



HAL
open science

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

Hajer Khefifi, Doriane Dumont, Gilles Costantino, Agnes Doligez, Anna Carla Brito, Aurélie Bérard, Raphael Morillon, Patrick Ollitrault, Luro François

► To cite this version:

Hajer Khefifi, Doriane Dumont, Gilles Costantino, Agnes Doligez, Anna Carla Brito, et al.. Mapping of QTLs for citrus quality traits throughout the fruit maturation process on clementine (*Citrus reticulata* x *C. sinensis*) and mandarin (*C. reticulata* Blanco) genetic maps. *Tree Genetics and Genomes*, 2022, 18 (6), pp.40. 10.1007/s11295-022-01571-w . hal-03857740

HAL Id: hal-03857740

<https://hal.inrae.fr/hal-03857740>

Submitted on 17 Nov 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License



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

Hajer Kheffi^{1,2,3} · Doriane Dumont⁴ · Gilles Costantino¹ · Agnès Doligez³ · Anna Carla Brito^{2,3} · Aurélie Bérard⁵ · Raphael Morillon^{2,3} · Patrick Ollitrault^{2,3} · François Luro¹

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

© The Author(s) 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

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

Communicated by C. Chen

✉ François Luro
francois.luro@inrae.fr

¹ INRAE, UMR AGAP Institut, Equipe SEAPAG,
Univ Montpellier, CIRAD, INRAE, Institut Agro,
20230 San Giuliano, France

² CIRAD, UMR AGAP Institut, Equipe SEAPAG,
20230 San Giuliano, France

³ UMR AGAP Institut, Univ Montpellier, CIRAD, INRAE,
Institut Agro, 34398 Montpellier, France

⁴ UR-PSH Plantes Et Systèmes de Culture Horticoles INRAE,
Avignon Cédex 9, 84914 Avignon, France

⁵ UR EPGV INRAE, 2 rue Gaston Cremieux, 91057 Evry,
France

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 (Minami-kawa 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

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.

157 Phenotyping

Clementine and mandarin maturation occurs in Corsica over the November and January–February periods, respectively (Jacquemon 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).

181 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 polyvinylpyrrolidone (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: $\sigma_G^2 / (\sigma_G^2 + \sigma_e^2)$.

240 Genotyping of the CxM population

241 Young leaves from each genotype were harvested to gen-
 242 otype the parents and progeny with molecular markers.
 243 Total DNA was extracted from leaf tissue using the method
 244 described by Doyle and Doyle (1987). Single-sequence
 245 repeats (SSRs) and single-nucleotide polymorphisms (SNPs)
 246 were used. SSR amplification and detection of amplified
 247 DNA fragments were performed according to Luro et al.
 248 (2008). The genetic map was constructed with 94 heterozy-
 249 gous SSR markers originated from genomic mandarin DNA
 250 library (Ci*****) (Froelicher et al. 2008) or clementine
 251 EST library (MEST***) (Luro et al. 2008). SNP markers
 252 (CiC****-**) were mined from the clementine BACend
 253 Sequence database, and 1536 SNPs were used for an Illu-
 254 mina GoldenGate assay (Terol et al. 2008; Ollitrault et al.
 255 2012b). Some SNP markers from genes involved in the pri-
 256 mary and secondary metabolite biosynthesis pathway and
 257 in salt tolerance—mined by Sanger sequencing of 44 geno-
 258 types representative of *Citrus* and relatives (Garcia-Lor et al.
 259 2012)—were added to the Illumina SNP set (CHI-*-***,
 260 LCY2-*-***, TScMI1331, HKT1c800F141, PSY-M-289,
 261 PKF-M-186). The SNP and SSR markers used in our study
 262 had been previously mapped on the clementine reference
 263 genetic map (Ollitrault et al. 2012a).

264 Genetic linkage maps

265 Genetic linkage analysis and map construction were per-
 266 formed with Join Map 4 (Van Ooijen 2006), and maps were
 267 drawn with Mapchart 2.3 (Voorrips 2002). Framework con-
 268 sensus and parental maps were constructed based on 645
 269 markers (Additional Table 1) and 105 CxM hybrid trees,
 270 with “CP” as population type. Segregation distortion for
 271 parental and consensus data was assessed with χ^2 tests
 272 according to the segregating type of each marker. These
 273 markers revealed three segregation patterns: 1:1 for mark-
 274 ers segregating only in one parent (ll × l m and nn × np),
 275 1:2:1 for markers segregating in male and female parents
 276 (hk × hk), and more informative 1:1:1:1 segregation in mark-
 277 ers segregating in both parents with three alleles (ef × eg).
 278 Grouping was achieved using a minimum LOD score of 4.
 279 The regression mapping algorithm (round 2) and Kosambi
 280 mapping function were used to establish the map order and
 281 distances in centiMorgans (Kosambi 1943; Stam 1993)
 282 within each linkage group. The linkage group nomenclature
 283 was the same as in the Clementine reference map (Ollitrault
 284 et al. 2012a). For subsequent QTL analysis, the number of
 285 markers was reduced in very dense map regions by main-
 286 taining only one marker for identical genetic positions and
 287 removing all other redundant ones with the same or a very
 288 close position (< 1 cM), resulting in what we called frame-
 289 work maps.

QTL detection

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

Results

Distribution of phenotypic traits

313 The distribution of raw phenotypic values for fruit attrib-
 314 utes in the progeny and parents at the three dates evalu-
 315 ated throughout fruit maturation was described based on
 316 the distribution of the number of genotypes by class of raw
 317 concentrations in fresh matter (Fig. 1) and by box plots
 318 (Additional Fig. 1). The concentration of primary metabo-
 319 lism compounds was also calculated on a dry matter basis
 320 and presented according to the distribution of the number
 321 of genotypes by class of raw concentrations (Fig. 2) and in
 322 box plots (Additional Fig. 2). During maturation, the average
 323 fruit mass and equatorial diameter of the progeny reached a
 324 maximum average value in December and then levelled off
 325 in clementine and CxM offspring, while these two param-
 326 eters continuously increased in mandarin. The juice percent-
 327 age increased until December and then decreased consider-
 328 ably in the two parents and the offspring. Otherwise, the
 329 acidity pattern was the same for the parents and the CxM
 330 offspring. It decreased until reaching low values especially
 331 for clementine and the 105 hybrids (0.3–0.6 g/100 g).

332 Sucrose was the major sugar detected during maturation
 333 in the CxM offspring and parents. Its mean concentration
 334 was about 3- to sixfold higher than that of glucose and fruc-
 335 tose ones, which had equivalent levels. All sugar mean con-
 336 centrations increased between October and December and
 337 then remained relatively constant. Minor differences were

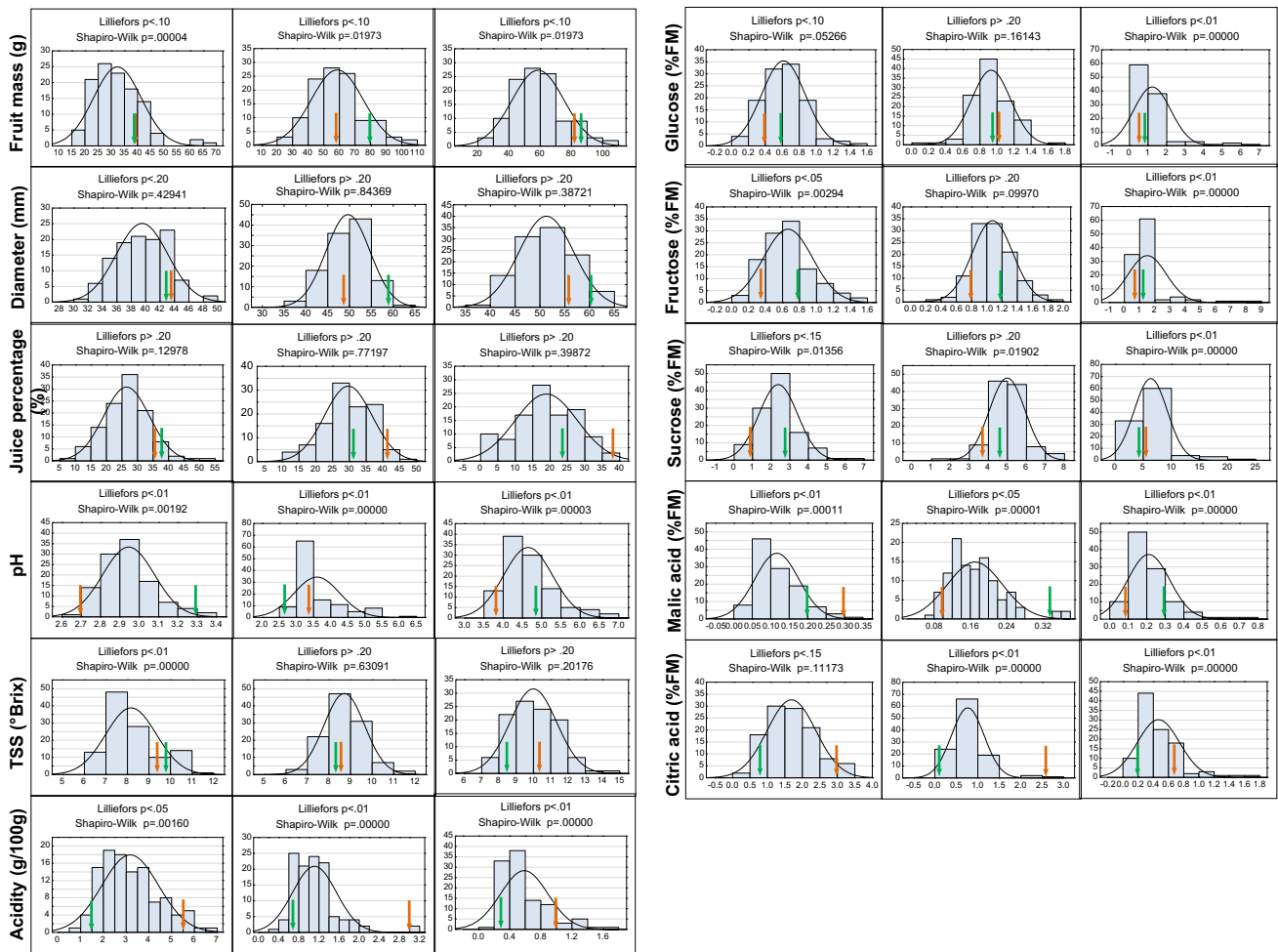


Fig. 1 Distribution of the number of genotypes by class of raw values for fruit attributes in the CxM population measured on 10/08/2012 (first column), 12/03/2012 (second column), and 02/27/2013 (third

