

# Mapping QTLs associated with fruit quality traits in peach [*Prunus persica* (L.) Batsch] using SNP maps

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**Abstract** Fruit quality is an essential criterion used to select new cultivars in peach breeding programs and is determined based on a combination of organoleptic and nutritional traits. The aim of this study was to identify quantitative trait loci (QTLs) for fruit quality traits in an F<sub>1</sub> nectarine population derived from ‘Venus’ and ‘Big Top’ cultivars. The progeny were evaluated over 4 years for agronomical and biochemical characteristics and genotyped using simple sequence repeat (SSR) markers and ‘IPSC 9K peach SNP array v1’. Two genetic maps were constructed using 411 markers. The ‘Venus’ map spanned 259 cM on nine linkage groups (LGs) with 104 markers. The ‘Big Top’ map spanned 464 cM on 10 LGs with 122 markers. Single or Multiple QTL models mapping was applied separately for each year and all years combined. A total of 54 QTLs mapped over 12 LGs belonged to seven peach chromosomes. Most of the QTLs were consistent over the 4 years of study and were validated with the multi-year analysis. QTLs for total phenolic, flavonoid, and anthocyanin contents were reported for the first time in peach. LG 4 in ‘Venus’ and LG 5 in ‘Big Top’ showed the highest numbers

of QTLs. This work represents the first study in an F<sub>1</sub> nectarine family to identify peach genomic regions that control fruit quality traits using ‘IPSC 9K SNP array v1’ and provides useful information for marker-assisted breeding to produce peaches with better antioxidant content and healthy attributes.

**Keywords** Peach physical map · Vitamin C · Total phenolics · Flavonoids · Anthocyanins · Sugars

## Introduction

Peach [*Prunus persica* (L.) Batsch] is the third most important fruit crop worldwide in terms of production (FAOSTAT 2015). In Spain, the first peach exporter to Europe, peach is an economically important crop; it covers a large area (84,400 ha in 2013), being the second in peach production in the European Union and the third worldwide (FAOSTAT 2015).

Fruit quality is important for the peach industry because it can modify consumer preference. Traits such as flesh texture, color, sweetness, acidity, and other organoleptic attributes may affect the consumption of specific varieties (Crisosto 2002). In recent years, consumers have attached greater importance to functional foods, which have health-promoting properties, such as antioxidant, antimutagenic, and anticarcinogenic effects (Orazem et al. 2011; Vizzotto et al. 2014). In conjunction, these traits represent food quality, which has become a primary goal in many international peach breeding programs in recent decades (Infante et al. 2008; Cantín et al. 2009a; Byrne et al. 2012). Unfortunately, most traits related to fruit quality are quantitatively inherited and the genetic control of many of these traits is still unknown (Eduardo et al. 2011). Determining the genetic basis of these traits is necessary to understand their genetic control and will provide necessary

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information to develop specific approaches to enhance breeding programs (Peace and Norelli 2009).

Peach is one of the best characterized fruit tree species which, due to its short juvenility period and the simplicity of its genome, serves as a model for genetic studies in Rosaceae (Zeballos 2012 and references therein). Moreover, the availability of the T × E *Prunus* reference map (Dirlewanger et al. 2004), the release of the peach genome v1.0 and v2.0 (Arús et al. 2012; Verde et al. 2013), and the recent development of single nucleotide polymorphism (SNP) genotyping platforms offer the opportunity to determine the inheritance of many qualitative and quantitative traits at the molecular level (Frett et al. 2014). Likewise, alignment of the updated physical map to the *Prunus* reference map would provide the *Prunus* research community with a basis for comparing the positions of the major genes and quantitative trait loci (QTLs) identified in several previous studies across different mapping populations.

However, as mentioned above, many agricultural traits are quantitative in nature, and determining their genetic basis is complicated because the majority of genes have little effect and few have substantial effects (Brem and Kruglyak 2005). Many studies have been carried out for QTL identification in *Prunus* (Zeballos 2012). Nevertheless, many important agronomic traits of *Prunus* species have not yet been mapped and only a few are currently being used for marker-assisted selection (including major genes for disease and pest resistance, self-incompatibility, slow ripening, and fruit quality traits such as flesh color, endocarp staining, flesh adherence to stone, non-acid fruit, skin pubescence, skin color, and fruit shape) (Dirlewanger et al. 2004; Eduardo et al. 2015; Ru et al. 2015 and references therein). Important QTLs that control fruit quality traits have been found for total sugar content, organic acid content, fruit weight, acidity, and blooming and harvest dates (Dirlewanger et al. 1999; Etienne et al. 2002; Quilot et al. 2004); blooming and ripening dates (Eduardo et al. 2011; Dirlewanger et al. 2012); chilling injury susceptibility (Cantín et al. 2010a); and other traits anchored in the T × E *Prunus* reference map that have been widely described by Arús et al. (2012).

Most previous studies have been limited because of the low marker density in the maps (Eduardo et al. 2013). However, the availability of SNP genotyping resources has assisted in fine mapping of peach (Martínez-García et al. 2013a, b; Zhebentyayeva et al. 2014). More recently, attempts have been made to map QTLs in peach using the newly developed SNP genotyping array v1 (Verde et al. 2012). Several QTLs that control traits such as chilling and heat requirements (Romeu et al. 2014), blush (Frett et al. 2014), maturity date (Pirona et al. 2013; Fresnedo-Ramírez et al. 2015; Nuñez-Lillo et al. 2015), or other pomological traits, such as fruit weight, soluble solid content, or pH (Fresnedo-Ramírez et al. 2015), have been mapped. Moreover, the current

analytical techniques are more powerful for large-scale phenotyping than older methods and new traits related to fruit quality are being incorporated in QTL analysis. For example, aroma and other volatile compounds were partially mapped onto the *Prunus* reference map (Illa et al. 2011; Eduardo et al. 2013) and were analyzed using a high-throughput gas chromatography-mass spectrometry (GC-MS)-based metabolomics approach (Sánchez et al. 2012). Some phenolic compounds (pigments) were mapped on the T × E reference map (Ogundiwin et al. 2009), and other phenolic compounds (Chagné et al. 2012; Verdu et al. 2014) and vitamin C (Davey et al. 2006) were identified in apple, but to our knowledge, no QTLs that control phenolic compounds (including total phenolic, flavonoid, or anthocyanin contents) or vitamin C have been mapped in peach. These antioxidant compounds are important and potentially beneficial to human health because they are involved in the prevention of degenerative diseases such as hypertension, coronary heart diseases, Alzheimer's disease, stroke, and cancer (Boeing et al. 2012; Martin et al. 2013; Vizzotto et al. 2014).

To our knowledge, there is no peach breeding program that enhances antioxidant contents, despite the importance of healthy nutraceutical compounds from peach and other fruits (Wargovich et al. 2012). The peach breeding program at the Experimental Station of Aula Dei-CSIC has studied, over a period of 10 years, a nectarine population derived from a cross between 'Venus' and 'Big Top' cultivars. This progeny has been phenotyped for agronomic and fruit quality attributes over a period of 4 years. Moreover, this population has been genotyped with simple sequence repeats (SSRs) and 'IPSC 9K peach SNP array v1' markers.

The main objective of this study was to identify genetic regions associated with the most important peach pomological traits using the 'Venus' × 'Big Top' mapping population. To achieve this goal, two genetic maps were constructed with 'IPSC 9K peach SNP array v1' and six SSR markers and the obtained maps were previously compared with the peach physical map and anchored to the *Prunus* reference map T × E. A QTL analysis was performed using the maps and the phenotypic data obtained during the 4 years of evaluation (2007–2010). In this paper, we describe the identification of genomic regions that regulate the main fruit quality traits in peach using the 'IPSC 9K peach SNP array v1'. Quantitative trait loci for total phenolic, flavonoid, and anthocyanin contents are reported for the first time in this species.

