a refractometer) and the titratable acidity, skin

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coloration and juice percentage have been used as indicators of citrus fruit maturity, e.g., in clementines, mandarins, and sweet oranges, and they are jointly taken into account when determining fruit harvest dates (Julhia et al. 2019).

A comprehensive understanding of the genetic determinism of fruit quality during maturation is necessary to facilitate the breeding of new varieties (Gmitter et al. 2007). However, conventional citrus breeding programs must cope with many constraints: (1) the juvenility phase, which generally extends from 5 to 7 years; (2) large plant size; (3) high heterozygosity of the main cultivars; and (4) polyembryony, which reduces the chance of obtaining zygotic seedlings and self-incompatibility (Ollitrault and Luro 1997). Marker-assisted selection (MAS) is potentially highly advantageous for citrus breeding since it enables selection at the seedling stage, thereby overcoming some of the mentioned breeding difficulties related to citrus reproductive constraints (Roose 2007). This approach depends on the development of molecular markers and genetic maps to detect linkage with economically important traits (Staub et al. 1996).

Due to the high heterozygosity of citrus germplasm, most of citrus genetic maps have been developed on the basis of F1 crosses, while segregation analyses have enabled the development of genetic maps for each parent and sometimes consensus genetic maps (Ollitrault 2019). Several saturated genetic maps have been published over the last 10 years. The first one is the reference clementine genetic map that was constructed with 961 co-dominant markers from a progeny between clementine and pummelo (Ollitrault et al. 2012a). A sweet orange genetic map (569 markers) was also published in the same paper. Saturated maps of sweet orange with 943 markers (Xu et al. 2013) and mandarin with 706 markers (Shimada et al. 2014) have also been released. More recently, NGS applied with complexity-reduced genomes was used to produce medium- to high-density genetic maps (Guo et al. 2015; Curtolo et al. 2017).

Although QTL mapping of fruit quality has received a surge of interest with regard to many species, such as apple (Calenge et al. 2005; Rymenants et al. 2020), peach (Quilot et al. 2005; Rawandoozi et al. 2020), grapevine (Doligez et al. 2013; Houel et al. 2015), and tomato (Ashrafi and Foolad 2015; Cabodevila et al. 2021), this technique has been developed to a lesser extent in citrus (Ollitrault 2019). The majority of published citrus studies have dealt with QTLs related to fruit yield (García et al. 2000) or tolerance/resistance to diseases such as tristeza (Asins et al. 2004) and *Phytophthora* (Siviero et al. 2006), as well as to salinity (Tozlu et al. 1999). Few reports have been published related to fruit quality traits such as acidlessness, acidity, soluble solids content, seediness, color index, carotenoid and flavonoid content, and some morphological fruit traits (Fang et al. 1997; Sugiyama et al. 2011; Asins et al. 2015; Yu et al. 2016; Imai et al. 2017; Curtolo et al. 2017, Mou et al. 2021). Most research on QTLs for fruit quality traits has been carried out on F1 populations involving one or two mandarin parents, but with a variable number of detected QTLs. Only two major QTLs have been consistently detected for fruit quality traits, including one on the *C. clementina* map contributing up to 21.3% to rind thickness (Asins et al. 2015). A total of 48 fruit quality QTLs have been identified, 10 of which were stable over two or more samplings, while a cluster of QTLs for flavedo and juice colors were detected in a single genomic region on linkage group 4 on the mandarin genetic map (Yu et al. 2016). A total of 19 QTLs were identified for 12 fruit quality traits on an integrated linkage map of Murcott tangor and Pera sweet orange (Curtolo et al. 2017), whereas four QTLs associated with fruit weight, one QTL associated with sugar content, three QTLs associated with peel puffing, and one QTL associated with water rot in mandarin were also identified (Imai et al. 2017). Genome-wide association mapping (GWAS) has also been used for the detection of QTLs of citrus fruit quality traits (Minamikawa et al. 2017; Imai et al. 2018).

Knowledge regarding factors controlling genetic variation in citrus fruit traits related to fruit maturation is still quite limited, mainly due to the lack of phenotypic data and the complexity of those traits.

In order to analyze the genetic determinants of citrus fruit quality during maturation, the phenotypic variations of physical and chemical attributes of fruit were studied in a backcross-like population derived from a cross between clementine (*C. reticulata* × *C. sinensis*) and mandarin (*C. reticulata*). The aim of this study was to map QTLs associated with citrus fruit quality traits. Genetic maps were built with codominant markers. Fruit attributes such as mass, equatorial diameter, pH, acidity, sugar, and acid contents were monitored at different dates during fruit maturation.

Material and methods

Experimental population

This study was based on a segregating population derived from a cross between clementine *cv* "Commun SRA 63" (*Citrus reticulata* × *C. sinensis*) (C) and mandarin *cv* "Willow leaf" (*C. reticulata* Blanco) (M), with clementine as female parent. The direction of this cross was chosen based on the gametic self-incompatibility and absence of apomictic reproduction in clementine, thereby enabling generation of only hybrids derived from cross hybridization. This cross closely resembles a backcross because clementine originates from a cross between "Willow leaf" mandarin and sweet orange and sweet orange probably emerged from a cross between two (mandarin × pummelo) hybrids (Ollitrault et al. 2012a; Wu et al. 2014). Due to its pedigree, clementine is

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close to the mandarin phenotype but displays interspecific heterozygous genomic regions with alleles inherited from pummelo (*C. maxima*) (Ollitrault et al. 2012a; Wu et al. 2014). This progeny consisted of 105 offspring grafted onto Carrizo citrange (*C. sinensis* × *Poncirus trifoliata*). The trees were 25 years old. The parents and offspring were grown under the same conditions. The orchard was located at the INRAE research station at San Giuliano (France), and there is no replicate in field design. Standard cultivation practices were applied regularly in order to maintain the orchard healthy and ensure good physiological growth. Fruit quality traits were evaluated during one maturation period between autumn and winter.

Phenotyping

Clementine and mandarin maturation occurs in Corsica over the November and January–February periods, respectively (Jacquemond and Agostini 2013), so fruit measurements were performed at three different periods, i.e., in October, December, and February. At each date, 10 random fruits per genotype were collected around the tree and their quality trait attributes were evaluated: 10 replicates for fruit mass, equatorial diameter, and five replicates for juice percentage, pH, titratable acidity, sugar content, citric and malic acids, and soluble sugar (glucose, fructose, sucrose) contents (expressed in % dry and fresh matter [DM and FM, respectively]).

Fruit diameter was measured using a digital caliper (Mitutoya, Absolute Digimatic, Kawazaki, Japan). Fruit juice was extracted with an electric press (Santos 52C, Vaulx-en-Velin, France), filtered and weighed, according to the standardized and normative method for citrus fruit marketing (CEE-ONU FFV-14). The pH and titratable acidity (TA expressed in g of citrate/100 g of juice) were determined for each fruit using an autotitrator (Mettler Toledo DL 50, Greifensee, Swiss), as described in Albertini et al. (2006). Sugar content (TSS in Brix), was measured using a digital refractometer (RFM710, Bellinghan Stanley, UK).

Measurement of sugar and organic acid contents

Organic acids and soluble sugars were extracted and analyzed by enzymatic assay according to Gomez et al. (2007) and Etienne et al. (2013a, b), adapted to citrus fruit. Briefly, fruit pulp was lyophilized at − 80 °C and 0.06 bar using a lyophilizer (Christ BETA 1–8-LD, Osterode Am Harz, Germany). The lyophilization period lasted 3 weeks, and then, the fruit pulp was ground into a powder using a TissueLyser II bead mill (QIAGEN). Two milliliters of water were added to 20 mg of lyophilized pulp powder. Samples were centrifuged for 5 min (17,000 g at 4 °C; Sigma 4-16 K). Supernatants (1650 µL) were recovered and supplemented with 10 mg of polyvinylpolypyrrolidone (PVPP) (part no. 25 249/54/1, Sigma-Aldrich Corp., Lyon, France) to eliminate residual phenols. After sample homogenization using a vortex for a few seconds and agitation for 20 min at 4 °C on a rotating wheel, the microtube was centrifuged (10 min, 17,000 g, at 4 °C). The supernatant was then recovered and stored at − 80 °C prior to analysis.

Soluble sugars and organic acids were quantified using an absorbance microplate reader (Biotek, ELx808, Vermont, USA) according to Gomez et al. (2007) and Etienne et al. (2013a, b), with some modifications to tailor it to the citrus fruit samples. The only difference relative to the initial protocol was the enzymatic reaction duration. For glucose and fructose, the nicotinamide adenine dinucleotide hydride (NADH) concentration became stable after 3 h instead of 2 h after starting the reaction. For the two organic acids, the NADH concentration plateaued 2 h after the onset of the reaction as compared to 3 h for citric acid and 2 h 45 min for malic acid. During the enzymatic reaction, a microplate was placed in an oven at 25 °C, i.e., the optimal temperature for all of the reagents used.