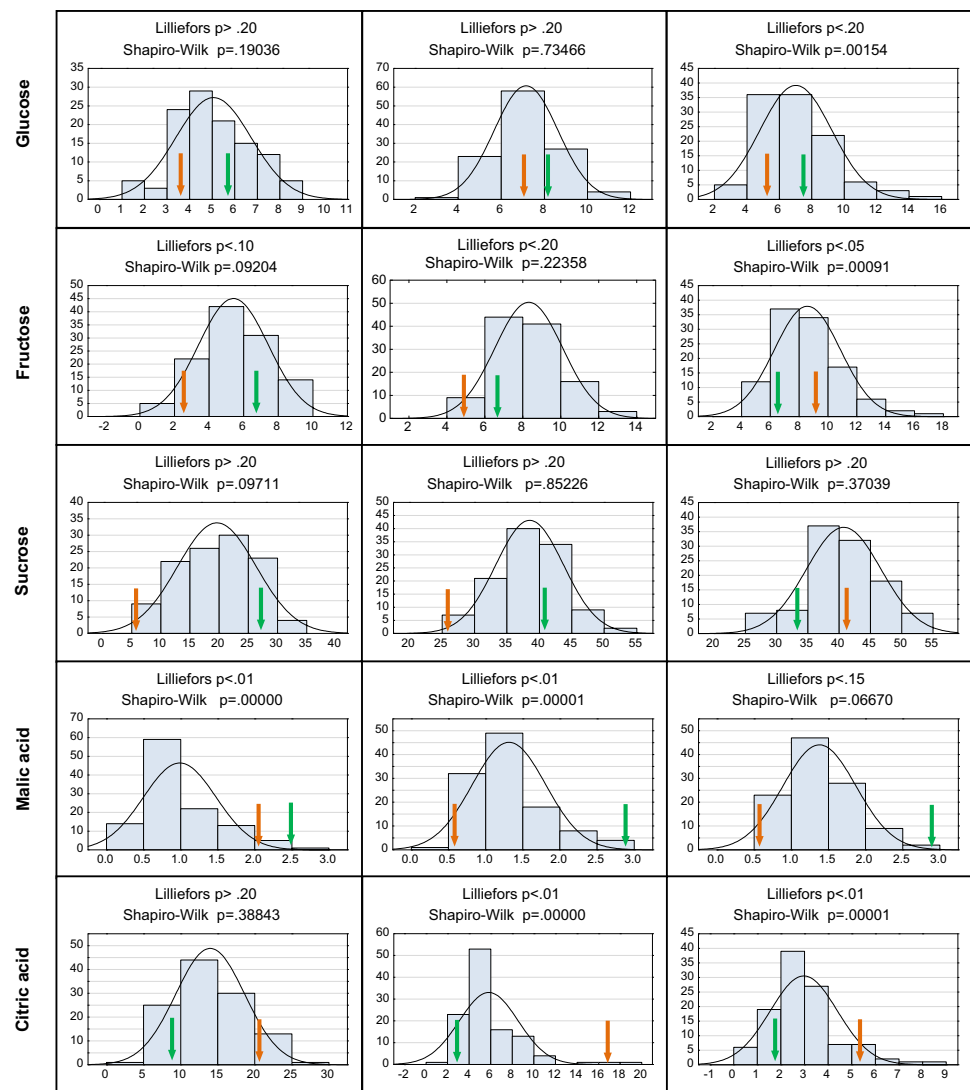
column). Mean values of the two parents are indicated by arrows: clementine (green) and mandarin (orange). FM, fresh matter

338 observed between the two parents regarding the glucose
 339 content (% of DM and % of FM). For acids, citric acid
 340 presented the highest mean concentration, especially in October
 341 where it was about 30-fold higher than that of malic acid.
 342 During maturation, citric acid decreased while malic acid
 343 increased, except in “Willow leaf” mandarin. Despite these
 344 variations during fruit maturation, citric acid remained the
 345 predominant organic acid. The pattern noted in the progeny
 346 was similar to that of clementine. The total acidity and cit-
 347 ric acid concentration continuously declined from December
 348 until February, especially for mandarin. Nevertheless,
 349 malic acid did not show the same variation pattern since its
 350 concentration increased slightly until December and then
 351 remained constant. During maturation, mandarin fruits
 352 were significantly more acidic than clementine fruits, which
 353 showed a lower malic acid concentration but a higher citric
 354 acid concentration. The mandarin acidity level reached in
 355 February was in line with the known maturity period for

this citrus fruit (January–February). The acidity of clementine
 356 in December reached its low marketing limit under the
 357 protected geographical identification label (IGP Clementine
 358 de Corse). For other traits such as TSS, differences were
 359 minor between clementine and mandarin for the first two
 360 dates (October and December) but they increased thereafter.
 361

High variability was observed within the population.
 362 Average trait means varied over the three dates. For fruit
 363 mass, equatorial diameter, juice percentage, pH, acidity, and
 364 TSS, the range of variation over the fruit maturation period
 365 was approximately 1.5- to twofold. The variability within the
 366 population evolved differently over time, depending on the
 367 parameter, while being almost stable for fruit mass, equato-
 368 rial diameter, juiciness, TSS, and malic acid. On the other
 369 hand, it increased for pH and the three soluble sugars, while
 370 it decreased for acidity and citric acid. For organic acids
 371 and sugars, the range of variation was 3- to 50-fold over the
 372 fruit maturation period. However, the variability decreased
 373

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)



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

376 Phenotypes with much higher and/or lower values than
377 the highest and lowest values estimated for the two parents
378 were observed for fruit mass, equatorial diameter, juice per-
379 centage, TSS, glucose, fructose, and sucrose. Indeed, the
380 majority of fruit traits segregated in a transgressive man-
381 ner. For example, in October, fruit mass ranged from 15.7
382 to 78.5 g in the population, despite the very small differ-
383 ence between parents (39.1–39.4 g). Conversely, acidity and
384 citric acid were distributed essentially within the range of
385 the parental values. Most traits, such as equatorial diameter,
386 juice percentage, and sucrose content (in % of DM), pre-
387 sented a normal distribution. However, some traits such as
388 acidity and pH deviated from normality. Therefore, appro-
389 priate transformations (ln or square root) were applied to
390 unskew their distributions (Tables 1 and 2). The contin-
391 uous variation pattern indicates that the studied traits were

controlled by several genes, so they were classified as quan- 392
titatively inherited. 393

Genetic correlation and heritability 394

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

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

408 December and February. Both acidity and pH were corre-
409 lated with citric and malic acid contents.

410 For all traits, broad-sense heritability (H^2) values
411 (repeatability among the 10 fruit replicates) were quite
412 high (> 0.5) (Tables 1 and 2). They ranged from 0.64 to
413 0.82, 0.50 to 0.94, and 0.61 to 0.89 for traits measured
414 in October, December, and February, respectively. In

October, fruit mass, acidity and TSS showed the highest
415 heritability (> 0.8). In December, the highest heritability
416 values (> 0.8) were observed for fruit mass, equatorial
417 diameter, pH, and malic acid. However, in February, the
418 traits showing > 0.8 heritability were fruit mass, juice per-
419 centage, pH, citric acid FM and DM, malic acid FM, and
420 glucose FM (Tables 1 and 2).
421