## Material and methods

### Plant material

The mapping population included 75 offspring of F<sub>1</sub> progeny from a cross between two diploid outbreed nectarine

cultivars, with ‘Venus’ as the female parent and ‘Big Top’ as the male parent. Both cultivars are nectarines with red skin and yellow flesh. ‘Venus’ is freestone and acidic, whereas ‘Big Top’ is clingstone and sub-acidic. The progeny were established in the Aula Dei Experimental Station orchards in 2002 as described by Cantín et al. (2009b). One tree per genotype was grafted on GF 677 and grown under standard irrigation, fertilization, and pest control conditions. Winter pruning and spring thinning were conducted as in commercial orchards.

### Agronomical and pomological evaluation

Over a period of 4 years (2007–2010), agronomic and pomological traits were measured in each seedling tree. Production (yield), fruit weight, flesh firmness, soluble solid concentration (SSC), pH, and titratable acidity (TA) were evaluated for 3 years (2007, 2009, and 2010); relative antioxidant capacity (RAC) and contents of vitamin C, total phenolic, flavonoids, anthocyanins, and individual sugars were evaluated for 4 years as previously reported (Cantín et al. 2009b, 2010b; Abidi et al. 2011, 2015). For production, all fruits from each tree were harvested and weighed (kg/tree). Then, a sub-sample of 20 fruits/tree was weighed to calculate the average fruit weight, which was used for subsequent analysis. Flesh firmness was measured in 10 fruits with a hand penetrometer. Five fruits were homogenized in a blender to determine the SSC of juice with a temperature-compensated refractometer (model ATC-1; Atago Co., Tokyo, Japan), and pH and TA were measured with an automatic titration system (862 Compact Titrosampler; Metrohm, Herisau, Switzerland). Ripening index (RI) was calculated as the ratio of SSC to TA. For biochemical analyses, five arbitrarily selected fruits were peeled and cut into small cubes (~1 cm<sup>3</sup>) to pool homogeneous sub-samples of 5 g flesh, immediately frozen in liquid nitrogen and then stored at –20 °C until analysis. To preserve ascorbic acid, sub-samples were frozen with 5 mL of meta-phosphoric acid (5 %) in liquid nitrogen and then stored at –20 °C. Then, samples were homogenized in a polytron for 2 min with 10 mL of the extraction solution of 0.5 mol L<sup>-1</sup> HCl in 800 mL L<sup>-1</sup> methanol for phenolic content, 800 mL L<sup>-1</sup> ethanol for sugar content, and 50 mL L<sup>-1</sup> meta-phosphoric for vitamin C and processed as previously described (Cantín et al. 2009b; Abidi et al. 2011). Vitamin C, total phenolic, flavonoid, and anthocyanin contents and RAC were evaluated with colorimetric methods and measured using a spectrophotometer (Beckman Coulter DU 800; Beckman Coulter, Brea, CA, USA) as previously described (Abidi et al. 2011, 2015). For the sugar profile, sugar composition and quantification were analyzed by high-performance liquid chromatography as described by Cantín et al. (2009a) with some modifications as described in Abidi et al. (2011).

### Statistical analysis

Descriptive statistics of all phenotypic data were calculated using SPSS® 22.0 (IBM®). Data were averaged and minimum and maximum values were identified. To evaluate whether the data followed a normal distribution, a normality analysis by Kolmogorov-Smirnov and Shapiro-Wilk tests was performed separately each year/trait. Histograms for each trait were constructed with all data set. Pearson’s correlation coefficients among years were calculated for 2007, 2008, 2009, and 2010. The number of records varied from year to year. Correlations among variables were performed with the mean value for all years. Since correlations between years for most of the traits were low or moderate, a QTL analysis was carried out separately for each year. The Box-Cox transformation method was used for non-normally distributed traits to perform multiple QTL model (MQM)-QTL analysis.

### Population genotyping and marker selection

For genotyping, total DNA was extracted from the young leaves of both parents, ‘Venus’ and ‘Big Top’, and each progeny using the DNeasy® Plant Mini Kit (QIAGEN Inc., Valencia, CA, USA) following the manufacturer’s instructions. DNA concentration and quality was checked using PicoGreen® dye and measured in a fluorospectrometer. Then, all samples were genotyped using the ‘IPSC 9K peach SNP array v1’, which includes 8144 SNP markers (Verde et al. 2012), using the single-base extension assay (Steemers et al. 2006) and Illumina® Infinium® HD Assay Ultra protocol (Illumina, San Diego, CA, USA). The analysis was conducted by the Endocrinology Laboratory Service at *Hospital Clínico Universitario de Valencia*.

For mapping, we also selected genotypic data of six SSR markers previously evaluated in the same progeny (BPPCT025, BPPCT033, BINEPPCU6377, pchcms5, UCDCH15, and UDP98-024) (Cantín et al. 2010a; Abidi et al. 2012).

Self-pollinated seedlings were identified using homozygous SNP markers with different alleles in both parents. Seedlings with the same genotype as the female parent were excluded from further analysis.

Markers with missing data (in one or both parents) that are non-polymorphic, redundant, or deviated from the expected segregation proportion were excluded. When markers had the same segregation pattern, only one marker was included to improve computational algorithm efficiency (Van Ooijen 1992).

For the segregation deviation test, a chi-square test was performed with  $p=0.05$  as the threshold (Zeballos et al. 2015). In a second round, the markers were adjusted to a 1:1 segregation ratio with a  $p=0.005$  threshold.

## Map construction

JoinMap® 4 software (Van Ooijen 2006) was used to construct the linkage maps as a cross-pollinated population, following the software manual instructions. To use the double pseudo-test cross strategy (Grattapaglia and Sederoff 1994), the option “Create maternal and paternal population nodes” command in JoinMap was used. Two mapping rounds were performed.

A preliminary number of groups and linkage groups (LGs) were established using the recombination fraction criterion (see details in Zeballos et al. 2015). A second mapping round was performed with the selected SNPs by including the SSR markers, the order of markers in each LG was established using the maximum likelihood mapping option, and map distances were calculated using Kosambi’s mapping function. Further details regarding genetic map construction can be found in Zeballos (2012).

## QTL analysis

A QTL analysis was carried out using R/qtl (v1.22-21) software with the single QTL model (SQM) or MQM procedures using the R platform (Broman et al. 2003). Single regression (Haley-Knott) was conducted for non-normalized (non-transformed) traits (pH, TA, RI, firmness (2007), and glucose (2009)). QTL analyses were performed for each trait separately for each year and with the overall mean (2007–2010). The likelihood of the presence of a QTL was expressed as a log of odds (LOD) score. LOD significance thresholds were

determined with the permutation test procedure; option settings included 1000 permutations, and significance was set to  $p=0.05$ . When the LOD score exceeded the significance threshold somewhere along an LG, a segregating QTL was declared. For the confidence interval, we used the *Bayesian command* in R/qtl with  $p=0.90$  and  $p=0.95$  for outer and inner interval bounds, respectively (Broman and Saunak 2009). Graphical representation of QTLs on maps was generated with MapChart® v2.2 software (Voorrips 2002).

A multi-year QTL analysis was carried out using QTLNetwork-2.1 software (<http://ibi.zju.edu.cn/software/qtlnetwork/>) to explore environmental effects and to increase the accuracy for QTL detection (Yang et al. 2008).