Statistical analysis and BLUPs

The statistical analysis was performed using the Statistica 10 (TIBCO Software Inc, Palo Alto, CA, USA; 2017), available from: https://www.tibco.com/products/tibco-statistica) and R 3.2.1 (RStudio: Integrated Development for R. RStudio, PBC, Boston, MA URL http://www.rstudio.com/) software packages. The mean and standard deviation of each trait were estimated separately for the two parents and their offspring. Distribution normality was evaluated based on a Shapiro–Wilk test (Royston 1995). As many traits did not follow a normal distribution, phenotypic correlations among traits were calculated using the non-parametric Spearman correlation coefficient. For traits with a distribution deviating from normality, several transformations (ln, square root and cubic root) were tested. The least-skewed transformed data were used to extract the best linear unbiased predictors (BLUPs) of genetic values at each date (Robinson 1991). A linear model with a random genotypic effect was fitted: $P_{ij} = \mu + G_i + e_{ij}$, where P_{ij} was the transformed phenotypic value of fruit j of genotype i, μ the overall mean, G_i the random effect of genotype i, and e_{ij} the residual error effect. BLUPs of genotypic values were used for genetic correlation estimation and QTL detection. Variance estimates were used to estimate the broad-sense heritability (H^2) as: $\sigma^2_G/(\sigma^2_G + \sigma^2_e)$.

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Genotyping of the CxM population

Young leaves from each genotype were harvested to genotype the parents and progeny with molecular markers. Total DNA was extracted from leaf tissue using the method described by Doyle and Doyle (1987). Single-sequence repeats (SSRs) and single-nucleotide polymorphisms (SNPs) were used. SSR amplification and detection of amplified DNA fragments were performed according to Luro et al. (2008). The genetic map was constructed with 94 heterozygous SSR markers originated from genomic mandarin DNA library (Ci*****) (Froelicher et al. 2008) or clementine EST library (MEST***) (Luro et al. 2008). SNP markers (CiC****-**) were mined from the clementine BACend Sequence database, and 1536 SNPs were used for an Illumina GoldenGate assay (Terol et al. 2008; Ollitrault et al. 2012b). Some SNP markers from genes involved in the primary and secondary metabolite biosynthesis pathway and in salt tolerance—mined by Sanger sequencing of 44 genotypes representative of *Citrus* and relatives (Garcia-Lor et al. 2012)—were added to the Illumina SNP set (CHI-*-***, LCY2-*-***, TScMI1331, HKT1c800F141, PSY-M-289, PKF-M-186). The SNP and SSR markers used in our study had been previously mapped on the clementine reference genetic map (Ollitrault et al. 2012a).

Genetic linkage maps

Genetic linkage analysis and map construction were performed with Join Map 4 (Van Ooijen 2006), and maps were drawn with Mapchart 2.3 (Voorrips 2002). Framework consensus and parental maps were constructed based on 645 markers (Additional Table 1) and 105 CxM hybrid trees, with "CP" as population type. Segregation distortion for parental and consensus data was assessed with χ^2 tests according to the segregating type of each marker. These markers revealed three segregation patterns: 1:1 for markers segregating only in one parent (ll × 1 m and nn × np), 1:2:1 for markers segregating in male and female parents (hk × hk), and more informative 1:1:1:1 segregation in markers segregating in both parents with three alleles (ef × eg). Grouping was achieved using a minimum LOD score of 4. The regression mapping algorithm (round 2) and Kosambi mapping function were used to establish the map order and distances in centiMorgans (Kosambi 1943; Stam 1993) within each linkage group. The linkage group nomenclature was the same as in the Clementine reference map (Ollitrault et al. 2012a). For subsequent QTL analysis, the number of markers was reduced in very dense map regions by maintaining only one marker for identical genetic positions and removing all other redundant ones with the same or a very close position < 1 cM), resulting in what we called framework maps.

QTL detection

Marker-phenotype associations per trait were tested by interval mapping (IM) and the multiple QTL model (MQM; composite interval mapping equivalent) using the Map QTL version 6 software package (Van Ooijen 2009). This analysis was performed on BLUPs of genotypic values at each date on parental and consensus framework maps. For each trait and map, we determined the IM LOD threshold through 1000 permutations of traits over marker data, for a genome-wide first type error rate of 5%. Thereafter, MQM was performed, using the same threshold level, by selecting markers nearest to the QTLs detected with IM as cofactors. This manual cofactor selection increased the number of identified QTLs. It allowed the detection of several QTLs which could not be detected by IM alone. The non-parametric Kruskal–Wallis rank-sum test was also used to check the MQM results, especially for QTLs detected in large intervals between adjacent markers, with a stringent significance level of 0.005. Confidence intervals of QTL positions were determined as one-LOD support intervals. The QTL results were plotted using MapChart 2.3 software.

Results

Distribution of phenotypic traits

The distribution of raw phenotypic values for fruit attributes in the progeny and parents at the three dates evaluated throughout fruit maturation was described based on the distribution of the number of genotypes by class of raw concentrations in fresh matter (Fig. 1) and by box plots (Additional Fig. 1). The concentration of primary metabolism compounds was also calculated on a dry matter basis and presented according to the distribution of the number of genotypes by class of raw concentrations (Fig. 2) and in box plots (Additional Fig. 2). During maturation, the average fruit mass and equatorial diameter of the progeny reached a maximum average value in December and then levelled off in clementine and CxM offspring, while these two parameters continuously increased in mandarin. The juice percentage increased until December and then decreased considerably in the two parents and the offspring. Otherwise, the acidity pattern was the same for the parents and the CxM offspring. It decreased until reaching low values especially for clementine and the 105 hybrids (0.3–0.6 g/100 g).

Sucrose was the major sugar detected during maturation in the CxM offspring and parents. Its mean concentration was about 3- to sixfold higher than that of glucose and fructose ones, which had equivalent levels. All sugar mean concentrations increased between October and December and then remained relatively constant. Minor differences were

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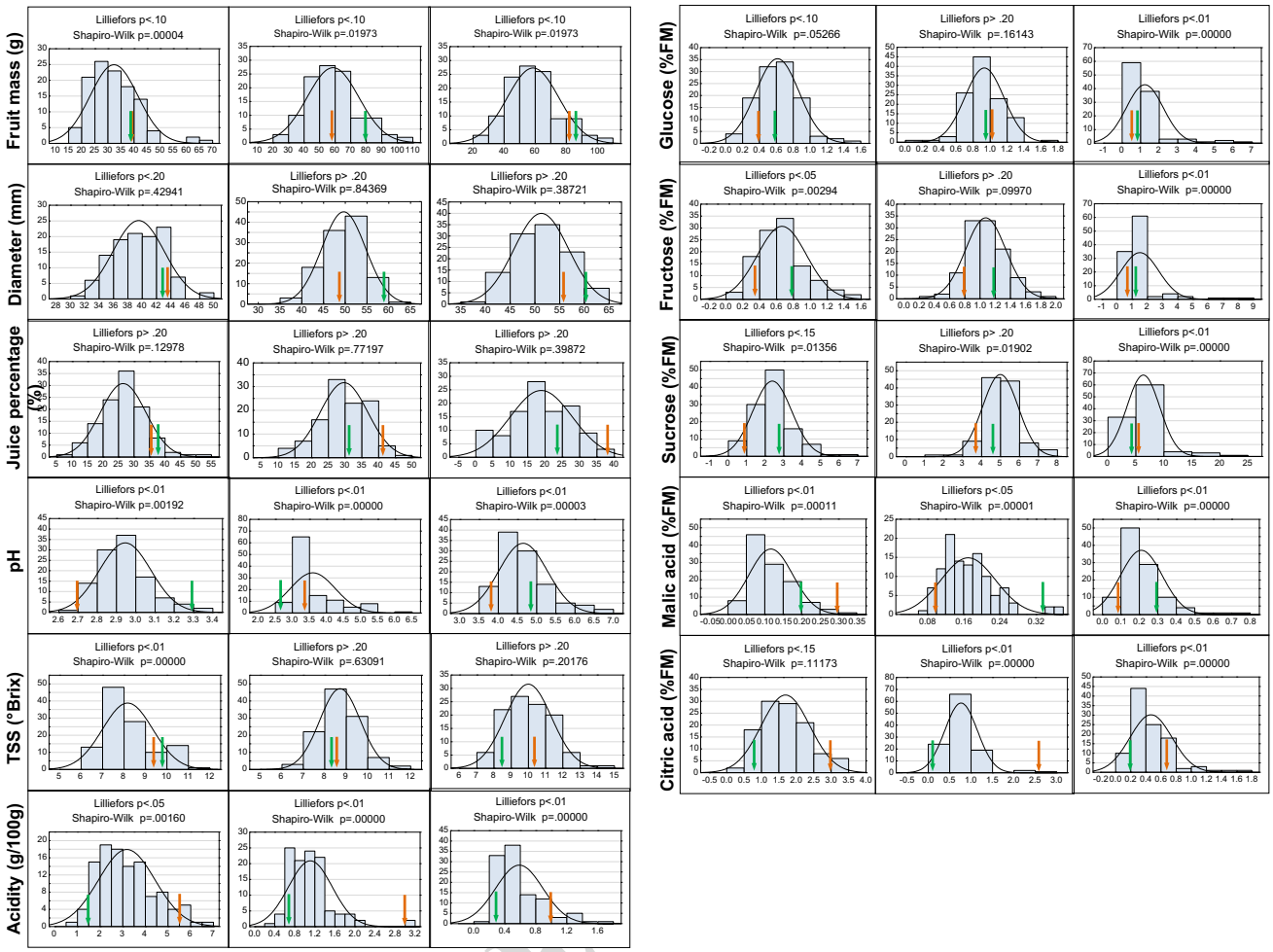

Fig. 1 Distribution of the number of genotypes by class of raw values for fruit attributes in the CxM population measured on 10/08/2012 (first column), 12/03/2012 (second column), and 02/27/2013 (third column). Mean values of the two parents are indicated by arrows: clementine (green) and mandarin (orange). FM, fresh matter

observed between the two parents regarding the glucose content (% of DM and % of FM). For acids, citric acid presented the highest mean concentration, especially in October where it was about 30-fold higher than that of malic acid. During maturation, citric acid decreased while malic acid increased, except in "Willow leaf" mandarin. Despite these variations during fruit maturation, citric acid remained the predominant organic acid. The pattern noted in the progeny was similar to that of clementine. The total acidity and citric acid concentration continuously declined from December until February, especially for mandarin. Nevertheless, malic acid did not show the same variation pattern since its concentration increased slightly until December and then remained constant. During maturation, mandarin fruits were significantly more acidic than clementine fruits, which showed a lower malic acid concentration but a higher citric acid concentration. The mandarin acidity level reached in February was in line with the known maturity period for this citrus fruit (January–February). The acidity of clementine in December reached its low marketing limit under the protected geographical identification label (IGP Clementine de Corse). For other traits such as TSS, differences were minor between clementine and mandarin for the first two dates (October and December) but they increased thereafter.