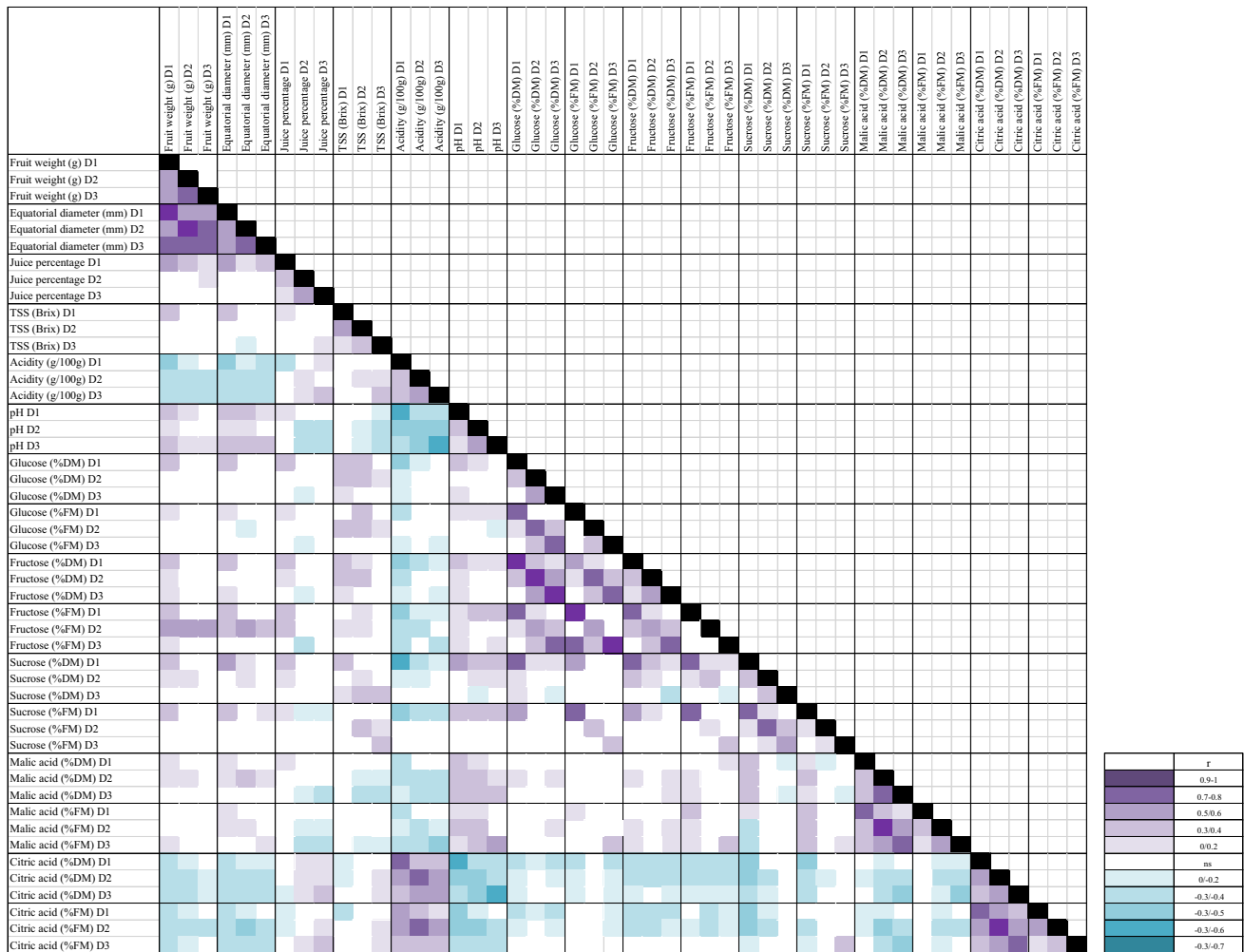


Fig. 3 Genotypic correlations (Spearman) between fruit characteristics measured during maturation on 10/08/2012 (D1), 12/03/2012 (D2), and 02/27/2013 (D3), based on genotypic BLUPs

422 Genetic linkage maps

423 A total of 551 SNP and 94 SSR markers were genotyped in
 424 clementine × mandarin offspring. Out of these, 622, 618,
 425 and 275 markers were selected to construct the consensus,
 426 clementine, and mandarin maps, respectively (Additional
 427 Table 1). Among these markers, 333 segregated at 1:2:1,
 428 268 segregated at 1:1, and 21 segregated at 1:1:1:1. The
 429 consensus map and the previously published clementine
 430 reference map (Ollitrault et al. 2012a) shared 551 com-
 431 mon markers. A comparative analysis of these two maps
 432 showed high synteny and colinearity, with very few inver-
 433 sions and distance differences (Additional Fig. 3). How-
 434 ever, LG3 on the reference clementine genetic maps was
 435 split into two sub-linkage groups on the consensus gen-
 436 etic map as well as on our clementine genetic map (Addi-
 437 tional Table 1). There is little resolution on the position of the
 438 markers due to the small size of the population, which

439 results in many co-locations of markers. The multiplic-
 440 ity of markers at the same locus is non-informative and
 441 lengthens the computer processing when detecting QTLS.
 442 To facilitate QTL analysis, the map density was reduced
 443 by removing markers with several missing data and with
 444 the same or very close positions (<0.1 cM), but without
 445 modifying the map coverage. These reduced density maps
 446 are hereafter called framework maps. The final numbers of
 447 markers retained for the framework consensus, clementine,
 448 and mandarin maps were 310, 277, and 147, respectively.
 449 SSR and SNP markers were grouped in 10 linkage groups
 450 on the consensus and clementine maps and in 12 linkage
 451 groups on the mandarin map. There was a greater number
 452 of chromosomes divided into several linkage groups on
 453 the mandarin map due to the lower number of markers:
 454 three referenced linkage groups were represented by two
 455 linkage groups each. The consensus, clementine, and man-
 456 darin maps, respectively, covered 795.7 cM, 809.8 cM,

457 and 629.7 cM, which corresponded to 74%, 75%, and 58%
 458 of the genome, respectively, compared to the clementine
 459 genetic reference map (Ollitrault et al. 2012a). Link-
 460 age groups had a mean distance of 2.6, 3.3, and 4.7 cM
 461 between adjacent markers on the consensus, clementine,
 462 and mandarin maps, respectively. Some linkage groups
 463 had large marker intervals. Linkage groups 6 and 7 had
 464 intervals ranging from 16 to 24 cM on the three maps.
 465 Moreover, linkage group 2 on the mandarin map had one
 466 18 cM interval. Another large interval was observed at
 467 the extremity of linkage group 8 on the clementine map.
 468 Common markers within the consensus and parental maps
 469 allowed a between-map comparison of their marker orders.
 470 Except for minor changes, strong collinearity was observed
 471 especially between the clementine and consensus maps.

QTL identification

473 QTL analysis overview: QTL detection was performed using
 474 a model with both additive and dominant effects and geno-
 475 typic BLUPs at each date on the consensus (Con) and both
 476 parental (C and M) framework maps. The LOD score of
 477 significant QTLs ranged from 3.6 to 8.3. We only retained
 478 QTLs detected by MQM and confirmed by a Kruskal–Wal-
 479 lis test. A total of 28 QTLs were identified on the consensus
 480 map for all traits except glucose FM and citric acid FM dur-
 481 ing maturation (Table 3), with 1–3 QTLs per trait and date.
 482 Nine QTLs were detected in October, 10 in December, and
 483 9 in February. QTLs were found on all LGs, except LG6 and
 484 LG1. The proportion of the total variation (R^2) ranged from
 485 13.1 to 34.1%. Sixteen QTLs showed an R^2 ranging from 10
 486 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	****
Equatorial diameter (mm)	October	8	6.9	4.2	CiC0100-04	44.7	41.0–48.7	22.7	*****
		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	*****
		9	6.8	4.2	MEST1201	52.7	46.7–56.3	27.3	*****
Juice percentage 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	*****
		8	5.9	4.2	CiC0598-01	11.8	8.6–13.7	24	*****
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

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

495 Fruit mass and equatorial diameter: For fruit mass,
 496 the greatest number of QTLs was found in February on
 497 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.

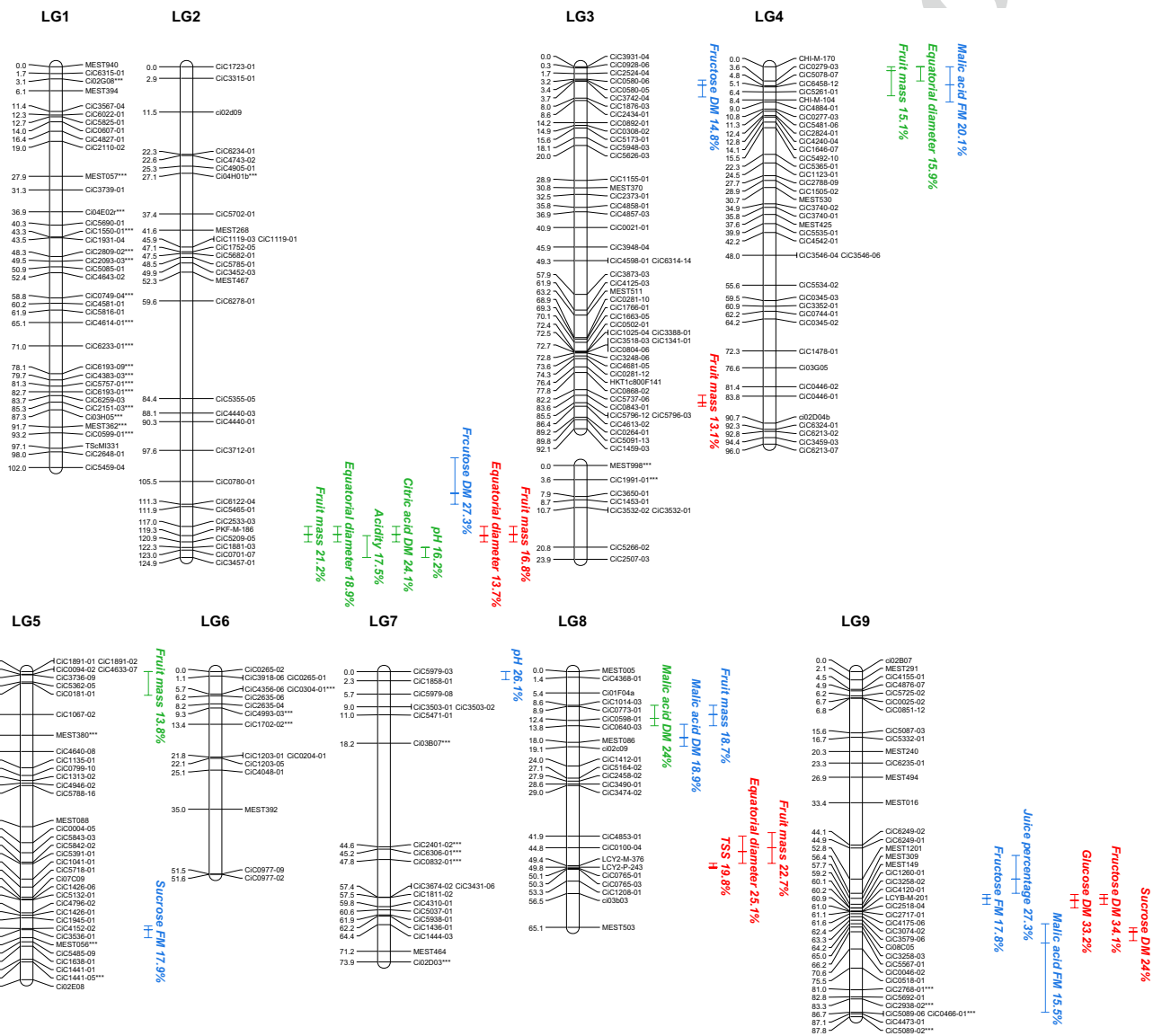


Fig. 4 QTL location on consensus genetic map for fruit attributes analyzed during three maturation dates and determined by interval mapping and multiple QTL model. Linkage groups are labelled as LG1–LG9. QTLs are listed on the right of each linkage group. Distances are in cM (Kosambi’s function). Vertical 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