## Results

### Phenotyping

Six out of the 75 initial seedlings of the ‘Venus’ × ‘Big Top’ progeny were identified as self-pollinating. With the SNP markers, a new genotype was identified in addition to the five previously reported as self-pollinated in the same population (Cantín et al. 2010a).

The results for the pomological traits evaluated over 4 years (2007–2010) in the remaining seedlings are summarized in Table 1. Wide phenotypic variation was found for most of the traits studied in this progeny (Abidi et al. 2011; Zeballos et al. 2015), which supports the quantitative nature of these traits. Distribution of the traits has

**Table 1** Units, minimum, maximum, and mean values for the pomological traits evaluated in the ‘Venus’ × ‘Big Top’ progeny during the four years (2007–2010) (partially presented in Zeballos et al. 2015)

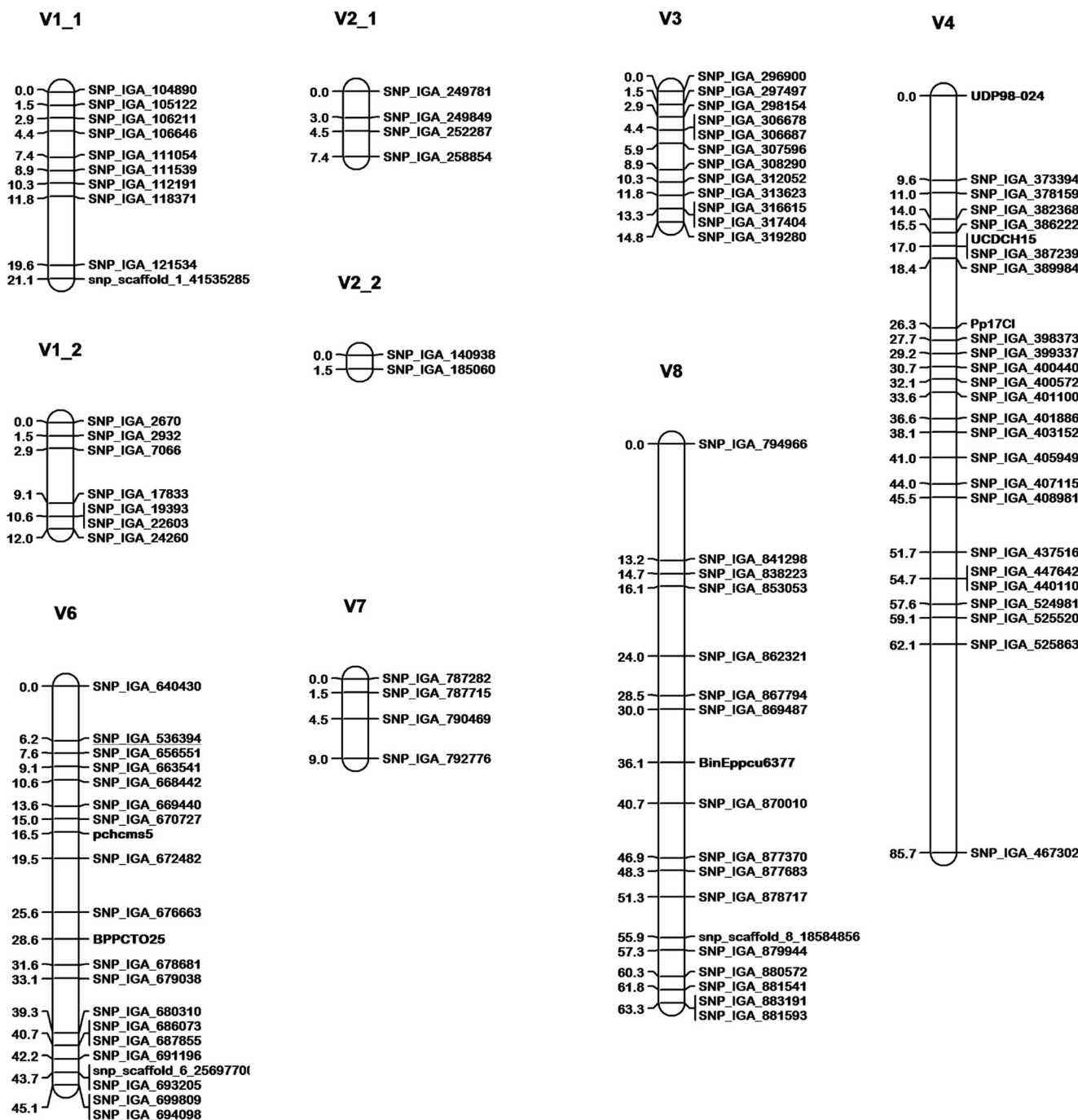
| Trait                               | Units                 | Min <sup>a</sup> | Max <sup>b</sup> | Mean ± SE      |
|-------------------------------------|-----------------------|------------------|------------------|----------------|
| Production/yield                    | kg/tree               | 0.83             | 19.70            | 7.09 ± 0.29    |
| Fruit weight (FW)                   | g                     | 69.44            | 375.87           | 185.22 ± 3.30  |
| Firmness                            | N                     | 6.23             | 60.76            | 40.78 ± 0.68   |
| Soluble solids concentration (SSC)  | °Brix (g SS/100 g FW) | 9.20             | 20.20            | 13.36 ± 0.13   |
| pH                                  | pH units              | 3.00             | 4.40             | 3.68 ± 0.02    |
| Titrateable acidity (TA)            | g malic acid/100 g FW | 0.24             | 1.52             | 0.64 ± 0.02    |
| Ripening index (RI) SSC/TA)         | g SS/g malic acid     | 7.55             | 66.98            | 25.60 ± 0.84   |
| Total sugars                        | g/kg FW               | 45.35            | 160.34           | 86.85 ± 1.14   |
| Sucrose                             | g/kg FW               | 23.16            | 109.79           | 57.18 ± 0.90   |
| Glucose                             | g/kg FW               | 6.58             | 24.00            | 11.58 ± 0.20   |
| Sorbitol                            | g/kg FW               | 1.00             | 18.79            | 5.83 ± 0.25    |
| Fructose                            | g/kg FW               | 7.43             | 21.53            | 12.07 ± 0.17   |
| Vitamin C                           | mg AsA/100 g FW       | 1.17             | 12.11            | 4.10 ± 0.13    |
| Total phenolics                     | mg GAE/100 g FW       | 12.10            | 58.85            | 32.32 ± 0.87   |
| Flavonoids                          | mg CE/100 g FW        | 1.58             | 60.13            | 12.69 ± 0.61   |
| Anthocyanins                        | mg C3GE/kg FW         | 0.32             | 25.72            | 3.14 ± 0.21    |
| Relative antioxidant Capacity (RAC) | mg TE/kg FW           | 125.31           | 1099.59          | 447.87 ± 11.26 |

Data are mean ± SE ( $n=198-257$ )

AsA ascorbic acid, GAE gallic acid equivalents, CE catechin equivalents, C3GE cyanidin-3-glucoside equivalents, TE Trolox equivalents, SE standard error

<sup>a</sup> Minimum value

<sup>b</sup> Maximum value



**Fig. 1** Genetic linkage map of ‘Venus’. Nine linkage groups of ‘Venus’. In each linkage group name, *V* refers to the ‘Venus’ parental, the *first number* to the scaffold that it represents and the *second one* to the subgroup when the scaffold is represented by more than one linkage group.