High variability was observed within the population. Average trait means varied over the three dates. For fruit mass, equatorial diameter, juice percentage, pH, acidity, and TSS, the range of variation over the fruit maturation period was approximately 1.5- to twofold. The variability within the population evolved differently over time, depending on the parameter, while being almost stable for fruit mass, equatorial diameter, juiciness, TSS, and malic acid. On the other hand, it increased for pH and the three soluble sugars, while it decreased for acidity and citric acid. For organic acids and sugars, the range of variation was 3- to 50-fold over the fruit maturation period. However, the variability decreased

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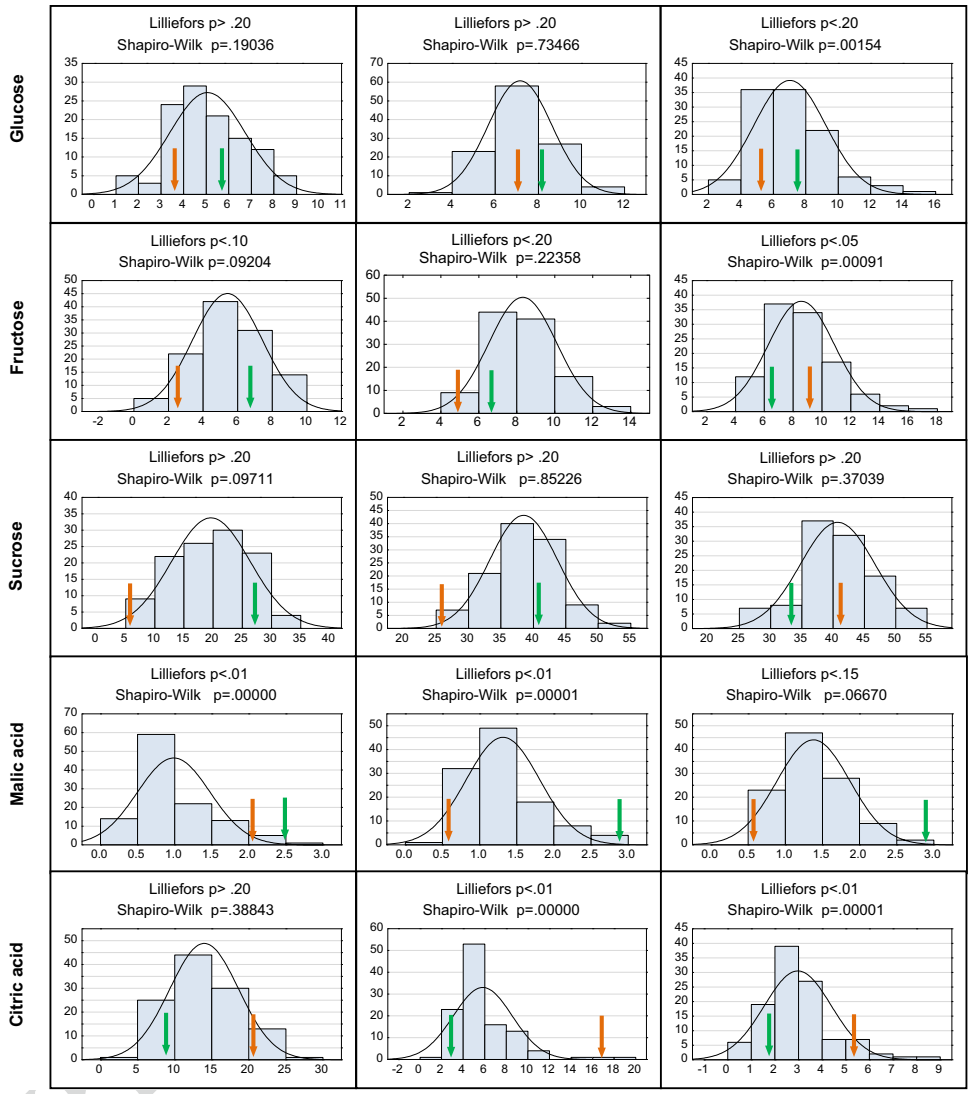

Fig. 2 Distribution of the number of genotypes by class of raw concentrations by 100 g of dry matter (%DM) for sugars and acids in the CxM population measured in 10/08/2012 (first column), 12/03/2012 (second column), and 02/27/2013 (third column). Arrows indicate mean values for clementine (green) and mandarin (orange)

during maturation for citric acid to the same extent in % of DM as in % of FM.

Phenotypes with much higher and/or lower values than the highest and lowest values estimated for the two parents were observed for fruit mass, equatorial diameter, juice percentage, TSS, glucose, fructose, and sucrose. Indeed, the majority of fruit traits segregated in a transgressive manner. For example, in October, fruit mass ranged from 15.7 to 78.5 g in the population, despite the very small difference between parents (39.1–39.4 g). Conversely, acidity and citric acid were distributed essentially within the range of the parental values. Most traits, such as equatorial diameter, juice percentage, and sucrose content (in % of DM), presented a normal distribution. However, some traits such as acidity and pH deviated from normality. Therefore, appropriate transformations (ln or square root) were applied to unskew their distributions (Tables 1 and 2). The continuous variation pattern indicates that the studied traits were controlled by several genes, so they were classified as quantitatively inherited.

Genetic correlation and heritability

Correlation coefficients calculated between BLUPs of the genetic values are detailed in Fig. 3. Several traits appeared to be jointly correlated and some correlations between traits varied during maturation. As expected, fruit mass and equatorial diameter—highly correlated with each other—were also correlated with most of the studied traits measured in October, such as juice percentage, fructose, and sucrose. Fruit mass and equatorial diameter were negatively correlated with acidity and citric acid throughout maturation. Among sugars, the strongest positive correlations were observed, throughout maturation, between glucose and fructose, i.e., ranging from 0.50 to 0.98 depending on the date. Sucrose and TSS were jointly positively correlated in

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Table 1 Transformations of raw phenotypic values of fruit attributes to unskew distributions, and broad sense heritability

Fruit traits	Sampling dates	Transformation	Heritability (H^2)
Fruit mass (g)	October	ln	0.81
	December	-	0.81
	February	ln	0.8
Equatorial diameter (mm)	October	ln	0.79
	December	-	0.8
	February	ln	0.73
Juice percentage	October	-	0.82
	December	-	0.75
	February	-	0.8
pH	October	-	0.67
	December	ln	0.94
	February	-	0.89
Acidity (g/100 g)	October	ln	0.81
	December	-	0.65
	February	ln	0.75
TSS	October	Square root	0.82
	December	ln	0.71
	February	Square root	0.61

ln neperian logarithm

Table 2 Transformations of raw phenotypic values of organic acids and sugars to unskew distributions, and broad sense heritability

Fruit trait	in	Sampling dates	Transformation	Heritability (H^2)	in	Sampling dates	Transformation	Heritability (H^2)
Citric acid	FM	October	Square root	0.78	DM	October	Square root	0.78
		December	ln	0.74		December	ln	0.73
		February	ln	0.82		February	ln	0.83
Malic acid	FM	October	ln	0.79	DM	October	ln	0.79
		December	ln	0.73		December	ln	0.81
		February	ln	0.85		February	Square root	0.77
Glucose	FM	October	Square root	0.67	DM	October	Square root	0.64
		December	-	0.56		December	-	0.6
		February	ln	0.82		February	Square root	0.75
Fructose	FM	October	Square root	0.7	DM	October	-	0.68
		December	-	0.59		December	-	0.63
		February	Square root	0.72		February	Square root	0.73
Sucrose	FM	October	Square root	0.74	DM	October	-	0.71
		December	-	0.54		December	-	0.5
		February	Square root	0.62		February	-	0.61

ln neperian logarithm, *FM* in % of fresh matter, *DM* dry matter

December and February. Both acidity and pH were correlated with citric and malic acid contents.

For all traits, broad-sense heritability (H^2) values (repeatability among the 10 fruit replicates) were quite high (> 0.5) (Tables 1 and 2). They ranged from 0.64 to 0.82, 0.50 to 0.94, and 0.61 to 0.89 for traits measured in October, December, and February, respectively. In October, fruit mass, acidity and TSS showed the highest heritability (> 0.8). In December, the highest heritability values (> 0.8) were observed for fruit mass, equatorial diameter, pH, and malic acid. However, in February, the traits showing > 0.8 heritability were fruit mass, juice percentage, pH, citric acid FM and DM, malic acid FM, and glucose FM (Tables 1 and 2).