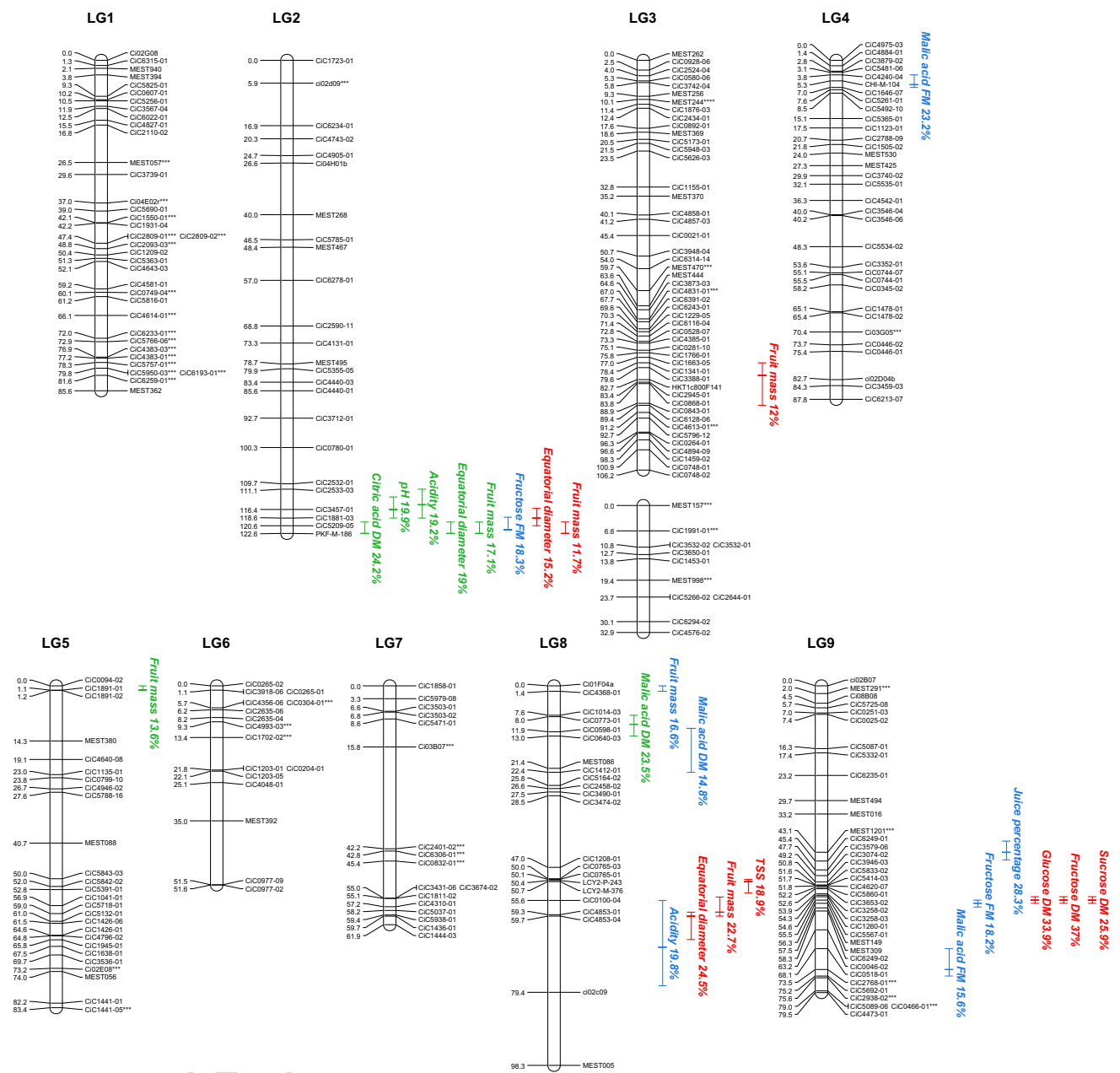


Fig. 5 QTL location on clementine genetic map for fruit attributes analyzed during three maturation dates and determined by interval mapping and multiple QTL model. Linkage groups are labelled as LG1–LG9. QTLs are listed on the right of each linkage group. Distances are in cM (Kosambi’s function). Vertical 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

509 Juiciness: Only one QTL, located on LG9, was associ-
 510 ated with the juice percentage in December on the consen-
 511 sus and clementine maps, and explained 27.3 and 28.3%,
 512 respectively, of the total variation (Figs. 4 and 5).

513 TSS: On the consensus and clementine maps, one QTL
 514 explaining 19.8 and 18.9%, respectively, of the total varia-
 515 tion was identified on LG8 in February (Figs. 4 and 5). On

the mandarin map, no QTL for TSS was detected regard-
 516 less of the maturation date (Fig. 6).

517
 518 pH and acidity: In October, one QTL for pH, explaining
 519 16.2 and 19.9% of the variance, was detected on LG2 on the
 520 consensus and clementine maps (Figs. 4 and 5). A second
 521 QTL accounting for 26.1% of the total variance was located
 522 on LG7 on the consensus map in December only. For acidity,

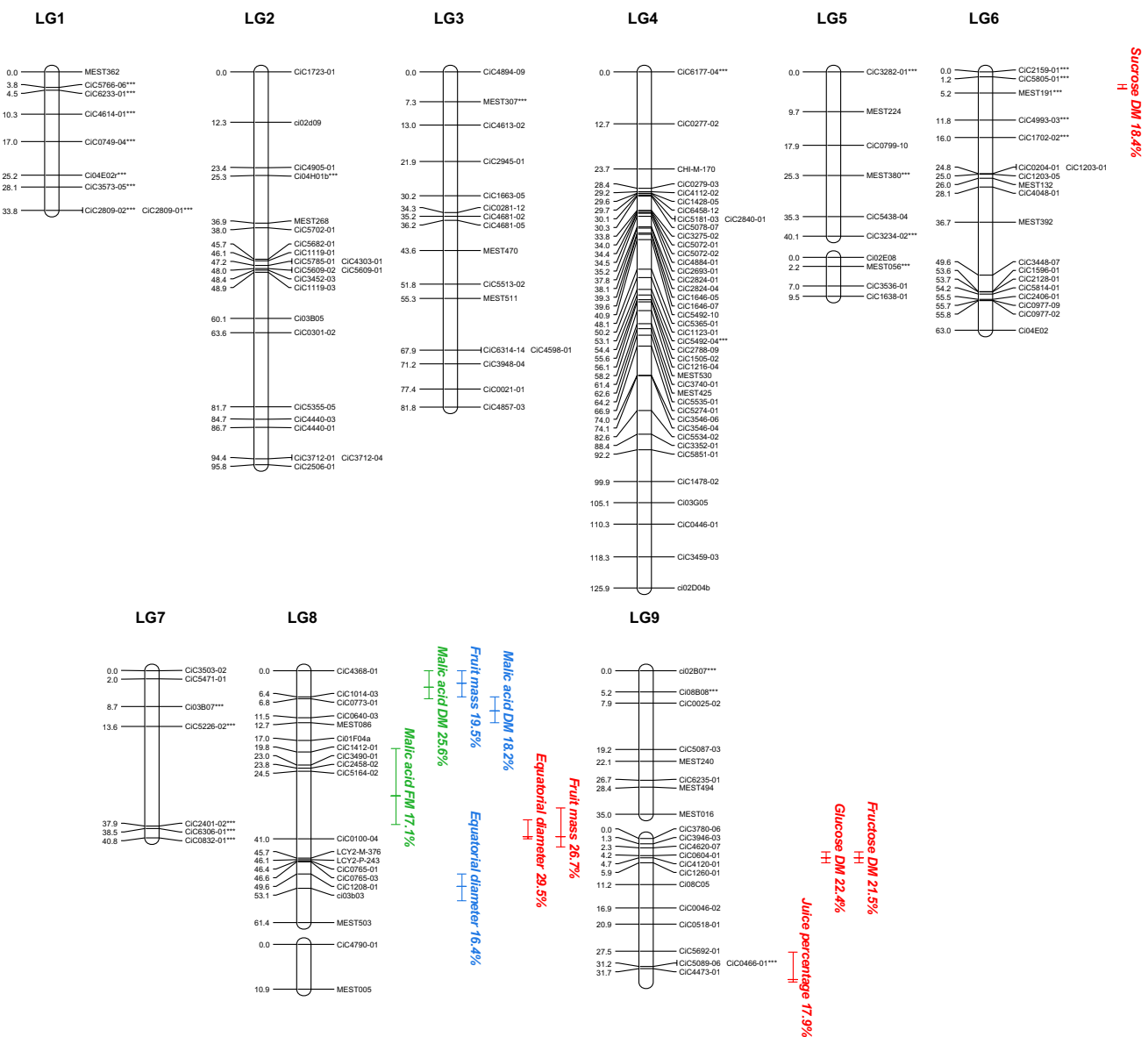


Fig. 6 QTL location on mandarin genetic map for fruit attributes analyzed during three maturation dates and determined by interval mapping and multiple QTL model. Linkage groups are labelled as LG1–LG9. Names of the markers and QTLs are listed on the right of each linkage group. Distances in cM (Kosombi’s function) are in the left of each linkage group. With respect to QTL localization, the high-

est 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

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

526 Sugars: In February, QTLs associated with glucose, fructose,
527 and sucrose expressed in DM were mapped in the same
528 linkage group (LG9) on the consensus and clementine maps.
529 The QTL for fructose DM overlapped that for glucose DM.
530 These QTLs showed the greatest effects in this study, contributing
531 more than 30% to the total variance (Additional Table 2). Other QTLs
532 were detected on the consensus map