See the absence of scaffold 5 and the separation in the two groups of scaffolds 1 and 2. SSR markers are in **bold** (UDP98-024 and UCDCH15 in V4, pchcms5 and BPPCTO25 in V6, and BINEPPCU6377 in V8), and the SNP\_IGA\_536394 in V6 is *underlined*

been reported as Supplementary Fig. 1. Pearson’s correlations between years and traits are summarized in Supplementary Tables 1 and 2. Significant correlations were found in years 2007, 2009, and 2010 (high for yield, fruit weight, pH, TA, and RI and moderate for firmness and SSC). Pearson’s coefficients were significant in 2007, 2008, and 2009 for glucose, sorbitol, and

fructose. The lower correlation values were found for antioxidants in 2007 and sugars (except sorbitol) in 2010, respectively. The evaluated traits showed significant and high correlations for SSC and sugars (total sugars, sucrose, glucose, sorbitol, and fructose). Titratable acidity and pH were highly negatively correlated ( $r=-0.804$ ). Higher Pearson’s correlations were

found between total sugars and sucrose ( $r=0.910$ ) and total phenolics and flavonoids ( $r=0.828$ ).

### Marker screening and linkage mapping

Out of the 8144 SNPs, 5323 were non-polymorphic (43 had missing data for either parent and 5280 were homozygous in both parents), 1808 showed the same segregation pattern, and 338 presented a distorted segregation. Finally, a total of 675 SNPs were informative, with GenTrain scores that ranged from 0.35 to 0.92; of these, 270 SNPs were heterozygous in both parents and therefore discarded from analysis because they were not suitable for this mapping strategy. The final number of selected markers was 405 (223 were used for the ‘Venus’ map and 182 for the ‘Big Top’ map). Two preliminary dense genetic maps that were constructed for ‘Venus’ and ‘Big Top’ with 160 and 208 markers, respectively, were found on 11 LGs (Zeballos et al. 2015). Final LG assignment was performed after comparison with the peach genome v1.0 physical map (GDR 2015), and markers with identical segregation patterns that were previously excluded were included for the next step (Zeballos 2012). This information was updated with the peach genome v2.0 physical map (<https://phytozome.jgi.doe.gov/pz/portal.html>, see Supplementary Table 3).

#### Genetic linkage map of the ‘Venus’ parent

The second mapping round included 102 SNPs and five SSRs (BPPCT025, BINEPCU6377, pchcms5, UCDC15, and UDP98-024). The resulting map grouped 99 SNPs (the remaining three markers were not linked) and five SSRs in nine LGs that spanned 259.9 cM (Fig. 1). The length of the LGs ranged from 1.47 to 85.7 cM, with an average distance between adjacent markers of 2.49 cM. Seven scaffolds were represented in this map (1, 2, 3, 4, 6, 7, and 8). Scaffolds 1 and 2 were split into two LGs, and scaffold 5 was not represented in the female parent map. The marker SNP\_IGA\_536394 was correctly mapped on LG V6 (Fig. 1) as it was assigned in peach v2.0 (Pp06, in bold in Supplementary Table 3).

#### Genetic linkage map of the ‘Big Top’ parent

After establishing the most suitable order of markers, the second mapping round placed 122 SNPs and one SSR (BPPCT033) on 10 LGs (Fig. 2). The map spanned 464.3 cM, the length of LGs ranged from 1.47 to 85.4 cM, and the average distance between adjacent markers was 3.8 cM. Nine scaffolds were represented in this map (1, 2, 3, 4, 5, 6, 7, 8, and 13). Scaffolds 1 and 6 were split into two LGs. The marker SNP\_IGA\_430365 was mapped on LG B7 in contrast with the position assigned on the physical map (scaffold 4 and Pp03, in bold in Supplementary Table 3). The other marker apparently mapped in different scaffold,

**Fig. 2** Genetic linkage map of ‘Big Top’. Ten linkage groups of ‘Big Top’. In each linkage group, *B* refers to the ‘Big Top’ parental, the *first number* to the scaffold that it represents and the *second one* to the sub-group when the scaffold is represented by more than one linkage group. See the separation of scaffolds 1 and 6 in the two linkage groups. The SSR marker (BPPCT033) in B8 is in *bold*, and the markers SNP\_IGA\_913769 in B3 and SNP\_IGA\_430365 in B7 are *underlined*

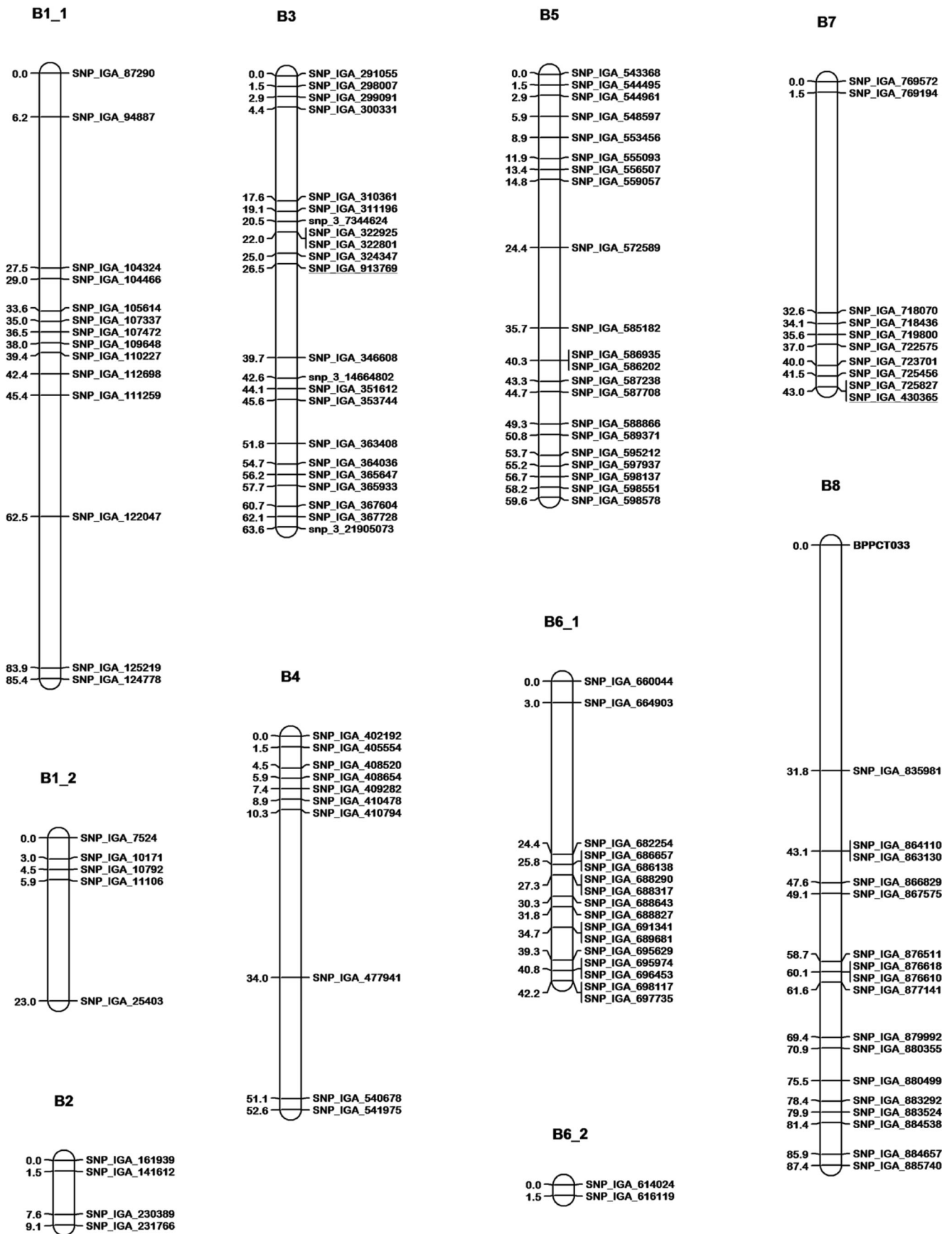
SNP\_IGA\_913769 on LG B3, was positioned in chromosome Pp03 in the peach physical genome v2.0 (in bold in Supplementary Table 3).