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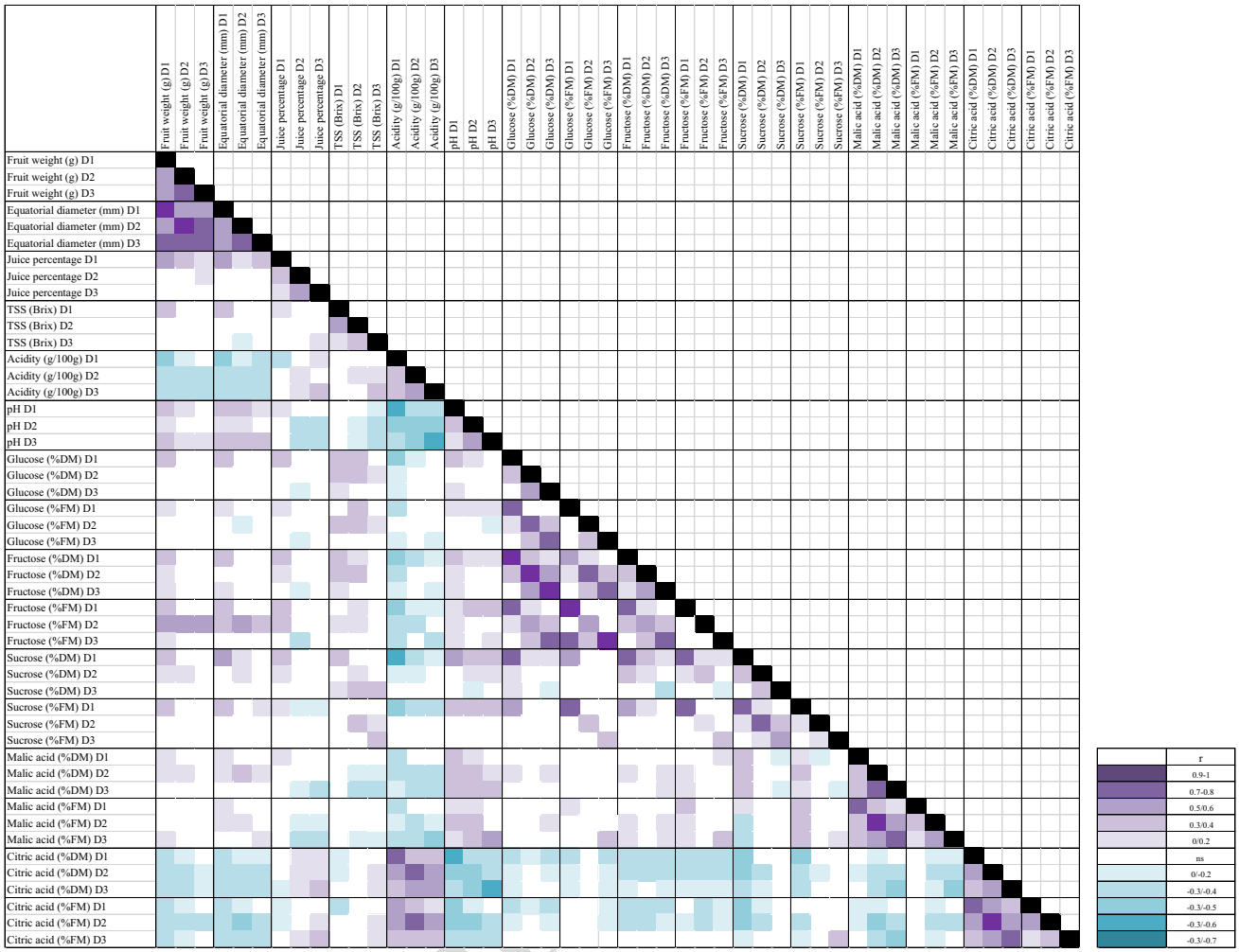

Fig. 3 Genotypic correlations (Spearman) between fruit characteristics measured during maturation on 10/08/2012 (D1), 12/03/2012 (D2), and 02/27/2013 (D3), based on genotypic BLUPs

Genetic linkage maps

A total of 551 SNP and 94 SSR markers were genotyped in clementine × mandarin offspring. Out of these, 622, 618, and 275 markers were selected to construct the consensus, clementine, and mandarin maps, respectively (Additional Table 1). Among these markers, 333 segregated at 1:2:1, 268 segregated at 1:1, and 21 segregated at 1:1:1:1. The consensus map and the previously published clementine reference map (Ollitrault et al. 2012a) shared 551 common markers. A comparative analysis of these two maps showed high synteny and colinearity, with very few inversions and distance differences (Additional Fig. 3). However, LG3 on the reference clementine genetic maps was split into two sub-linkage groups on the consensus genetic map as well as on our clementine genetic map (Additional Table 1). There is little resolution on the position of the markers due to the small size of the population, which results in many co-locations of markers. The multiplicity of markers at the same locus is non-informative and lengthens the computer processing when detecting QTLS. To facilitate QTL analysis, the map density was reduced by removing markers with several missing data and with the same or very close positions (< 0.1 cM), but without modifying the map coverage. These reduced density maps are hereafter called framework maps. The final numbers of markers retained for the framework consensus, clementine, and mandarin maps were 310, 277, and 147, respectively. SSR and SNP markers were grouped in 10 linkage groups on the consensus and clementine maps and in 12 linkage groups on the mandarin map. There was a greater number of chromosomes divided into several linkage groups on the mandarin map due to the lower number of markers: three referenced linkage groups were represented by two linkage groups each. The consensus, clementine, and mandarin maps, respectively, covered 795.7 cM, 809.8 cM,

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and 629.7 cM, which corresponded to 74%, 75%, and 58% of the genome, respectively, compared to the clementine genetic reference map (Ollitrault et al. 2012a). Linkage groups had a mean distance of 2.6, 3.3, and 4.7 cM between adjacent markers on the consensus, clementine, and mandarin maps, respectively. Some linkage groups had large marker intervals. Linkage groups 6 and 7 had intervals ranging from 16 to 24 cM on the three maps. Moreover, linkage group 2 on the mandarin map had one 18 cM interval. Another large interval was observed at the extremity of linkage group 8 on the clementine map. Common markers within the consensus and parental maps allowed a between-map comparison of their marker orders. Except for minor changes, strong collinearity was observed especially between the clementine and consensus maps.

QTL identification

QTL analysis overview: QTL detection was performed using a model with both additive and dominant effects and genotypic BLUPs at each date on the consensus (Con) and both parental (C and M) framework maps. The LOD score of significant QTLs ranged from 3.6 to 8.3. We only retained QTLs detected by MQM and confirmed by a Kruskal–Wallis test. A total of 28 QTLs were identified on the consensus map for all traits except glucose FM and citric acid FM during maturation (Table 3), with 1–3 QTLs per trait and date. Nine QTLs were detected in October, 10 in December, and 9 in February. QTLs were found on all LGs, except LG6 and LG1. The proportion of the total variation (R^2) ranged from 13.1 to 34.1%. Sixteen QTLs showed an R^2 ranging from 10 to 20%, 9 QTLs from 20 to 30%, while 2 QTLs had an R^2 of

Table 3 List of QTLs detected on the consensus map

Traits	Dates	LG	Max LOD peak	LOD GW	Nearest marker	Map position (cM)	Confidence interval (cM)	R^2 (%)	Kruskal–Wallis analysis
Fruit mass (g)	October	2	5.1	4.1	CiC5209-05	118.9	116.9–120.8	21.2	*******
		4	4.6	4.1	CiC0279-03	1.0	0.0–7.3	15.11	****
		5	4.1	4.1	CiC1891-02	0.0	0.0–5.9	13.8	*******
	December	8	5.2	4.1	CiC0598-01	10.8	8.6–13.7	18.7	*****
	February	2	5.3	4.2	PKF-M-186	118.9	116.9–120.8	16.8	*******
		3	5.3	4.2	CiC5796-12	85.4	83.6–86.4	13.1	****
		8	6.9	4.2	CiC0100-04	44.7	41.0–48.7	22.7	******
Equatorial diameter (mm)	October	2	4.5	4.2	PKF-M-186	118.9	116.9–120.8	18.9	*******
		4	4.7	4.2	CHI-M-170	0.0	0.0–3.6	15.9	****
	February	2	4.4	4.2	PKF-M-186	119.3	116.9–120.8	13.7	******
		8	7.6	4.2	CiC0100-04	45.7	41.8–48.7	25.1	*******
Juice percentage	December	9	6.8	4.2	MEST1201	52.7	46.7–56.3	27.3	******
pH	October	2	3.7	3.5	CiC3457-01	124.8	122.3–124.8	16.2	*****
	December	7	6.5	4.4	CiC5979-03	0.0	0.0–2.0	26.1	****
TSS (°Brix)	February	8	4.3	4.3	LCY2-P-243	49.8	48.7–50.1	19.8	******
Acidity (g/100 g)	October	2	4.0	4	CiC3457-01	124.8	119.3–124.8	17.5	*******
Malic acid DM	October	8	5.9	4.2	CiC0598-01	11.8	8.6–13.7	24	*******
	December	8	4.4	4.2	MEST086	16.7	13.3–18.9	18.9	****
Citric acid DM	October	2	5.9	4.2	PKF-M-186	118.9	116.9–120.8	24.2	*******
Malic acid FM	December	4	4.7	3.5	CiC5078-07	4.6	0.0–9.0	20.1	****
		9	3.6	3.5	CiC2768-01	69.0	64.1–86.6	15.5	******
Glucose DM	February	9	8.1	4.3	MEST149	58.2	56.6–60.0	33.2	*******
Fructose DM	December	2	4.7	4.2	CiC6122-04	108.4	99.5–111.2	27.3	******
		3	4.6	4.2	CiC3742-04	4.6	3.3–7.6	14.8	****
	February	9	8.3	4.3	MEST149	57.6	56.6–59.2	34.1	*******
Sucrose DM	February	9	5.4	4.3	CiC5567-01	66.2	65.2–68.5	24.0	*******
Fructose FM	December	9	4.2	4.2	MEST149	57.6	56.6–59.2	17.8	******
Sucrose FM	December 2012	5	4.2	4.2	CiC3536-01	65.7	64.6–67.7	17.9	****