533 for sucrose FM on LG5 and fructose DM on LGs 2 and 3
534 in December, yet only the LG2 QTL was also found on the
535 clementine map. On the mandarin map, while fructose DM
536 and glucose DM QTLs colocalized on LG9 in February, one
537 QTL for sucrose DM was detected on LG6 at the same date
538 (Fig. 6). Two QTLs for fructose DM were found on LG2 and
539 LG3 on the consensus map in December, which accounted for
540 27.3 and 14.8% of the total variance, respectively. QTLs
541 controlling fructose FM and sucrose FM in December were
542 found on LG9 and LG5 on the consensus maps, respectively,

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

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

556 Discussion

557 QTL detection accuracy and power

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

581 We used MQM to optimize QTL detection, especially
582 in the case of two linked QTLs, since it increases the QTL
583 detection power and the QTL position estimation precision,
584 while allowing us to map additional QTLs located on the
585 same chromosome (Paterson 1997). Twenty-eight QTLs
586 of quality attributes in citrus were found on the consen-
587 sus map, most of which (24) had a relatively marked effect
588 ($R^2 > 15\%$). Both parental and consensus maps were used to
589 yield complementary results. Indeed, QTLs with dominant
590 allelic effects like fructose DM identified on chromosomes 2
591 and 3 and pH mapped on chromosome 7 in December were

592 detected only on the consensus map. Conversely, the detec-
593 tion accuracy could be higher in parental maps for QTLs
594 with additive effects only, as shown by the 6 additional
595 QTLs identified only on the parental maps and not on the
596 consensus map. Overall, 34 QTLs were identified throughout
597 the maturation period.

598 The effectiveness of molecular markers associated with
599 detected QTLs should be determined as the percentage of
600 the explained genetic variance, instead of the phenotypic
601 variance, because fluctuations in phenotypic values as a
602 result of environmental variations blurs the marker effects
603 (Nishio et al. 2011). The use of BLUP values thus improved
604 the QTL detection power by removing part of the environ-
605 mental variance per tree since the variance between fruits
606 might be at slightly different stages of maturity at harvest
607 time. For instance, this variability in fruit maturity was
608 studied for clementine and used to determine the “harvest-
609 ability window” (Julhia et al. 2019). Indeed, several studies
610 have demonstrated the effectiveness of the BLUP method
611 for species such as apple (Segura et al. 2009) or grapevine
612 (Doligez et al. 2013) where the experimental material had
613 been phenotyped over several years or under different grow-
614 ing conditions.

615 QTL detection

616 In our case, all traits had high heritability ($H^2 > 0.56$) during
617 maturation, which increased our chances of detecting QTLs.
618 However, the high heritability was also due to the absence of
619 annual variation and the low variance between fruits. A main
620 limitation of this experimentation plot was related to the
621 number of individuals present in the population. With 105
622 individuals, it could be assumed that the detected QTLs were
623 those with marked and possibly significant effects (Staub
624 et al. 1996; Beavis 1998). The percentage of total genotypic
625 variation explained by QTLs detected throughout maturation
626 ranged from 11.7 to 37%. Fruit mass, equatorial diameter,
627 malic acid FM, and fructose DM were controlled by more
628 than one QTL. The presence of several QTLs showed the
629 complexity of the metabolic pathways. QTLs of fruit mass
630 detected during maturation did not explain 100% of the total
631 variance, which suggests the presence of other undetectable
632 QTLs and/or epistatic effects that could explain the remain-
633 ing percentage of total variation. To our knowledge, this is
634 the first report of QTL mapping of fruit attributes in cit-
635 rus at three sampling dates during maturation. This QTL
636 study showed that the traits were probably not controlled by
637 the same QTLs during maturation. In our case, the use of
638 more complete maps and a high number of measurements
639 (replicates) provided more accurate QTL detection results.
640 Indeed, QTL detection is known to be affected by environ-
641 mental conditions, which represent a major source of vari-
642 ability (Rousseaux et al. 2005; Kenis et al. 2008).

643 **Trait correlations and QTL co-location**

644 Several traits were clustered mainly on linkage groups 2, 4,
645 8, and 9 irrespective of the evaluation dates. These clusters
646 may have reflected a pleiotropic effect of one QTL or tight
647 linkage between at least two QTLs. A QTL with a pleio-
648 tropic effect indicates the segregation of a single QTL con-
649 trolling several traits due to related metabolisms or causal
650 relationships between traits (De Vienne and Causse 1998).
651 Several QTL clusters for fruit maturation and agronomic
652 traits were detected in previous studies in many species,
653 including tomato (Monforte et al. 1999), peach (Etienne
654 et al. 2002), apple (Liebhard et al. 2003), and citrus (Sugiy-
655 ama et al. 2011). Common or close QTL locations have often
656 been observed for correlated attributes (Paterson et al. 1991).
657 These correlations could suggest candidate regions for future
658 studies to gain further insight into these traits. In this study,
659 QTL co-locations were observed for the majority of the stud-
660 ied fruit traits, including fruit mass, equatorial diameter, pH,
661 acidity, sugar, and acid contents. QTL clusters varied during
662 maturation. Indeed, some of them were stable throughout
663 maturation, while others were identified for only one or two
664 maturation dates. Fruit quality traits vary with the degree
665 of maturation in citrus. Therefore, our QTL analysis results
666 might also have been affected by fruit maturity heterogeneity
667 (Ladanyia 2008). This lack of stability of fruit quality QTLs
668 during maturation thus suggests that some fruit traits are not
669 governed by the same locus during maturation.

670 **Fruit mass and size:** In our study, fruit mass QTLs were
671 detected on LGs 2, 3, 4, 5, and 8. Some of them had been
672 detected in other studies. For instance, the QTL for fruit
673 mass located on LG4 may have corresponded to the previ-
674 ously reported FW4.2 QTL (Yu et al. 2016) and FWq3 QTL
675 (Imai et al. 2017). Besides, the fruit mass QTL detected
676 on LG3 may have corresponded to FWq1 detected in 2013
677 and FWq2 detected in 2013 and 2014 (Imai et al. 2017).
678 Moreover, another fruit mass QTL was mapped in the same
679 region of LG8 in two QTL mapping studies, confirming that
680 this is a single major QTL (Yu et al. 2016; Minamikawa
681 et al. 2017). On another hand, Imai et al. (2018) found four
682 fruit mass QTLs situated on LGs 2, 3, 5, and 7. However,
683 although three of them were detected on the same LG in
684 our study, they were not mapped in the same region (Imai
685 et al., 2018). This comparison with the findings of the four
686 mentioned studies was possible because the QTLs were posi-
687 tioned on the same scaffolds 2, 3, 4, 5, and 8 on the clemen-
688 tine reference map, which presented high synteny with our
689 consensus linkage map.

690 Ting and Attaway (1971) reported that fruit mass and
691 equatorial diameter variation patterns are interrelated dur-
692 ing fruit development and maturation, thus highlighting that
693 it was normal to find overlapping QTLs between these two
694 traits: one QTL co-location on different chromosomes at

695 two maturation dates on the consensus map, i.e., 2 and 4 in
696 October and 2 and 8 in February. This correlation between
697 fruit mass and fruit diameter was also found in mandarin (Yu
698 et al. 2016). Moreover, common genomic regions may con-
699 trol both fruit mass and size in *C. volkameriana* × *P. trifo-*
700 *liata* (Garcia et al. 2000) and *C. clementina* hybrid × *C. gran-*
701 *dis* hybrid (Asins et al. 2015) populations. The involvement
702 of several regions of different chromosomes in the control of
703 these two traits revealed their complex genetic determinism,
704 as already noted in other species such as apple (Kenis et al.
705 2008) and tomato (Grandillo et al. 1999).

706 **Acidity and fruit mass:** QTLs of titratable acidity and
707 fruit mass measured in October were mapped at the end of
708 LG2 on the consensus map, and negative correlations were
709 obtained between acidity and fruit mass. In citrus, the first
710 fruit development stage is characterized by slow fruit growth
711 rates but high cell division (Bain 1958), whereas phase II
712 constitutes a fast growth period where fruit increases in size
713 mostly by cell enlargement and water accumulation (Iglesias
714 et al. 2007; Tadeo et al. 2008). QTLs for fruit mass could
715 thus be associated mainly with the cell expansion process,
716 as shown in tomato (Bertin et al. 2007). Cell growth and
717 enlargement depend on water and carbon compound accu-
718 mulation (Yakushiji et al. 1996). Admittedly, citrus fruit act
719 as carbohydrate storage sinks during the cell enlargement
720 stage and thereafter (Mehouachi et al. 1995; Cercós et al.
721 2006). Fruit accumulates a considerable amount of organic
722 acids in juice sac cell vacuoles (Etienne et al. 2013a, b).
723 The high acid concentration could result in enhanced sink
724 strength, thus facilitating carbohydrate accumulation (Hock-
725 ema and Etxeberria 2001) and increased fruit size (Agustí
726 et al. 2002). Nevertheless, October, i.e., the period when
727 our research began, corresponds to phase III under Mediter-
728 ranean conditions (Jacquemond and Agostini 2013). Dur-
729 ing this stage, accumulated organic acids are progressively
730 catabolized, thereby implying acid reduction. Meanwhile,
731 fruits continue to increase in size. If fruit mass is positively
732 linked to acidity during the first half of stage II and nega-
733 tively from the second half of stage II and during stage III,
734 this may be explained by the high carbohydrate supplies
735 on acidity. In fact, according to Etienne et al. (2013a, b),
736 abundant carbohydrate supplies result in higher fruit mass
737 and increased respiration. Yet, at this physiological stage,
738 sugars stored in the vacuole may no longer be available as
739 a respiratory substrate. Consequently, organic acids sustain
740 respiration, thus leading to a decline in acidity. Antoine et al.
741 (2016) demonstrated that smaller and more acidic fruits were
742 the result of carbohydrate depletion (through water stress or
743 modification of leaf/fruit ratio), which could confirm and
744 explain the negative correlation between acidity and fruit
745 mass in our study.