### QTL analysis

Using the SQM and MQM methods, at least one QTL was found for 16 out of 17 traits evaluated. No significant QTL was found for vitamin C, although on LG V4, two QTLs that did not overcome the threshold explained more than 10 % of the phenotypic variance (11.5 and 12.8 %, respectively). The list of QTLs with their magnitude of impact across years is presented in Table 2. Analyzing the data by year, 54 QTLs were detected and mapped over 12 LGs that represented seven scaffolds (Figs. 3 and 4). The portion of phenotypic variance explained by each significant QTL ranged between 7.7 and 85.3 % of the total variance (Table 2). When using the multi-year approach, QTLs were detected for all traits except for flavonoids (Table 2). For the multi-year analysis, the explained variation was between 0.9 and 71.1 %. The fraction of the variation explained for the genotype-environment interaction (VGE) ranged from 0.1 to 12.0 %. VGE was below 6 % except for firmness (12 %) and anthocyanin content (8 %).

In both parental maps, QTLs were found for fruit weight; firmness; SSC; total sugar, sucrose, sorbitol, fructose, total phenolic, and flavonoid contents; and RAC (Table 2). Production (yield, kg/tree) and glucose content were mapped only onto the ‘Venus’ map (Table 2, Fig. 3), whereas QTLs for pH, TA, RI, and anthocyanin content were only mapped onto the ‘Big Top’ map (Table 2, Fig. 4). QTLs detected for fruit weight, pH, TA, RI, and sorbitol content explained more than 50 % of the variance and had LOD score values up to 18.0, 28.0, 27.6, 21.6, and 22.2, respectively (Table 2). The number of QTLs found over each LG in the different years of evaluation varied between 1 and 20. In the ‘Venus’ map (Table 2, Fig. 3), LGs V1\_1, V2\_1, V3, V7, and V8 had only one QTL; LG V2\_2 had four QTLs; and LG V4 had 19 QTLs. In the ‘Big Top’ map (Table 2, Fig. 4), LGs B1\_2 and B4 had only one QTL, LGs B2 and B8 had two QTLs, and LG B5 had 20 QTLs that controlled different traits.

With regard to the QTLs identified based on the mean value (not shown in Figs. 3 and 4), most of the traits were consistently significant over time (Table 2, Supplementary Table 4). For example, for fruit weight, QTLs identified on LG V4 in the yearly analysis were in the same region as QTLs were detected with the mean or the multi-year analysis.



**Table 2** QTLs found for agronomic and fruit quality traits detected in the ‘Venus’ × ‘Big Top’ progeny

| Trait               | QTL name           | QTL code | cM   | LOD score | Threshold | VG (%) | VGE (%) | Additive effect (a) | Flanking Markers               | Dist cM |
|---------------------|--------------------|----------|------|-----------|-----------|--------|---------|---------------------|--------------------------------|---------|
| <b>Production</b>   | qPRD.V-Ch1_2-MYear |          | 11.6 |           |           | 8.0    | 0.6     | 1.03                | SNP_IGA_22603-SNP_IGA_24260    |         |
|                     | qPRD.V-Ch3-Mean    | PRD X-b  | 9    | 3.34      | 2.13      | 16.3   |         | 2.46                | SNP_IGA_308290                 | 8.87    |
|                     | qPRD.V-Ch3-MYear   |          | 14.3 |           |           | 8.6    | 0.3     | -1.00               | SNP_IGA_317404-SNP_IGA_319280  |         |
|                     | qPRD.V-Ch4-MYear   |          | 24.4 |           |           | 10.1   | 0.4     | -1.06               | SNP_IGA_389984-Pp17Cl          |         |
|                     | qPRD.V-Ch8-2010    | PRD 10   | 13   | 2.90      | 2.14      | 17.0   |         | 2.62                | SNP_IGA_841298                 | 13.19   |
|                     | qPRD.V-Ch8-Mean    | PRD X-a  | 24   | 3.74      | 2.13      | 18.1   |         | 2.62                | SNP_IGA_862321                 | 23.96   |
| <b>Fruit weight</b> | qFW.V-Ch1_1-2010   | FW 10-c  | 20   | 2.66      | 2.00      | 7.7    |         | 30.28               | SNP_IGA_121534                 | 19.64   |
|                     | qFW.V-Ch4-2007     | FW 07    | 60   | 3.97      | 2.10      | 22.1   |         | 39.21               | SNP_IGA_525520                 | 59.11   |
|                     | qFW.V-Ch4-2009     | FW 09    | 46   | 7.54      | 2.08      | 34.9   |         | 42.53               | SNP_IGA_408981                 | 45.50   |
|                     | qFW.V-Ch4-2010     | FW 10-a  | 47   | 17.97     | 2.00      | 50.1   |         | 68.15               | SNP_IGA_408981                 | 45.50   |
|                     | qFW.V-Ch4-Mean     | FW X-a   | 46   | 17.38     | 2.13      | 54.7   |         | 49.50               | SNP_IGA_408981                 | 45.50   |
|                     | qFW.V-Ch4-MYear    |          | 46.5 |           |           | 33.6   | 3.2     | -16.36              | SNP_IGA_408981-SNP_IGA_437516  |         |
|                     | qFW.V-Ch4-MYear    |          | 62.1 |           |           | 29.9   | 1.5     | -10.67              | SNP_IGA_5558633-SNP_IGA_467302 |         |
|                     | qFW.BT-Ch8-2010    | FW 10-b  | 25   | 3.45      | 2.19      | 19.9   |         | 40.81               | SNP_IGA_835981                 | 31.76   |
|                     | qFW.BT-Ch8-Mean    | FW X-b   | 9    | 3.07      | 2.21      | 15.3   |         | 28.90               | BPPCT033                       | 0.00    |
|                     | qFW.BT-Ch8-MYear   |          | 16.0 |           |           | 11.6   | 2.0     | 16.80               | BPPCT033-SNP_IGA_835981        |         |
| <b>Firmness</b>     | qFF.V-Ch4-2007     | FIR 07   | 48   | 8.37      | 2.33      | 43.2   |         | 12.95               | SNP_IGA_408981                 | 45.50   |
|                     | qFF.V-Ch4-2009     | FIR 09-a | 55   | 3.37      | 2.12      | 19.5   |         | 5.89                | SNP_IGA_440110                 | 54.65   |
|                     | qFF.V-Ch4-Mean     | FIR X-a  | 54   | 8.70      | 2.15      | 37.2   |         | 6.59                | SNP_IGA_440110                 | 54.65   |
|                     | qFF.V-Ch4-MYear    |          | 48.5 |           |           | 18.3   | 12.0    | -3.28               | SNP_IGA_408981-SNP_IGA_437516  |         |
|                     | qFF.BT-Ch5-2009    | FIR 09-b | 12   | 4.74      | 2.21      | 24.9   |         | 6.56                | SNP_IGA_555093                 | 11.90   |
|                     | qFF.BT-Ch5-2010    | FIR 10   | 3    | 3.42      | 2.17      | 19.9   |         | 4.47                | SNP_IGA_544961                 | 2.94    |
|                     | qFF.BT-Ch5-Mean    | FIR X-b  | 12   | 2.27      | 2.23      | 14.6   |         | 4.01                | SNP_IGA_585182                 | 35.73   |
|                     | qFF.BT-Ch5-MYear   |          | 10.9 |           |           | 7.0    | 2.3     | 1.90                | SNP_IGA_553456-SNP_IGA_555093  |         |
| <b>SSC</b>          | qSSC.V-Ch2_2-2009  | SSC 09-d | 0    | 2.52      | 2.15      | 10.7   |         | 1.34                | SNP_IGA_140938                 | 0.00    |
|                     | qSSC.V-Ch4-2007a   | SSC 07-a | 29   | 3.45      | 2.06      | 18.5   |         | -0.29               | SNP_IGA_399337                 | 29.19   |
|                     | qSSC.V-Ch4-2007b   | SSC 07-b | 44   | 5.29      | 2.06      | 28.0   |         | 0.99                | SNP_IGA_407115                 | 44.03   |
|                     | qSSC.V-Ch4-2009a   | SSC 09-a | 32   | 2.61      | 2.15      | 11.3   |         | 0.30                | SNP_IGA_400572                 | 32.13   |
|                     | qSSC.V-Ch4-2009b   | SSC 09-b | 44   | 6.49      | 2.15      | 27.6   |         | 1.57                | SNP_IGA_407115                 | 44.03   |
|                     | qSSC.V-Ch4-2010    | SSC 10-a | 54   | 3.85      | 2.10      | 21.4   |         | 1.78                | SNP_IGA_440110                 | 54.65   |