DM dry matter, *FM* fresh matter, *LG* linkage group, *GW* genome wide, R^2% total variance explained/ Significance levels
****0.005; *****0.001; ******0.0005; *******0.0001

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more than 30%. Co-localization between QTLs for different traits was observed at several locations (Fig. 4). The majority of QTLs detected on the consensus map were also detected on the clementine map (Figs. 4 and 5; Table 3; Additional Table 2). Fewer QTLs were detected on the mandarin map than on the consensus and clementine maps (Figs. 5 and 6). Six additional QTLs were detected on the two parental maps but not on the consensus map (Figs. 4, 5, and 6).

Fruit mass and equatorial diameter: For fruit mass, the greatest number of QTLs was found in February on LG2 (Con and C maps), LG3 (Con and C maps), and LG8 (bottom part, on the 3 maps) (Figs. 4, 5, and 6). The QTL on LG2 was also present in October. On the upper part of LG8, an additional QTL for fresh mass was detected in December on the 3 maps. In October, 2 QTLs were found on LG4 and 5 on the consensus map and on LG5 on the clementine map. The percentage of total variance explained by each of these fruit mass QTLs ranged from 13.1 to 26.7%. QTLs for equatorial diameter colocalized with QTLs for fresh mass at the same dates on LG 2, 4, and 8 (bottom part). Diameter QTLs explained from 13.7 to 29.5% of total variance.

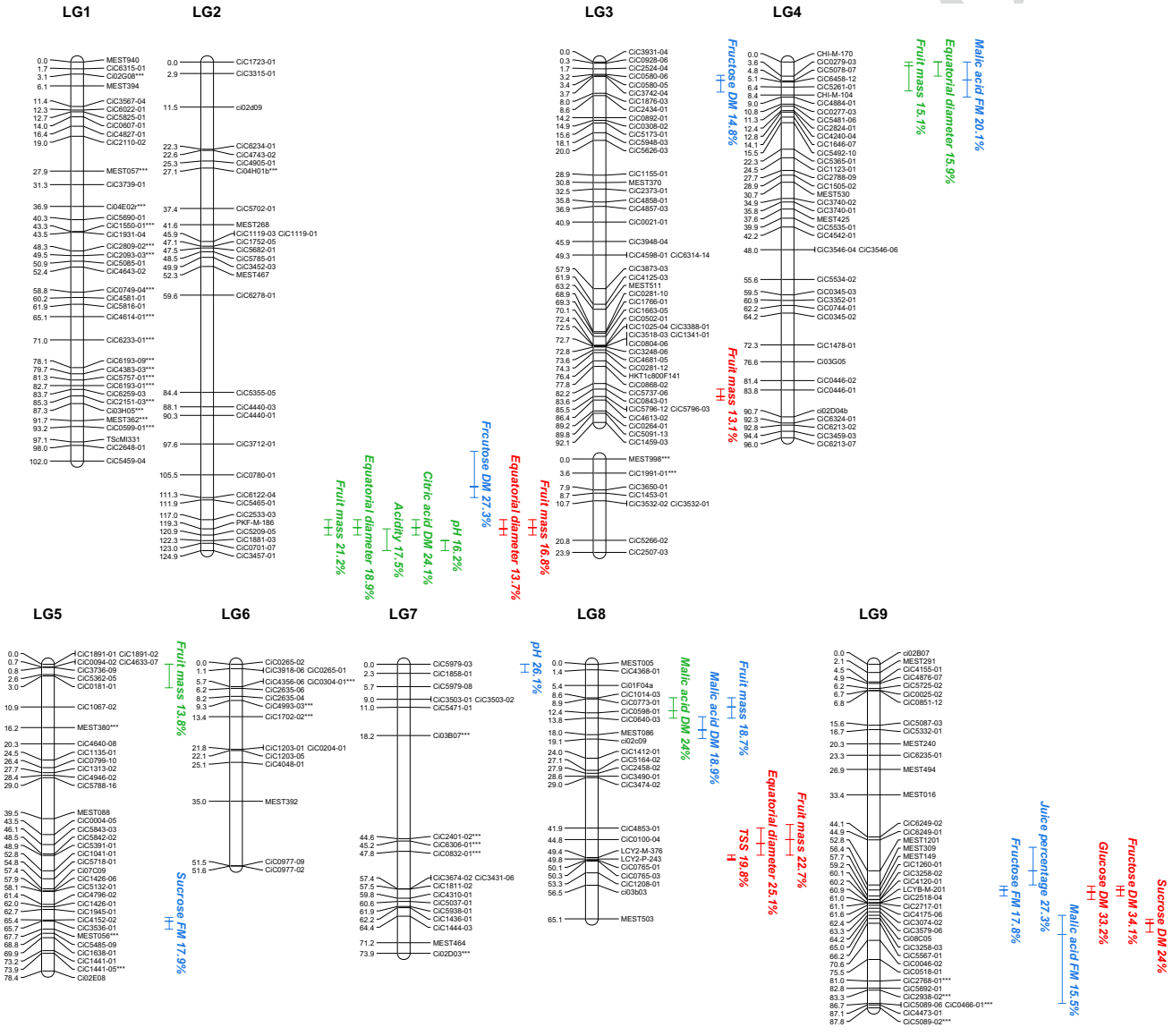

Fig. 4 QTL location on consensus genetic map for fruit attributes analyzed during three maturation dates and determined by interval mapping and multiple QTL model. Linkage groups are labelled as LG1–LG9. QTLs are listed on the right of each linkage group. Distances are in cM (Kosambi's function). Vertical lines represent 1-LOD confidence intervals, and horizontal ticks indicate the positions of the LOD peaks. For each confidence interval, the trait is followed by the percentage of total variance explained by the QTL. QTLs detected in October, December, and February are drawn in green, blue and red, respectively

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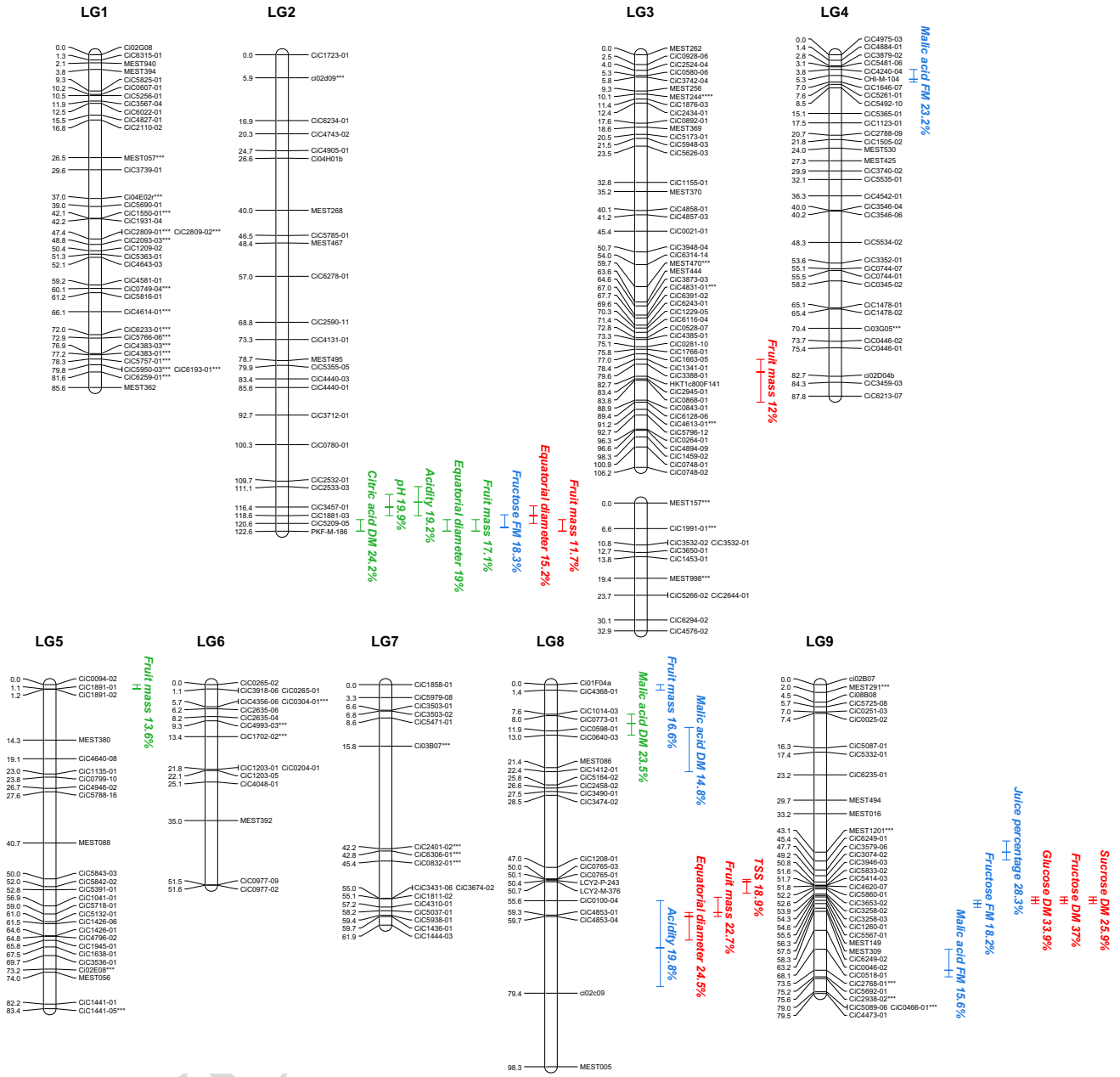