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

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.

854 FM vs DM: It is noteworthy that in the current study,
855 QTLs for sugars and acids expressed in DM and QTLs for
856 sugars and acids expressed in FM did not co-localize despite
857 the high correlation between these traits. Interestingly, QTLs
858 detected for sugars and acids expressed in DM were twice
859 as numerous as QTLs expressed in FM. This difference was
860 also found in a study by Prudent et al. (2009) in tomato.
861 These latter authors detected 11 QTLs for sugar DM vs 6
862 QTLs for sugar FM with only two overlapping QTLs. This
863 confirms the fact that traits expressed in fresh and dry matter
864 are not always correlated (Woodward and Clearwater 2008).
865 The reason for this difference in QTL detection could be
866 explained by the fact that fresh matter depends on the water
867 content (Bolarin et al. 2001), which is largely influenced
868 by environmental factors such as the soil water status and
869 climatic factors (Jones and Tardieu 1998). Moreover, envi-
870 ronmental factors represent a major source of variability that
871 affects QTL detection (Rousseaux et al. 2005; Kenis et al.
872 2008).

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

898 Coincidence of QTL position with other citrus studies:
899 Our study is the only one in citrus that assessed the position
900 and number of QTLs of a trait over the course of fruit ripen-
901 ing (3 dates spaced about 6 weeks apart and QTLs differed
902 between measurement dates. Mainly the other citrus studies
903 on QTLs of fruit traits were assessed at a single date, without
904 considering the fruit maturity evolution of each hybrids of
905 the progenies (Curtolo et al. 2017; Imai et al. 2017). There-
906 fore, we find very little similarity between the different

907 studies. Curtolo et al. 2017 studied the QTLs of fruit quality
908 traits by using a progeny combining Murcott tangor and Pera
909 sweet orange and DArTseq™ molecular markers. They did
910 not visualize the position of the QTLs in the genetic map but
911 they gave a physical position on the sweet orange genome of
912 the markers related to the QTLs. The position of QTLs for
913 common quality traits (acidity, TSS and juiciness) between
914 their and our study is different. Only one QTL of fruit diam-
915 eter localizes on the same linkage group 8. Imai et al. (2017)
916 studied the QTLs of sugar content and fruit diameter of two
917 Japanese mandarins (Imai et al. 2017). Only the position of
918 one of the fruit mass QTLs at the beginning of GL 3 seems
919 to coincides between that study and ours. Comparatively
920 to Yu et al. (2016) among the 48 detected QTLs, only the
921 QTL of TSS seems to be located at the same position in
922 our study at the beginning of the LG 8. None of the other
923 QTLs from their study matches ours. The low coincidence
924 between the genetic maps can be explained by different
925 reasons.. Many previous studies based on reduced genome
926 complexity representation (GBS, DartSeq) were based on a
927 sequence mapping on the orange genome with a numbering
928 and orientation of the chromosomes different from those
929 of the reference genome (the clementine tree) used in the
930 present study. In the absence of a pan genome, it is difficult
931 to make a link between our results and those resulting from
932 mapping on other references and many studies did not make
933 the link with the genomic positions on a given reference but
934 only in relation to the genetic map.

935 Conclusion

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

952 **Supplementary Information** The online version contains supplemen-
953 tary material available at <https://doi.org/10.1007/s11295-022-01571-w>.

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

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, synergy 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.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Agustí M, Martínez-Fuentes A, Mesejo C (2002) Citrus fruit quality. Physiological basis and techniques of improvement. *Agrociencia* VI 2:1–16
- Albertini M-V, Carcouet E, Pailly O, Gambotti C, Luro F, Berti L (2006) Changes in organic acids and sugars during early stages of development of acidic and acidless citrus fruit. *J Agric Food Chem* 54:8335–8339
- Antoine S, Pailly O, Gibon Y, Luro F, Santini J, Giannettini J, Berti L (2016) Short- and long-term effects of carbohydrate limitation on sugar and organic acid accumulation during mandarin fruit growth. *J Sci Food Agric* 96:3906–3914
- Ashrafi H, Foolad MR (2015) Characterization of early blight resistance in a recombinant inbred line population of tomato: II. Identification of QTLs and their co-localization with candidate resistance. *Genes Adv Stud Biol* 7:149–168
- Asins MJ, Bernet GP, Ruiz C, Cambra M, Guerri J, Carbonell EA (2004) QTL analysis of citrus tristeza virus-citradia interaction. *Theor Appl Genet* 108:603–611
- Asins MJ, Raga V, Bernet GP, Carbonell EA (2015) Genetic analysis of reproductive, vegetative and fruit quality traits to improve Citrus varieties. *Tree Genet Genomes* 11:117
- Bain JM (1958) Morphological, anatomical, and physiological changes in the developing fruit of the Valencia orange, *Citrus sinensis* (L) Osbeck. *Aust J Bot* 6:1–23. <https://doi.org/10.1071/BT9580001>
- Baldwin EA (1993) Citrus fruit. In: Seymour G, Taylor J, Tucker G (eds) *Biochemistry of fruit ripening*. Springer Netherlands, 107–149. https://doi.org/10.1007/978-94-011-1584-1_4
- Beavis WD (1998) QTL analyses: power, precision, and accuracy. In: Patterson HA (ed) *Molecular dissection of complex traits*. CRC Press, Boca Raton, Boston, London, New York, Washington, pp 145–162

- Bertin N, Lecomte A, Brunel B, Fishman S, Génard M (2007) A model describing cell polyploidization in tissues of growing fruit as related to cessation of cell proliferation. *J Exp Bot* 58:1903–1913
- Bolarin MC, Estan MT, Caro M, Romero-Aranda R, Cuartero J (2001) Relationship between tomato fruit growth and fruit osmotic potential under salinity. *Plant Sci* 160:1153–1159
- Cabodevila VG, Cambiaso V, Rodríguez GR et al (2021) A segregating population from a tomato second cycle hybrid allows the identification of novel QTL for fruit quality traits. *Euphytica* 217:6
- Calenge F, Drouet D, Denancé C, Van de Weg W, Brisset MN, Paulin JP, Durel CE (2005) Identification of a major QTL together with several minor additive or epistatic QTLs for resistance to fire blight in apple in two related progenies. *Theoret Appl Genetics* 111:128–135
- Causse M, Saliba-Colombani V, Lesschaeve I, Buret M (2001) Genetic analysis of organoleptic quality in fresh market tomato. 2. Mapping QTLs for sensory attributes. *Theor Appl Genet* 102:273–283
- Cercós M, Soler G, Iglesias DJ, Gadea J, Forment J, Talón M (2006) Global analysis of gene expression during development and ripening of citrus fruit flesh. A proposed mechanism for citric acid utilization. *Plant Mol Biol* 62:513–527
- Charmet G (2000) Power and accuracy of QTL detection: simulation studies of one-QTL models. *Agronomie* 20:309–323
- Chen J, Wang N, Fang LC, Liang ZC, Li SH, Wu BH (2015) Construction of a high-density genetic map and QTLs mapping for sugars and acids in grape berries. *BMC Plant Biol* 15:1–14
- Curtolo M, Cristofani-Yaly M, Gazaffi R, Takita MA, Figueira A, Machado MA (2017) QTL mapping for fruit quality in Citrus using DArTseq markers. *BMC Genomics* 18(1):289
- De Vienne D, Causse M (1998) La cartographie et la caractérisation des locus contrôlant la variation des caractères quantitatifs. In :D. de Vienne (ed) *Les marqueurs moléculaires en génétique et biotechnologies végétales*. 2nd edn. INRA, Paris pp 89–118
- Doligez A, Bertrand Y, Farnos M, Grolier M, Romieu C, Esnault F, Dias S, Berger G, François P, Pons T, Ortigosa P, Roux C, Houel C, Laucou V, Bacilieri R, Péros JP, This P (2013) New stable QTLs for berry weight do not colocalize with QTLs for seed traits in cultivated grapevine (*Vitis vinifera* L.). *BMC Plant Biol* 13:217
- Doyle JJ, Doyle JL (1987) A Rapid DNA Isolation Procedure for Small Quantities of Fresh Leaf Tissue. *Phytochem Bull* 19:11–15
- Echeverria E, Valich J (1989) Enzymes of sugar and acid metabolism in stored 'Valencia' oranges *Journal of the American Society for Horticultural Science* (USA)
- Etienne C, Rothan C, Moing A, Plomion C, Bodénès C, Svanella-Dumas L, Cosson P, Pronier V, Monet R, Dirlwanger E (2002) Candidate genes and QTLs for sugar and organic acid content in peach [*Prunus persica* (L.) Batsch]. *Theor Appl Genet* 105:145–159
- Etienne A, Génard M, Bancel D, Benoit S, Bugaud C (2013a) A model approach revealed the relationship between banana pulp acidity and composition during growth and post harvest ripening. *Sci Hortic* 162:125–134
- Etienne A, Génard M, Lobit P, Mbéguié-A-Mbéguié D, Bugaud C (2013b) What controls fleshy fruit acidity? A review of malate and citrate accumulation in fruit cells. *J Exp Bot* 64:1451–1469
- Fang DQ, Federici CT, Roose ML (1997) Development of molecular markers linked to a gene controlling fruit acidity in citrus. *Genome* 40:841–849
- Froelicher Y, Dambier D, Bassene JB, Costantino G, Lotfy S, Didout C, Beaumont V, Brottier P, Risterucci AM, Luro F, Ollitrault P (2008) Characterization of microsatellite markers in mandarin orange (*Citrus reticulata* Blanco). *Mol Ecol Resour* 8:119–122
- García MR, Asins MJ, Carbonell EA (2000) QTL analysis of yield and seed number in Citrus. *Theor Appl Genet* 101:487–493