Trait, QTL name and QTL code, position, LOD score, threshold, genetic variance and additive effect of the QTLs detected, and position of the nearest SNP marker are presented. QTLs from the multi-year analysis are colored in gray

SSC soluble solids concentration, TA titratable acidity, RI ripening index, RAC relative antioxidant capacity. See Table 1 for units

<sup>a</sup> Genetic variance (VG) = fraction of the total variation explained by the QTL

<sup>b</sup> Genetic environmental interaction (VGE) = fraction of the total variation explained by the genetic environmental interaction and marker ranges of QTL

<sup>c</sup> Marker position on the genetic linkage map

Similar situations were observed in QTLs for firmness, SSC, and sorbitol content, which were mapped in the same position

on LG V4 (Table 2, Supplementary Table 4). On LG B5, QTLs for the traits pH, TA, RI, and sucrose and anthocyanin



**Table 2** (continued)

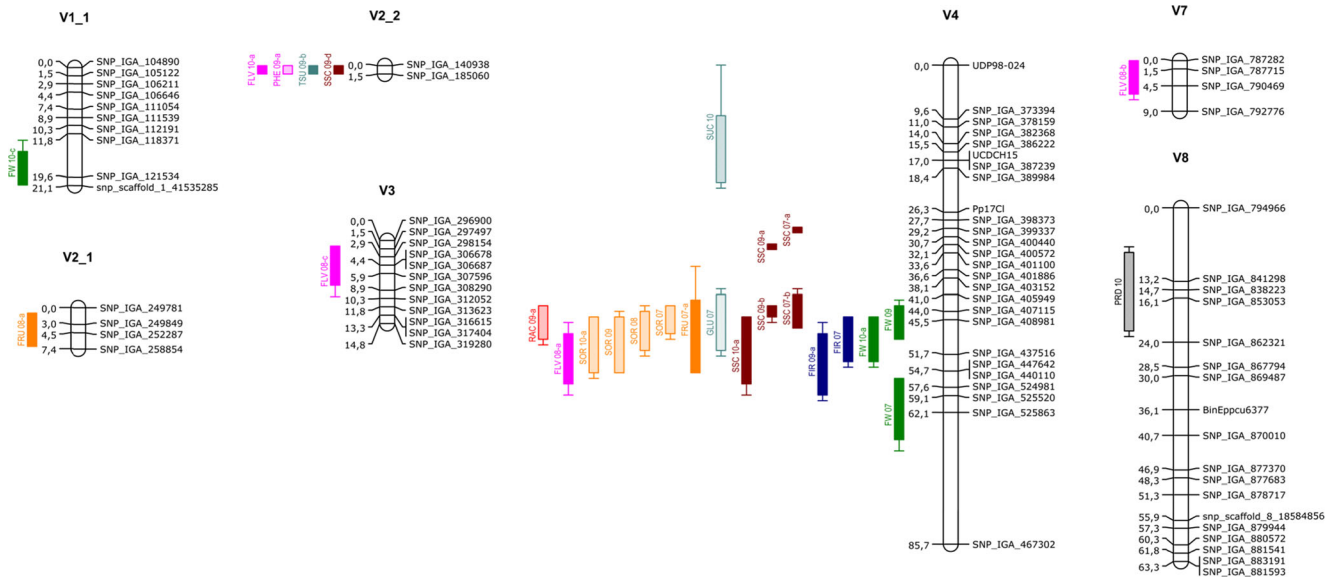
| Trait               | QTL name          | QTL code | cM   | LOD score | Threshold | VG (%) | VGE (%) | Additive effect (a) | Flanking Markers              | Dist cM |
|---------------------|-------------------|----------|------|-----------|-----------|--------|---------|---------------------|-------------------------------|---------|
|                     | qSSC.V-Ch4-Mean   | SSC X-a  | 44   | 4.02      | 2.20      | 22.0   |         | 1.37                | SNP_IGA_407115                | 44.03   |
|                     | qSSC.V-Ch4-MYear  |          | 44.0 |           |           | 16.3   | 0.8     | -071                | SNP_IGA_407115-SNP_IGA_408981 |         |
| <b>SSC</b>          | qSSC.BT-Ch5-2009  | SSC 09-c | 21   | 3.02      | 2.23      | 17.1   |         | 1.48                | SNP_IGA_572589                | 24.39   |
|                     | qSSC.BT-Ch5-2010  | SSC 10-b | 33   | 2.60      | 2.14      | 15.2   |         | 1.18                | SNP_IGA_585182                | 35.73   |
|                     | qSSC.BT-Ch5-Mean  | SSC X-b  | 29   | 2.32      | 2.18      | 13.5   |         | 1.04                | SNP_IGA_572589                | 24.39   |
|                     | qSSC.BT-Ch5-MYear |          | 28.4 |           |           | 7.5    | 1.7     | 0.51                | SNP_IGA_572589-SNP_IGA_585182 |         |
| <b>pH</b>           | qpH.BT-Ch5-2007   | pH 07    | 4    | 15.17     | 2.36      | 52.2   |         | 0.40                | SNP_IGA_544961                | 2.94    |
|                     | qpH.BT-Ch5-2009   | pH 09    | 3    | 27.96     | 2.48      | 85.3   |         | 0.58                | SNP_IGA_544961                | 2.94    |
|                     | qpH.BT-Ch5-2010   | pH 10    | 4    | 21.42     | 2.55      | 75.7   |         | 0.53                | SNP_IGA_544961                | 2.94    |
|                     | qpH.BT-Ch5-Mean   | pH X     | 3    | 26.66     | 2.62      | 84.0   |         | 0.51                | SNP_IGA_544961                | 2.94    |
|                     | qpH.BT-Ch5-MYear  |          | 3.9  |           |           | 71.1   | 1.5     | 0.25                | SNP_IGA_544961-SNP_IGA_548597 |         |
| <b>TA</b>           | qTA.BT-Ch5-2007   | TA 07-a  | 3    | 12.67     | 2.63      | 60.4   |         | 0.43                | SNP_IGA_544961                | 2.94    |
|                     | qTA.BT-Ch5-2009   | TA 09    | 3    | 25.4      | 2.56      | 82.5   |         | -0.37               | SNP_IGA_544961                | 2.94    |
|                     | qTA.BT-Ch5-2010   | TA 10    | 2    | 14.8      | 2.54      | 62.6   |         | -0.59               | SNP_IGA_544961                | 2.94    |
|                     | qTA.BT-Ch5-Mean   | TA X     | 3    | 27.63     | 2.53      | 84.6   |         | -0.46               | SNP_IGA_544961                | 2.94    |
|                     | qTA.BT-Ch5MYear-a |          | 2.9  |           |           | 62.9   | 0.2     | -0.23               | SNP_IGA_544961-SNP_IGA_548597 |         |
|                     | qTA.BT-Ch5MYear-b |          | 53.7 |           |           | 0.9    | 2.7     | 0.01                | SNP_IGA_595212-SNP_IGA_597937 |         |
| <b>RI (SSC/TA)</b>  | qRI.BT-Ch5-2007   | RI 07    | 0    | 7.55      | 2.45      | 42.4   |         | 14.57               | SNP_IGA_543368                | 0.00    |
|                     | qRI.BT-Ch5-2009   | RI 09    | 3    | 17.41     | 2.56      | 69.8   |         | 17.06               | SNP_IGA_544961                | 2.94    |
|                     | qRI.BT-Ch5-2010   | RI 10    | 4    | 11.38     | 2.54      | 53.1   |         | 19.58               | SNP_IGA_548597                | 5.93    |
|                     | qRI.BT-Ch5-Mean   | RI X     | 3    | 21.55     | 2.51      | 76.8   |         | 17.52               | SNP_IGA_544961                | 2.94    |
|                     | qRI.BT-Ch5-MYear  |          | 3.9  |           |           | 53.0   | 1.1     | 8.71                | SNP_IGA_544961-SNP_IGA_548597 |         |
| <b>Total sugars</b> | qTSU.V-Ch2_2-2009 | TSU 09-b | 0    | 2.52      | 2.14      | 15.5   |         | 11.66               | SNP_IGA_140938                | 0.00    |
|                     | qTSU.BT-Ch4-2010  | TSU 10   | 34   | 2.20      | 2.05      | 14.9   |         | 15.20               | SNP_IGA_477941                | 33.97   |
|                     | qTSU.BT-Ch5-2009  | TSU 09-c | 19   | 3.15      | 2.23      | 16.6   |         | 12.01               | SNP_IGA_559057                | 14.84   |
|                     | qTSU.BT-Ch5-Mean  | TSU X-c  | 23   | 2.43      | 2.01      | 15.1   |         | 10.10               | SNP_IGA_572589                | 24.39   |
|                     | qTSU.BT-Ch5-MYear |          | 20.8 |           |           | 12.2   | 0.1     | 6.08                | SNP_IGA_559057-SNP_IGA_572589 |         |
| <b>Sucrose</b>      | qSUC.V-Ch4-2010   | SUC 10   | 11   | 2.37      | 2.13      | 18.9   |         | -11.73              | SNP_IGA_378159                | 11.02   |
|                     | qSUC.BT-Ch5-2007  | SUC 07   | 5    | 2.87      | 2.16      | 18.6   |         | -12.86              | SNP_IGA_548597                | 5.93    |
|                     | qSUC.BT-Ch5-2009  | SUC 09   | 17   | 4.21      | 2.19      | 22.0   |         | 10.89               | SNP_IGA_559057                | 14.84   |
|                     | qSUC.BT-Ch5-Mean  | SUC X    | 3    | 6.14      | 2.13      | 30.4   |         | 10.61               | SNP_IGA_544961                | 2.94    |
|                     | qSUC.BT-Ch5-MYear |          | 4.9  |           |           | 16.8   | 0.5     | 5.70                | SNP_IGA_544961-SNP_IGA_548597 |         |
| <b>Glucose</b>      | qGLU.V-Ch4-2007   | GLU 07   | 48   | 4.07      | 2.06      | 23.8   |         | 2.33                | SNP_IGA_408981                | 45.50   |