Fig. 5 QTL location on clementine genetic map for fruit attributes analyzed during three maturation dates and determined by interval mapping and multiple QTL model. Linkage groups are labelled as LG1–LG9. QTLs are listed on the right of each linkage group. Distances are in cM (Kosambi's function). Vertical lines represent 1-LOD confidence intervals, and horizontal ticks indicate the positions of the LOD peaks. For each confidence interval, the trait is followed by the percentage of total variance explained by the QTL. QTLs detected in October, December, and February are drawn in green, blue and red, respectively

Juiciness: Only one QTL, located on LG9, was associated with the juice percentage in December on the consensus and clementine maps, and explained 27.3 and 28.3%, respectively, of the total variation (Figs. 4 and 5).

TSS: On the consensus and clementine maps, one QTL explaining 19.8 and 18.9%, respectively, of the total variation was identified on LG8 in February (Figs. 4 and 5). On the mandarin map, no QTL for TSS was detected regardless of the maturation date (Fig. 6).

pH and acidity: In October, one QTL for pH, explaining 16.2 and 19.9% of the variance, was detected on LG2 on the consensus and clementine maps (Figs. 4 and 5). A second QTL accounting for 26.1% of the total variance was located on LG7 on the consensus map in December only. For acidity,

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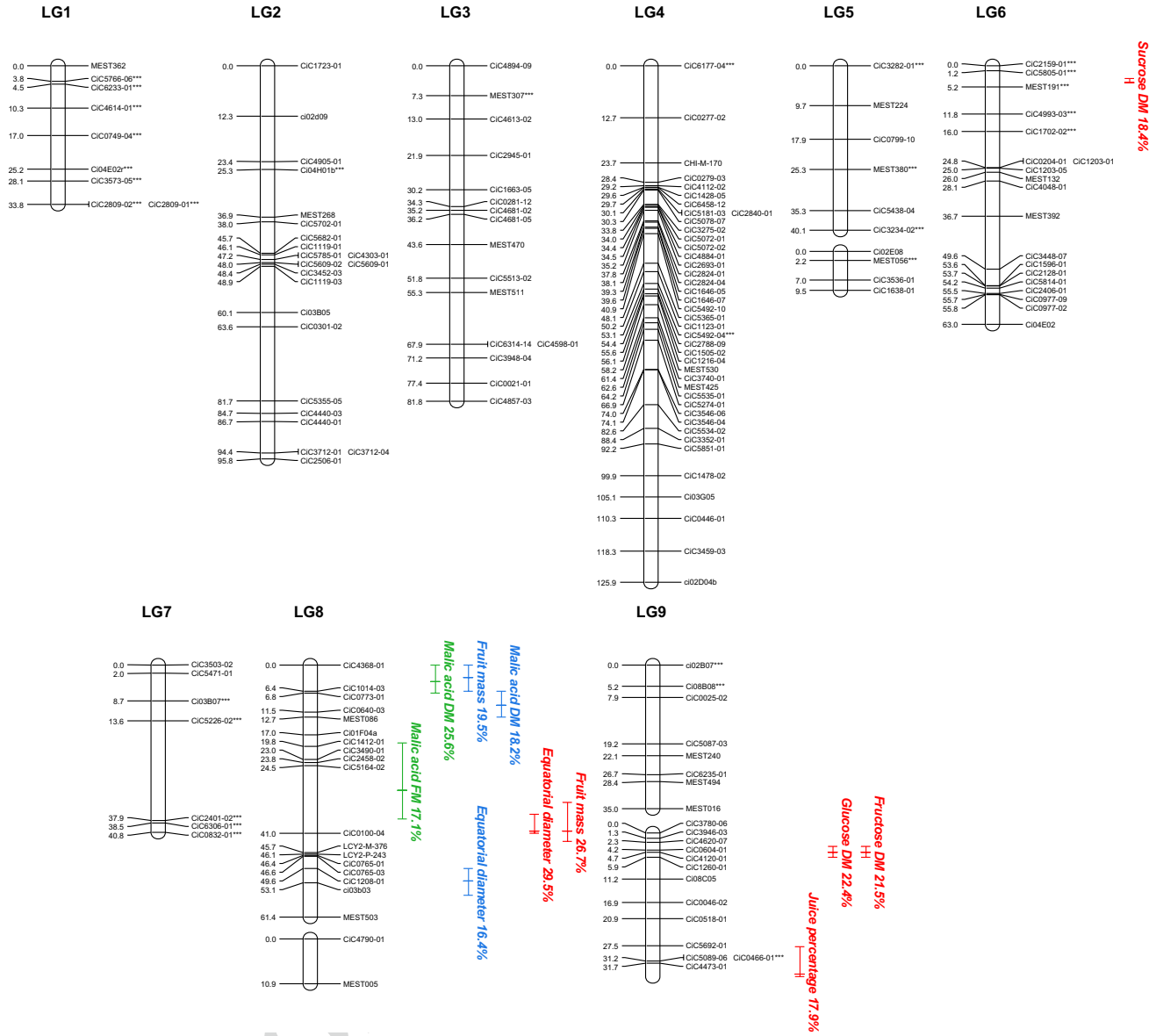

Fig. 6 QTL location on mandarin genetic map for fruit attributes analyzed during three maturation dates and determined by interval mapping and multiple QTL model. Linkage groups are labelled as LG1–LG9. Names of the markers and QTLs are listed on the right of each linkage group. Distances in cM (Kosombi's function) are in the left of each linkage group. With respect to QTL localization, the highest probability is indicated by vertical line within the 1-LOD confidence intervals situated between two vertical lines. For each confidence interval, the trait is followed by the percentage of total variance explained by the QTL. QTLs detected in October, December, and February are drawn in green, blue, and red, respectively

one QTL was collocated with the pH QTL on LG2 on the consensus and clementine maps in October. A single acidity QTL was found in December on LG8 for clementine only.

Sugars: In February, QTLs associated with glucose, fructose, and sucrose expressed in DM were mapped in the same linkage group (LG9) on the consensus and clementine maps. The QTL for fructose DM overlapped that for glucose DM. These QTLs showed the greatest effects in this study, contributing more than 30% to the total variance (Additional Table 2). Other QTLs were detected on the consensus map for sucrose FM on LG5 and fructose DM on LGs 2 and 3 in December, yet only the LG2 QTL was also found on the clementine map. On the mandarin map, while fructose DM and glucose DM QTLs colocalized on LG9 in February, one QTL for sucrose DM was detected on LG6 at the same date (Fig. 6). Two QTLs for fructose DM were found on LG2 and LG3 on the consensus map in December, which accounted for 27.3 and 14.8% of the total variance, respectively. QTLs controlling fructose FM and sucrose FM in December were found on LG9 and LG5 on the consensus maps, respectively,

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while only the LG9 QTL was also found on the clementine map. About 18% of the variance was explained on the three maps (Table 3; Additional Table 2).

Organic acids: One QTL for citric acid DM was identified in October on LG2 on the consensus and clementine maps. This QTL overlapped the single QTL for acidity and explained 24.2% of the total variance. Two QTLs were detected for malic acid DM on the consensus and both parental maps, i.e., one in October and the other in December. These two QTLs overlapped on LG8. For malic acid expressed in FM, two QTLs were located on LG 4 (20.1 and 23.2%) and LG9 (15.5 and 15.6%) on the consensus and clementine maps in December (Table 3; Additional Table 2).

Discussion

QTL detection accuracy and power

The number of markers is insufficient to saturate the genetic map and to have a large coverage of the genome, but the low number of markers does not decrease the power of detection because the LD is extended in a bi-parental progeny; it only decreases the precision of localization of the QTL in case of too big gap.Charmet carried out simulations to evaluate the effect of marker density on QTL detection for one-QTL models. Detection power and length of confidence intervals of both QTL location and QTL effect were not affected by marker density between 5 and 20 cM for a population size of $N = 200$ (Charmet 2000). The number of markers is low because we sorted out the markers that were very close to each other (at the same locus) and therefore were not useful for the localization of QTLs since the locus was tagged by another marker. Moreover, for mandarin, the low number of markers is explained by the fact that this citrus variety is weakly heterozygous and so could explain some large gaps on maps, and thus, it was difficult to obtain markers that were both heterozygous for mandarin and homozygous for clementine, because this cross is a backcross. Nevertheless, we detected and localized QTLs for fruit quality traits with strong effect and a larger population of hybrids would be more profitable to detect more QTLs with smaller effects.