- 1077 Garcia-Lor A, Curk F, Morillon R, Ancillo G, Luro F, Navarro L, Ollitrault P (2012) Nuclear phylogeny within Citrus (Rutaceae) and four related genera. *Ann Bot* 111:1–9 1143
- 1078 1144
- 1079 Garcia-Luis A, Didehvar F, Guardiola J, Baker D (1991) The transport of sugars in developing fruits of satsuma mandarin. *Ann Bot* 68:349–357 1145
- 1080 1146
- 1081 1147
- 1082 Gmitter F Jr, Grosser J, Castle W, Moore G, Khan I (2007) A comprehensive citrus genetic improvement programme. Citrus genetics, breeding and biotechnology. Cabi, Cambridge, pp 9–18 1148
- 1083 1149
- 1084 Goldenberg L, Yaniv Y, Kaplunov T, Doron-Faigenboim A, Porat R, Carmi N (2014) Genetic diversity among mandarins in fruit-quality traits. *J Agric Food Chem* 62:4938–4946. <https://doi.org/10.1021/jf5002414> 1150
- 1085 1151
- 1086 Goldman IL, Paran I, Zamir D (1995) Quantitative trait locus analysis of a recombinant inbred line population derived from a *Lycopersicon esculentum* × *Lycopersicon cheesmanii* cross. *Theoret Appl Genetics* 90:925–932 1152
- 1087 1153
- 1088 Goldschmidt EE, Koch KE (1996) Citrus. In: Photoassimilate distribution in plants and crops. 1st edition New York, pp 928–<https://doi.org/10.1201/9780203743539> 1154
- 1089 1155
- 1090 Gomez L, Bancel D, Rubio E, Vercambre G (2007) The microplate reader: an efficient tool for the separate enzymatic analysis of sugars in plant tissues—validation of a micro-method. *J Sci Food Agric* 87:1893–1905 1156
- 1091 1157
- 1092 Grandillo S, Ku H, Tanksley S (1999) Identifying the loci responsible for natural variation in fruit size and shape in tomato. *Theor Appl Genet* 99:978–987 1158
- 1093 1159
- 1094 Guo F, Yu H, Tang Z, Jiang X, Wang L, Wang X, Xu Q, Deng X (2015) Construction of a SNP-based high-density genetic map for pummelo using RAD sequencing. *Tree Genet Genomes* 11:2 1160
- 1095 1161
- 1096 Hockema BR, Etxeberria E (2001) Metabolic contributors to drought-enhanced accumulation of sugars and acids in oranges. *J Amer Soc Hort Sci.* 126:599–605 1162
- 1097 1163
- 1098 Houel C, Chatbanyong R, Doligez A, Rienth M, Foria S, Luchaire N, Roux C, Adivèze A, Lopez G, Farnos M, Pellegrino A, This P, Romieu R, Torregrosa L (2015) Identification of stable QTLs for vegetative and reproductive traits in the microvine (*Vitis vinifera* L.) using the 18 K Infinium chip. *BMC Plant Biol* 15:205 1164
- 1099 1165
- 1100 Hussain SB, Shi CY, Guo LX, Kamran HM, Sadka A, Liu YZ (2017) Recent advances in the regulation of citric acid metabolism in citrus fruit. *Crit Rev Plant Sci* 36:241–256 1166
- 1101 1167
- 1102 Iglesias DJ, Cercós M, Colmenero-Flores JM, Naranjo MA, Ríos G, Carrera E, Ruiz-Rivero O, Lliso I, Morillon R, Tadeo FR, Talon M (2007) Physiology of citrus fruiting Brazilian. *J Plant Physiol* 19:333–362 1168
- 1103 1169
- 1104 Imai A, Yoshioka T, Hayashi T (2017) Quantitative trait locus (QTL) analysis of fruit-quality traits for mandarin breeding in Japan. *Tree Genet Genomes* 13:79 1170
- 1105 1171
- 1106 Imai A, Nonaka K, Kuniga T, Yoshioka T, Hayashi T (2018) Genome-wide association mapping of fruit-quality traits using genotyping-by-sequencing approach in citrus landraces, modern cultivars, and breeding lines in Japan. *Tree Genet Genomes* 14:24 1172
- 1107 1173
- 1108 Jacquemond C, Agostini D (2013) Connaitre l'arbre et son fonctionnement. In: Les clémentiniers et autres petits agrumes. Eds: Jacquemond C, Curk F, Heuzet M. Quae Versailles, pp.165–182. 1174
- 1109 1175
- 1110 Jones HG, Tardieu F (1998) Modelling water relations of horticultural crops: a review. *Sci Hortic* 74:21–46 1176
- 1111 1177
- 1112 Julhia L, Belmin R, Meynard JM, Pailly O, Casabianca F (2019) Acidity drop and coloration in clementine: implications for fruit quality and harvesting practices. *Archive Ouverte d'INRAE*. <https://doi.org/10.3389/fpls.2019.00754> 1178
- 1113 1179
- 1114 Kenis K, Keulemans J, Davey MW (2008) Identification and stability of QTLs for fruit quality traits in apple. *Tree Genet Genomes* 4:647–661 1180
- 1115 1181
- 1116 Kosambi D (1943) The estimation of map distances from recombination values. *Ann Eugen* 12:172–175 1182
- 1117 1183
- 1118 Ladanyia M (2008) Citrus fruit: biology, technology and evaluation. Elsevier, USA 1184
- 1119 1185
- 1120 Li S, Liu X, Xie X, Sun C, Grierson D, Yin X, Chena K (2015) CrMYB73, a PH-like gene, contributes to citric acid accumulation in citrus fruit. *Sci Hortic* 197:212–217 1186
- 1121 1187
- 1122 Liebhard R, Kellerhals M, Pfammatter W, Jertmini M, Gessler C (2003) Mapping quantitative physiological traits in apple (*Malus* × domestica Borkh.). *Plant Mol Biol* 52:511–526 1188
- 1123 1189
- 1124 Lin PQ, Wang C, Dong W, Jiang Q, Wang D, Li S, Chen M, Liu C, Sun C, Chen K (2015) Transcriptome and metabolome analyses of sugar and organic acid metabolism in Ponkan (*Citrus reticulata*) fruit during fruit maturation. *Gene* 554:64–74 1190
- 1125 1191
- 1126 Luro F, Costantino G, Terol J, Argout X, Allario T, Wincker P, Talon M, Ollitrault P, Morillon R (2008) Transferability of the EST-SSRs developed on Nules clementine (*Citrus clementina* Hort ex Tan) to other Citrus species and their effectiveness for genetic mapping. *BMC Genomics* 9:287 1192
- 1127 1193
- 1128 Mehouchi J, Zaragoza S, Agusti M, Talon M, Primo-Millo E (1995) Defoliation increases fruit abscission and reduces carbohydrate levels in developing fruits and woody tissues of Citrus unshiu. *Plant Sci* 107:189–197 1194
- 1129 1195
- 1130 Minamikawa MF, Nonaka K, Kaminuma E, Kajiya-Kanegae H, Onogi A, Goto S, Yoshioka T, Imai A, Hamada H, Hayashi T, Matsumoto M, Katayose Y, Toyoda A, Fujiyama A, Nakamura Y, Shimizu T, Iwata H (2017) Genome-wide association study and genomic prediction in citrus: potential of genomics-assisted breeding for fruit quality traits. *Sci Rep* 7:4721 1196
- 1131 1197
- 1132 Monforte AJ, Asíns MJ, Carbonell EA (1999) Salt tolerance in *Lycopersicon* spp. VII. Pleiotropic action of genes controlling earliness on fruit yield. *Theor Appl Genet* 98:593–601 1198
- 1133 1199
- 1134 Monselise S (1986) Citrus. In: Monselise S (ed) Handbook of fruit set development. CRC Press, USA, pp 87–108 1200
- 1135 1201
- 1136 Mou J, Zhang Z, Qiu H, Lu Y, Zhu X, Fan Z, Zhang Q, Ye J, Fernie AR, Cheng Y, Deng X, Wen W (2021) Multiomics-based dissection of citrus flavonoid metabolism using a citrus *reticulata* × *Poncirus trifoliata* population. *Hortic Res* 8:56 1202
- 1137 1203
- 1138 Nishawy E, Sun X, Ewas M, Ziaf K, Xu R, Wang D, Amar M, Zeng Z, Cheng Y (2015) Overexpression of Citrus grandis DREB gene in tomato affects fruit size and accumulation of primary metabolites. *Sci Hortic* 192:460–467 1204
- 1139 1205
- 1140 Nishio S, Yamada M, Awamura Y, Takada N, Saito T (2011) Environmental variance components of fruit ripening date as used in both phenotypic and marker-assisted selection in Japanese pear breeding. *HortScience* 46:1540–1544 1206
- 1141 1207
- 1142 Ollitrault P, Terol J, Chen C, Federici CT, Lotfy S, Hippolyte I, Ollitrault F, Bérard A, Chauveau A, Cuenca J, Costantino G, Kacar Y, Mu L, Garcia-Lor A, Froelicher Y, Aleza P, Boland A, Billot C, Navarro L, Luro F, Roose ML, Gmitter FG, Talon M, Brunel D (2012) A reference genetic map of *C. clementina* hort. ex Tan.; citrus evolution inferences from comparative mapping. *BMC Genomics* 13:593 1207
- 1143 1208
- 1144 Ollitrault P, Terol J, Garcia-Lor A, Bérard A, Chauveau A, Froelicher Y, Belzile C, Morillon R, Navarro L, Brunel D, Talon M (2012) SNP mining in *C. clementina* BAC end sequences; transferability in the Citrus genus (Rutaceae), phylogenetic inferences and perspectives for genetic mapping. *BMC Genomics* 13:13 1209
- 1145 1210
- 1146 Ollitrault P (2019) Mapping and exploiting the citrus genome. In: Yahia EM (ed) Achieving sustainable cultivation of tropical fruits. Burleigh Dodds Science Publishing, Cambridge, pp 3–42. (Burleigh Dodds Series in Agricultural Science) 1211
- 1147 1212
- 1148 Ollitrault P and Luro F (1997) Les agrumes. In: Charrier A, Jacquot M, Hamon S, Nicolas D (eds) Amélioration des plantes tropicales. CIRAD, Orstom, pp 13–36 1213
- 1149 1214
- 1150 Paterson AH (1997) Molecular dissection of complex traits. CRC-Press, Boca Raton 1215