contents were mapped at the same position as the mean and the multi-year analysis (Table 2). Some QTLs, such as those for glucose and flavonoid contents, did not appear when using the mean value, even though they were identified in analysis across multiple years. However, some QTLs were mapped

based only on the mean value such as for production and total phenolic content (qPDR.V-Ch3-Mean and qPHE.V-Ch4-Mean, respectively) or were only detected by the multi-year analysis (qPDR.V-Ch1\_2-MYear, qPDR.V-Ch4-MYear, qGLU.BT-Ch3-MYear, and qFRU.BT-Ch8-MYear).

**Table 2** (continued)

| Trait                  | QTL name           | QTL code | cM   | LOD score | Threshold | VG (%) | VGE (%) | Additive effect (a) | Flanking Markers              | Dist cM |
|------------------------|--------------------|----------|------|-----------|-----------|--------|---------|---------------------|-------------------------------|---------|
|                        | qGLU.V-Ch4-MYear   |          | 47.5 |           |           | 9.0    | 6.0     | -0.67               | SNP_IGA_408981-SNP_IGA_437516 |         |
|                        | qGLU.BT-Ch3-MYear  |          | 26.5 |           |           | 5.4    | 2.0     | 0.50                | SNP_IGA_913739-SNP_IGA_346608 |         |
| <b>Sorbitol</b>        | qSOR.V-Ch4-2007    | SOR 07   | 46   | 12.88     | 2.14      | 51.2   |         | 4.50                | SNP_IGA_408981                | 45.50   |
|                        | qSOR.V-Ch4-2008    | SOR 08   | 49   | 5.75      | 2.02      | 27.3   |         | 4.35                | SNP_IGA_437516                | 51.66   |
|                        | qSOR.V-Ch4-2009    | SOR 09   | 46   | 18.59     | 2.27      | 57.7   |         | 6.48                | SNP_IGA_408981                | 45.50   |
|                        | qSOR.V-Ch4-2010    | SOR 10a  | 54   | 5.62      | 2.16      | 36.8   |         | 3.82                | SNP_IGA_440110                | 54.65   |
|                        | qSOR.V-Ch4-Mean    | SOR X    | 47   | 22.22     | 2.12      | 60.5   |         | 4.71                | SNP_IGA_408981                | 45.50   |
|                        | qSOR.V-Ch4-MYear   |          | 45.5 |           |           | 48.6   | 2.1     | -2.42               | SNP_IGA_408981-SNP_IGA_437516 |         |
|                        | qSOR.BT-Ch5-2010   | SOR 10b  | 40   | 2.56      | 2.16      | 20.5   |         | 2.72                | SNP_IGA586202                 | 40.28   |
|                        | qSOR.BT-Ch5-MYear  |          | 32.4 |           |           | 8.6    | 0.2     | 1.07                | SNP_IGA_572589-SNP_IGA_585182 |         |
| <b>Fructose</b>        | qFRU.V-Ch2_1-2008  | FRU 08-a | 3    | 2.90      | 2.00      | 13.2   |         | -2.13               | SNP_IGA_249781                | 0.00    |
|                        | qFRU.V-Ch4-2007    | FRU 07-a | 48   | 2.73      | 2.14      | 17.6   |         | 1.80                | SNP_IGA_408981                | 45.50   |
|                        | qFRU.V-Ch4-MYear   |          | 47.5 |           |           | 6.0    | 2.8     | -0.56               | SNP_IGA_408981-SNP_IGA_437516 |         |
|                        | qFRU.BT-Ch1_2-2008 | FRU 08-b | 23   | 2.62      | 2.21      | 16.5   |         | 2.27                | SNP_IGA_25403                 | 23.04   |
|                        | qFRU.BT-Ch1_2-Mean | FRU X-b  | 23   | 2.48      | 2.26      | 15.9   |         | 1.41                | SNP_IGA_25403                 | 23.04   |
|                        | qFRU.BT-Ch5-2007   | FRU 07-b | 54   | 3.57      | 2.28      | 22.7   |         | 2.03                | SNP_IGA_595212                | 53.74   |
|                        | qFRU.BT-Ch5-MYear  |          | 53.7 |           |           | 9.9    | 2.7     | 0.61                | SNP_IGA_595212-SNP_IGA_597937 |         |