We used MQM to optimize QTL detection, especially in the case of two linked QTLs, since it increases the QTL detection power and the QTL position estimation precision, while allowing us to map additional QTLs located on the same chromosome (Paterson 1997). Twenty-eight QTLs of quality attributes in citrus were found on the consensus map, most of which (24) had a relatively marked effect ($R^2 > 15\%$). Both parental and consensus maps were used to yield complementary results. Indeed, QTLs with dominant allelic effects like fructose DM identified on chromosomes 2 and 3 and pH mapped on chromosome 7 in December were detected only on the consensus map. Conversely, the detection accuracy could be higher in parental maps for QTLs with additive effects only, as shown by the 6 additional QTLs identified only on the parental maps and not on the consensus map. Overall, 34 QTLs were identified throughout the maturation period.

The effectiveness of molecular markers associated with detected QTLs should be determined as the percentage of the explained genetic variance, instead of the phenotypic variance, because fluctuations in phenotypic values as a result of environmental variations blurs the marker effects (Nishio et al. 2011). The use of BLUP values thus improved the QTL detection power by removing part of the environmental variance per tree since the variance between fruits might be at slightly different stages of maturity at harvest time. For instance, this variability in fruit maturity was studied for clementine and used to determine the "harvestability window" (Julhia et al. 2019). Indeed, several studies have demonstrated the effectiveness of the BLUP method for species such as apple (Segura et al. 2009) or grapevine (Doligez et al. 2013) where the experimental material had been phenotyped over several years or under different growing conditions.

QTL detection

In our case, all traits had high heritability ($H^2 > 0.56$) during maturation, which increased our chances of detecting QTLs. However, the high heritability was also due to the absence of annual variation and the low variance between fruits. A main limitation of this experimentation plot was related to the number of individuals present in the population. With 105 individuals, it could be assumed that the detected QTLs were those with marked and possibly significant effects (Staub et al. 1996; Beavis 1998). The percentage of total genotypic variation explained by QTLs detected throughout maturation ranged from 11.7 to 37%. Fruit mass, equatorial diameter, malic acid FM, and fructose DM were controlled by more than one QTL. The presence of several QTLs showed the complexity of the metabolic pathways. QTLs of fruit mass detected during maturation did not explain 100% of the total variance, which suggests the presence of other undetectable QTLs and/or epistatic effects that could explain the remaining percentage of total variation. To our knowledge, this is the first report of QTL mapping of fruit attributes in citrus at three sampling dates during maturation. This QTL study showed that the traits were probably not controlled by the same QTLs during maturation. In our case, the use of more complete maps and a high number of measurements (replicates) provided more accurate QTL detection results. Indeed, QTL detection is known to be affected by environmental conditions, which represent a major source of variability (Rousseaux et al. 2005; Kenis et al. 2008).

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Trait correlations and QTL co-location

Several traits were clustered mainly on linkage groups 2, 4, 8, and 9 irrespective of the evaluation dates. These clusters may have reflected a pleiotropic effect of one QTL or tight linkage between at least two QTLs. A QTL with a pleiotropic effect indicates the segregation of a single QTL controlling several traits due to related metabolisms or causal relationships between traits (De Vienne and Causse 1998). Several QTL clusters for fruit maturation and agronomic traits were detected in previous studies in many species, including tomato (Monforte et al. 1999), peach (Etienne et al. 2002), apple (Liebhard et al. 2003), and citrus (Sugiyama et al. 2011). Common or close QTL locations have often been observed for correlated attributes (Paterson et al. 1991). These correlations could suggest candidate regions for future studies to gain further insight into these traits. In this study, QTL co-locations were observed for the majority of the studied fruit traits, including fruit mass, equatorial diameter, pH, acidity, sugar, and acid contents. QTL clusters varied during maturation. Indeed, some of them were stable throughout maturation, while others were identified for only one or two maturation dates. Fruit quality traits vary with the degree of maturation in citrus. Therefore, our QTL analysis results might also have been affected by fruit maturity heterogeneity (Ladanyia 2008). This lack of stability of fruit quality QTLs during maturation thus suggests that some fruit traits are not governed by the same locus during maturation.

Fruit mass and size: In our study, fruit mass QTLs were detected on LGs 2, 3, 4, 5, and 8. Some of them had been detected in other studies. For instance, the QTL for fruit mass located on LG4 may have corresponded to the previously reported FW4.2 QTL (Yu et al. 2016) and FWq3 QTL (Imai et al. 2017). Besides, the fruit mass QTL detected on LG3 may have corresponded to FWq1 detected in 2013 and FWq2 detected in 2013 and 2014 (Imai et al. 2017). Moreover, another fruit mass QTL was mapped in the same region of LG8 in two QTL mapping studies, confirming that this is a single major QTL (Yu et al. 2016; Minamikawa et al. 2017). On another hand, Imai et al. (2018) found four fruit mass QTLs situated on LGs 2, 3, 5, and 7. However, although three of them were detected on the same LG in our study, they were not mapped in the same region (Imai et al., 2018). This comparison with the findings of the four mentioned studies was possible because the QTLs were positioned on the same scaffolds 2, 3, 4, 5, and 8 on the clementine reference map, which presented high synteny with our consensus linkage map.

Ting and Attaway (1971) reported that fruit mass and equatorial diameter variation patterns are interrelated during fruit development and maturation, thus highlighting that it was normal to find overlapping QTLs between these two traits: one QTL co-location on different chromosomes at two maturation dates on the consensus map, i.e., 2 and 4 in October and 2 and 8 in February. This correlation between fruit mass and fruit diameter was also found in mandarin (Yu et al. 2016). Moreover, common genomic regions may control both fruit mass and size in *C. volkameriana* × *P. trifoliata* (Garcia et al. 2000) and *C. clementina* hybrid × *C. grandis* hybrid (Asins et al. 2015) populations. The involvement of several regions of different chromosomes in the control of these two traits revealed their complex genetic determinism, as already noted in other species such as apple (Kenis et al. 2008) and tomato (Grandillo et al. 1999).

Acidity and fruit mass: QTLs of titratable acidity and fruit mass measured in October were mapped at the end of LG2 on the consensus map, and negative correlations were obtained between acidity and fruit mass. In citrus, the first fruit development stage is characterized by slow fruit growth rates but high cell division (Bain 1958), whereas phase II constitutes a fast growth period where fruit increases in size mostly by cell enlargement and water accumulation (Iglesias et al. 2007; Tadeo et al. 2008). QTLs for fruit mass could thus be associated mainly with the cell expansion process, as shown in tomato (Bertin et al. 2007). Cell growth and enlargement depend on water and carbon compound accumulation (Yakushiji et al. 1996). Admittedly, citrus fruit act as carbohydrate storage sinks during the cell enlargement stage and thereafter (Mehouachi et al. 1995; Cercós et al. 2006). Fruit accumulates a considerable amount of organic acids in juice sac cell vacuoles (Etienne et al. 2013a, b). The high acid concentration could result in enhanced sink strength, thus facilitating carbohydrate accumulation (Hockema and Etxeberria 2001) and increased fruit size (Agustí et al. 2002). Nevertheless, October, i.e., the period when our research began, corresponds to phase III under Mediterranean conditions (Jacquemond and Agostini 2013). During this stage, accumulated organic acids are progressively catabolized, thereby implying acid reduction. Meanwhile, fruits continue to increase in size. If fruit mass is positively linked to acidity during the first half of stage II and negatively from the second half of stage II and during stage III, this may be explained by the high carbohydrate supplies on acidity. In fact, according to Etienne et al. (2013a, b), abundant carbohydrate supplies result in higher fruit mass and increased respiration. Yet, at this physiological stage, sugars stored in the vacuole may no longer be available as a respiratory substrate. Consequently, organic acids sustain respiration, thus leading to a decline in acidity. Antoine et al. (2016) demonstrated that smaller and more acidic fruits were the result of carbohydrate depletion (through water stress or modification of leaf/fruit ratio), which could confirm and explain the negative correlation between acidity and fruit mass in our study.

Furthermore, Nishawy et al. (2015) reported that citrus fruit size was inversely associated with the organic acid

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level. In fact, they demonstrated that overexpression of the dehydration responsive element binding gene (*CgDREB*) led to a dramatic decrease in citrus fruit mass, in turn prompting higher organic acid accumulation. This gene is located on scaffold 1 of the clementine reference genome (between bases 24 667 693 and 24 668 568) (Wu et al. 2014). In the present study, no QTLs were detected in this scaffold, so this gene did not co-localize with the fruit mass and acidity QTL that was positioned on linkage group 2, corresponding to scaffold 2 of the reference genome. Sadka et al. (2000) previously showed that the citrus fruit organic acid content was affected by fruit size. Taken together, these results confirmed and explained the negative correlation between acidity and fruit mass.

The negative correlation between acidity and fruit mass and the colocation of their QTLs had also been observed in mandarin (Yu et al. 2016). Selection in regions containing QTLs for several traits should therefore be carefully considered to avoid raising conflicts between breeding objectives. Fine mapping in these regions with larger populations could help distinguish between real pleiotropy and very close linkage.