- 1208 Paterson AH, Damon S, Hewitt JD, Zamir D, Rabinowitch HD, Lin- 1268
 1209 coln SE, Lander ES, Tanksley SD (1991) Mendelian factors 1269
 1210 underlying quantitative traits in tomato: comparison across 1270
 1211 species, generations, and environments. *Genetics* 127:181–197 1271
 1212 Prudent M, Causse M, Genard M, Tripodi P, Grandillo S, Bertin N 1272
 1213 (2009) Genetic and physiological analysis of tomato fruit weight 1273
 1214 and composition: influence of carbon availability on QTL detec- 1274
 1215 tion. *J Exp Bot* 60:923–937 1275
 1216 Quilot B, Kervella J, Génard M, Lescourret F (2005) Analysing 1276
 1217 the genetic control of peach fruit quality through an ecophysio- 1277
 1218 logical model combined with a QTL approach. *J Exp Bot* 1278
 1219 56:3083–3092 1279
 1220 Rawandoozi ZJ, Hartmann TP, Carpenedo S et al (2020) Identifica- 1280
 1221 tion and characterization of QTLs for fruit quality traits in peach 1281
 1222 through a multi-family approach. *BMC Genomics* 21:522 1282
 1223 Robinson GK (1991) That BLUP is a good thing: the estimation of 1283
 1224 random effects. *Statist Sci* 6:15–32. [https://doi.org/10.1214/ss/](https://doi.org/10.1214/ss/1177011926) 1284
 1225 [1177011926](https://doi.org/10.1214/ss/1177011926) 1285
 1226 Roose ML (2007) Mapping and marker-assisted selection. In: Khan 1286
 1227 I (ed) *Citrus genetics, breeding and biotechnology*. CABI, UK, 1287
 1228 pp 275–285 1288
 1229 Rousseaux MC, Jones CM, Adams D, Chetelat R, Bennett A, Pow- 1289
 1230 ell A (2005) QTL analysis of fruit antioxidants in tomato using 1290
 1231 *Lycopersicon pennellii* introgression lines. *Theor Appl Genet* 1291
 1232 111:1396–1408 1292
 1233 Royston P (1995) Remark AS R94: a remark on algorithm AS 181: the 1293
 1234 W-test for normality. *Appl Stat* 44:547–551 1294
 1235 Rymenants M, Van de Weg E, Auwerkerken A, De Wit I, Czech A, Nij- 1295
 1236 land B, Heuven H, De Storme N, Keulemans W (2020) Detection 1296
 1237 of QTL for apple fruit acidity and sweetness using sensorial evalua- 1297
 1238 tion in multiple pedigreed full-sib families. *Tree Genet Genomes* 1298
 1239 16:71. <https://doi.org/10.1007/s11295-020-01466-8> 1299
 1240 Sadka A, Dahan E, Or E, Cohen L (2000) NADP+-isocitrate dehydro- 1300
 1241 genase gene expression and isozyme activity during citrus fruit 1301
 1242 development. *Plant Sci* 158:173–181 1302
 1243 Saliba-Colombani V, Causse M, Langlois D, Philouze J, Buret M 1303
 1244 (2001) Genetic analysis of organoleptic quality in fresh market 1304
 1245 tomato. 1. Mapping QTLs for physical and chemical traits. *Theor* 1305
 1246 *Appl Genetics* 102:259–272 1306
 1247 Segura V, Durel C-E, Costes E (2009) Dissecting apple tree archi- 1307
 1248 tecture into genetic, ontogenetic and environmental effects: QTL 1308
 1249 mapping. *Tree Genet Genomes* 5:165–179 1309
 1250 Shimada T, Fujii H, Endo T, Ueda T, Sugiyama A, Nakano M, Kita 1310
 1251 M, Yoshioka T, Shimizu T, Nesumi H, Ikoma Y, Moriguchi T, 1311
 1252 Omura M (2014) Construction of a citrus framework genetic 1312
 1253 map anchored by 708 gene-based markers. *Tree Genet Genomes* 1313
 1254 10(4):1001–1013 1314
 1255 Siviero A, Cristofani M, Furtado E, Garcia AF, Coelho AG, Machado 1315
 1256 M (2006) Identification of QTLs associated with citrus resistance 1316
 1257 to *Phytophthora gummosis*. *J Appl Genet* 47:23–28 1317
 1258 Spiegel-Roy P, Goldschmidt EE (1996) *The biology of citrus*. Cam- 1318
 1259 bridge University Press, Israel 1319
 1260 Stam P (1993) Construction of integrated genetic linkage maps by 1320
 1261 means of a new computer package: Join Map. *Plant J* 3:739–744 1321
 1262 Staub JE, Serquen FC, Gupta M (1996) Genetic markers, map con- 1322
 1263 struction, and their application in plant breeding. *HortScience* 1323
 1264 31:729–741 1324
 1265 Sugiyama A, Omura M, Matsumoto H (2011) Quantitative trait loci 1325
 1266 (QTL) analysis of carotenoid content in citrus fruit. *J Jpn Soc* 1326
 1267 *Hortic Sci* 80:136–144 1327
- Tadeo F, Cercos M, Colmenero-Flores JM, Iglesias D, Naranjo MA, 1268
 Rios G, Carrera E, Ruiz-Rivero O, Lliso I, Morillon R, Ollitrault 1269
 P, Talon M (2008) Molecular physiology of development and 1270
 quality of citrus. *Adv Bot Res* 4:147–223 1271
 Terol J, Naranjo MA, Ollitrault P, Talon M (2008) Development of 1272
 genomic resources for *Citrus clementina*: characterization of three 1273
 deep-coverage BAC libraries and analysis of 46,000 BAC end 1274
 sequences. *BMC Genomics* 9:423 1275
 Ting S, Attaway J (1971) *Citrus fruits*. In: *The biochemistry of fruits* 1276
 and their products 2 Academic Press, London, New York, pp 1277
 107–169 1278
 Tozlu I, Guy CL, Moore GA (1999) QTL analysis of Na⁺ and Cl⁻ 1279
 accumulation related traits in an intergeneric BC1 progeny of 1280
Citrus and *Poncirus* under saline and nonsaline environments. 1281
Genome 42:692–705 1282
 Van Ooijen J (2006) JoinMap® 4, Software for the calculation of 1283
 genetic linkage maps in experimental populations. Kyazma BV, 1284
 Wageningen, Netherlands 1285
 Van Ooijen J (2009) MapQTL 6 Software for the mapping of quan- 1286
 titative trait loci in experimental populations of diploid species. 1287
 Kyazma BV, Wageningen, Netherlands 1288
 Voorrips RE (2002) MapChart: software for the graphical presentation 1289
 of linkage maps and QTLs. *J Hered* 93:77–78 1290
 Woodward TJ, Clearwater MJ (2008) Relationships between ‘Hayward’ 1291
 kiwifruit weight and dry matter content. *Postharvest Biol Technol* 1292
 48:378–382 1293
 Wu GA, Prochnik S, Jenkins J, Salse J, Hellsten U, Murat FX, Ruiz 1294
 M, Scalabrin SJ, Takita MA, Labadie K, Poulain J, Couloux A, 1295
 Jabbari I, Cattonaro F, Fabbro CD, Pinosio S, Zuccolo A, Chap- 1296
 man J, Grimwood J, Tadeo FR, Estornell LH, Muñoz-Sanz JV, 1297
 Ibanez V, Herrero-Ortega A, Aleza P, Pérez-Pérez J, Ramón D, 1298
 Brunel D, Luro F, Chen C, Farmerie WG, Desany B, Kodira C, 1299
 Mohiuddin M, Harkins T, Fredrikson K, Burns P, Lomsadze A, 1300
 Borodovsky M, Reforgiato G, Freitas-Astúa J, Quetier F, Navarro 1301
 L, Roose M, Wincker P, Schmutz J, Morgante M, Machado MA, 1302
 Talon M, Jaillon O, Ollitrault P, Gmitter G, Rokhsa D (2014) 1303
 Sequencing of diverse mandarin, pummelo and orange genomes 1304
 reveals complex history of admixture during citrus domestication. 1305
Nat Biotechnol 32:656–662 1306
 Xu Q, Chen LL, Ruan X, Chen D, Zhu A, Chen C, Bertrand D, Jiao 1307
 WB, Hao BH, Lyon MP et al (2013) The draft genome of sweet 1308
 orange (*Citrus sinensis*). *Nat Genet* 45(1):59–66 1309
 Yakushiji H, Nonami H, Fukuyama T, Ono S, Takagi N, Hashimoto 1310
 Y (1996) Sugar Accumulation Enhanced by Osmoregulation in 1311
 Satsuma Mandarin Fruit. *J Amer Soc Hort Sci*. 121(3):466–472 1312
 Yu Y, Chen C, Gmitter FG (2016) QTL mapping of mandarin (*Citrus* 1313
reticulata) fruit characters using high-throughput SNP mark- 1314
 ers. *Tree Genet and Genomes* 12:77. [https://doi.org/10.1007/](https://doi.org/10.1007/s11295-016-1034-7) 1315
[s11295-016-1034-7](https://doi.org/10.1007/s11295-016-1034-7) 1316
 Zhou Y, He W, Zheng W, Tan Q, Xie Z, Zheng C, Hu C (2018) Fruit 1317
 sugar and organic acid were significantly related to fruit Mg of 1318
 six citrus cultivars. *Food Chem* 259:278–285 1319
- Publisher's Note** Springer Nature remains neutral with regard to 1320
 jurisdictional claims in published maps and institutional affiliations. 1321