Acidity and pH: The fact that a single QTL was found to control both pH and titratable acidity was an expected result since titration highlights the quantity of all acid functions and ions while the pH measures only the hydrogen potential, thereby indicating that these two features were correlated. This QTL is also involved in the control of citric acid, which is the major organic acid in citrus juice (Monselise 1986; Iglesias et al. 2007; Zhou et al. 2018). A gene encoding pH, i.e., a pH-like gene (*CrMYB73*), whose expression was found to be positively correlated with citric acid accumulation, was isolated from citrus fruit (Li et al. 2015). This gene is located in scaffold 2 of the reference genome like the mapped acidity QTL, but at the opposite end. Li et al. (2015) identified a citrus transcription factor (CitERF13), regulating citric acid accumulation in citrus fruit cells. The location of the CitERF13 corresponding gene in scaffold 1 did not match the position of the acidity QTL on the map in this study.

Acidity and organic acids: In the current study, acidity and citric acid shared a common QTL on the consensus map. In addition to their high correlation, the malate concentration range was much lower than that of citric acid. This suggests that citric acid markedly contributes to the acidity. Indeed, citrus fruit acidity is primarily determined by the citric acid concentration, representing 80–90% of total organic acids (Baldwin 1993). Note that while only one QTL was found for citric acid and acidity, malic acid seemed to be controlled by a much more complex biochemical determinism, since five QTLs located in different linkage groups during maturation were identified. This confirmed the complexity of malate accumulation in fruit cells, with a large number of metabolic pathways involved. Malic acid is either converted via oxaloacetate (OAA) from phosphoenolpyruvic (PEP) in the cytosol or produced through the cycle in mitochondria (Etienne et al. 2013a, b).

Fruit size, organic acids, and sugars: PKF-M-186 was the SNP marker with the highest LOD score for fruit mass, diameter, and citric acid DM located on LG2. The PKF-M-186 marker is located in a gene coding for phosphofructokinase, i.e., an enzyme involved in sugar and acid pathways (Echeverria and Valich, 1989). Organic acids are derived from sugars (Hussain et al. 2017). Furthermore, low pH, the main determinant of malate and citrate accumulation, increases sucrose hydrolysis into fructose and glucose (Etienne et al. 2013a, b). Cleaving sucrose enables the sink to amplify the existing sugar gradient between the sink and phloem, thereby allowing continued sucrose movement toward the sink cell (Hockema and Etxeberria 2001) and resulting in increased fruit size (Agustí et al. 2002). Moreover, Lin et al. (2015) demonstrated that the fructokinase gene was upregulated during maturation, indicating that the sucrose metabolism to organic acid metabolism flux change was enhanced. This highlights the close link between sugar and acid pathways. We hence think that the phosphofructokinase gene including the PKF-M-186 SNP marker is a candidate for controlling citric acid and fruit size variations.

On the other hand, our results showed that QTLs for acidity did not co-localize with sugar QTLs. Note that the same observation was reported in studies on tomato (Causse et al. 2001), mandarin varieties (Goldenberg et al. 2014), and a population derived from a cross between "Murcott" tangor and "Pera" sweet orange, where sugar and acidity QTLs were mapped in two different linkage groups (Curtolo et al. 2017). However, Asins et al. (2015) showed a colocation between QTLs of acidity and sugars on genetic maps developed from a mandarin × pummelo progeny. This discrepancy could be due to differences in parental genotypes assessed in the studies. The different locations of QTLs for sweetness and acidity suggest that it could be possible to improve both traits independently.

TSS and fruit mass: For sugar content (TSS), one QTL was mapped on LG8 at 49.8 cM with an $R^2 = 19.8\%$. This QTL may correspond to that previously detected on linkage group 8 in a bi-parental QTL mapping study that used a mandarin F1 population derived from "Fortune" × "Murcott" (Yu et al. 2016). On linkage group 8, the QTL for TSS was very near to but did not overlap a QTL for fruit mass in February, whereas these traits were not significantly correlated. In agreement with our results, these two traits were also reportedly independent in mandarin (Imai et al. 2017). However, in tomato, a clear co-localization between QTLs of fruit mass and TSS was shown, suggesting pleiotropy (Goldman et al. 1995; Saliba-Colombani et al. 2001). Our results suggest that TSS and fruit mass could be independently modified in citrus.

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FM vs DM: It is noteworthy that in the current study, QTLs for sugars and acids expressed in DM and QTLs for sugars and acids expressed in FM did not co-localize despite the high correlation between these traits. Interestingly, QTLs detected for sugars and acids expressed in DM were twice as numerous as QTLs expressed in FM. This difference was also found in a study by Prudent et al. (2009) in tomato. These latter authors detected 11 QTLs for sugar DM vs 6 QTLs for sugar FM with only two overlapping QTLs. This confirms the fact that traits expressed in fresh and dry matter are not always correlated (Woodward and Clearwater 2008). The reason for this difference in QTL detection could be explained by the fact that fresh matter depends on the water content (Bolarin et al. 2001), which is largely influenced by environmental factors such as the soil water status and climatic factors (Jones and Tardieu 1998). Moreover, environmental factors represent a major source of variability that affects QTL detection (Rousseaux et al. 2005; Kenis et al. 2008).

The very high correlation between fructose and glucose DM ($r = 0.95$) was consistent with the co-location of QTLs for those traits on linkage group 9 in February, with both QTLs presenting major effects ($R^2 > 30\%$). In grape berries, one QTL was detected for these two hexoses, which were highly correlated (Chen et al. 2015). These QTLs could facilitate breeding programs by helping control fruit sweetness. While fructose and glucose contribute to total sugars, their QTLs were not co-located with the QTL for TSS, whereas they were expected to be related. In February, both fructose and glucose were negatively correlated with sucrose DM. A QTL for sucrose was found in the same chromosome region as glucose and fructose QTLs. Sucrose is the main form of translocated carbon in citrus (Garcia-Luis et al. 1991). It is transported from leaves to the juice sac head, where it is partitioned into glucose and fructose (Goldschmidt and Koch 1996). The sucrose degradation pathway activated during fruit maturation to generate fructose and glucose (Lin et al. 2015) could explain the negative correlation between sucrose and the two other sugars and the co-localization of their QTLs. To the best of our knowledge, QTLs for organic acids and soluble sugars in this study were the first such QTLs to be mapped in citrus, while not corresponding to any previously reported QTLs. Therefore, at least 15 of the QTLs reported in this paper are novel QTLs.

Coincidence of QTL position with other citrus studies: Our study is the only one in citrus that assessed the position and number of QTLs of a trait over the course of fruit ripening (3 dates spaced about 6 weeks apart and QTLs differed between measurement dates. Mainly the other citrus studies on QTLs of fruit traits were assessed at a single date, without considering the fruit maturity evolution of each hybrids of the progenies (Curtolo et al. 2017; Imai et al. 2017). Therefore, we find very little similarity between the different studies. Curtolo et al. 2017 studied the QTLs of fruit quality traits by using a progeny combining Murcott tangor and Pera sweet orange and DArTseq™ molecular markers. They did not visualize the position of the QTLs in the genetic map but they gave a physical position on the sweet orange genome of the markers related to the QTLs. The position of QTLs for common quality traits (acidity, TSS and juiciness) between their and our study is different. Only one QTL of fruit diameter localizes on the same linkage group 8. Imai et al. (2017) studied the QTLs of sugar content and fruit diameter of two Japanese mandarins (Imai et al. 2017). Only the position of one of the fruit mass QTLs at the beginning of GL 3 seems to coincides between that study and ours. Comparatively to Yu et al. (2016) among the 48 detected QTLs, only the QTL of TSS seems to be located at the same position in our study at the beginning of the LG 8. None of the other QTLs from their study matches ours. The low coincidence between the genetic maps can be explained by different reasons.. Many previous studies based on reduced genome complexity representation (GBS, DartSeq) were based on a sequence mapping on the orange genome with a numbering and orientation of the chromosomes different from those of the reference genome (the clementine tree) used in the present study. In the absence of a pan genome, it is difficult to make a link between our results and those resulting from mapping on other references and many studies did not make the link with the genomic positions on a given reference but only in relation to the genetic map.

Conclusion

Fruit quality traits showed major variation in progeny during maturation. QTLs related to fruit quality traits were localized on several linkage groups using consensus and parental genetic maps. Many of these traits were correlated. Thirty-four QTLs for the major physical and chemical components of fruit were detected at three different fruit maturation dates. Notably, we detected at least 15 novel QTLs for sugars and acids. Malic acid was controlled by several QTLs during maturation, revealing a more complex genetic determinism than citric acid, for which only one QTL was detected. Several QTL clusters were identified. The majority of QTLs were mapped in three linkage groups (2, 8, and 9). This suggests that some QTLs may have pleiotropic effects. Although fine mapping is required to decipher such clusters, they could be useful in marker-assisted selection and thus increase the efficiency of future breeding programs.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11295-022-01571-w.

Acknowledgements The authors thank Laurent Gomez of INRAE Avignon for giving us access to his laboratory.

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Funding This work is part of Hajer Khefifi's PhD granted by CIRAD and INRA and the University of Carthage (Tunisia). This work received financial support from the European Regional Development Fund under the framework PO FEDER-FSE Corse 2014–2020 number 247SAEUFEDER1A, project called Innov'Agrumes (ARR-18/517 CE, synergie number: CO 0009083).

Data availability All the markers used for genetic mapping were previously published by Ollitrault et al. (2012a), and their corresponding GenBank accession numbers can be found in the Additional file 1 of this publication.

Declarations

Conflict of interest The authors declare no competing interests.

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