|                        | qFRU.BT-Ch8-MYear  |          | 34.8 |           |           | 6.7    | 4.0     | 0.44                | SNP_IGA_835981-SNP_IGA_864110 |         |
| <b>Total Phenolics</b> | qPHE.V-Ch2_2-2009  | PHE 09-a | 0    | 2.32      | 2.16      | 14.3   |         | 4.18                | SNP_IGA_140938                | 0.00    |
|                        | qPHE.V-Ch4-Mean    | PHE X    | 45   | 2.67      | 2.18      | 15.8   |         | 4.71                | SNP_IGA_408981                | 45.50   |
|                        | qPHE.BT-Ch2-2009   | PHE 09-b | 1    | 2.50      | 2.21      | 15.0   |         | -4.18               | SNP_IGA_141612                | 1.47    |
|                        | qPHE.BT-Ch2-MYear  |          | 8.6  |           |           | 9.2    | 1.4     | -2.63               | SNP_IGA_230389-SNP_IGA_231766 |         |
| <b>Flavonoids</b>      | qFLV.V-Ch2_2-2010  | FLV 10-a | 1.5  | 2.76      | 1.82      | 17.0   |         | 4.59                | SNP_IGA_185060                | 1.47    |
|                        | qFLV.V-Ch3-2008    | FLV 08-c | 3    | 2.18      | 2.02      | 10.1   |         | -4.97               | SNP_IGA_298154                | 2.98    |
|                        | qFLV.V-Ch4-2008    | FLV 08-a | 53   | 4.40      | 2.02      | 17.8   |         | 7.22                | SNP_IGA_437516                | 51.66   |
|                        | qFLV.V-Ch7-2008    | FLV 08-b | 0    | 4.04      | 2.02      | 18.6   |         | -7.45               | SNP_IGA_787282                | 0.00    |
|                        | qFLV.BT-Ch2-2010   | FLV 10-b | 0    | 2.86      | 1.93      | 17.1   |         | -4.59               | SNP_IGA_161939                | 0.00    |
| <b>Anthocyanins</b>    | qANT.BT-Ch5-2009   | ANT 09   | 3    | 3.24      | 1.93      | 18.7   |         | -3.84               | SNP_IGA_544961                | 2.94    |
|                        | qANT.BT-Ch5-2010   | ANT 10   | 3    | 5.28      | 2.19      | 27.6   |         | -4.15               | SNP_IGA_544961                | 2.94    |
|                        | qANT.BT-Ch5-Mean   | ANT X    | 3    | 6.02      | 2.14      | 30.0   |         | 1.98                | SNP_IGA_544961                | 2.94    |
|                        | qANT.BT-Ch5-MYear  |          | 2.9  |           |           | 13.2   | 8.0     | -1.07               | SNP_IGA_544961-SNP_IGA_548597 |         |
| <b>RAC</b>             | qRAC.V-Ch4-2009    | RAC 09-a | 46   | 7.47      | 2.14      | 34.9   |         | 117.17              | SNP_IGA_408981                | 45.50   |
|                        | qRAC.V-Ch4-Mean    | RAC X    | 54   | 3.58      | 2.03      | 20.2   |         | 82.55               | SNP_IGA_440110                | 54.65   |
|                        | qRAC.V-Ch4-MYear   |          | 45.0 |           |           | 11.6   | 6.0     | -26.80              | SNP_IGA_407115-SNP_IGA_408981 |         |
|                        | qRAC.BT-Ch8-2009   | RAC 09-b | 82   | 2.72      | 2.28      | 9.3    |         | -59.61              | SNP_IGA_884538                | 81.39   |

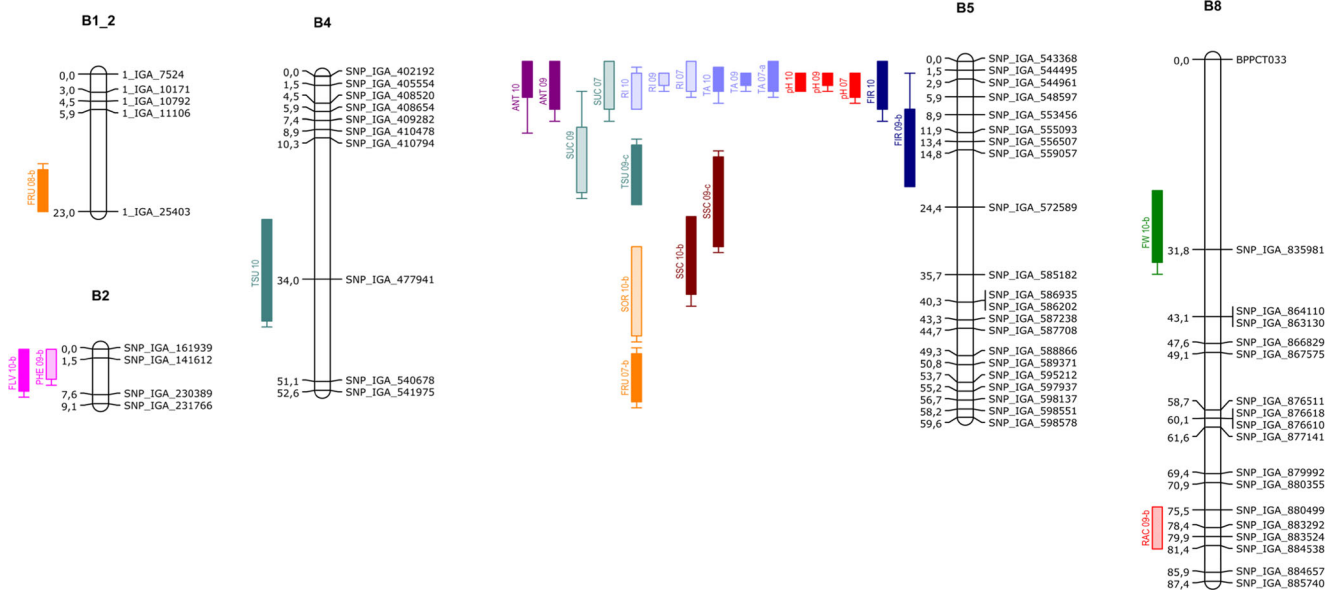


**Fig. 3** QTL map of ‘Venus’. Location of putative QTLs controlling fruit quality traits analyzed by year in the ‘Venus’ map and determined by SQM or MQM mapping. *V* refers to the ‘Venus’ parental, the *first number* to the scaffold that it represents and *second one* to the sub-group when the scaffold is represented by more than one linkage group. The markers are listed at the *right side* of each LG, and the genetic

distances are listed at the *left side*. The QTLs are drawn at the *left* of each corresponding LG and were represented in such a way that the *thick line* represents the inner confidence interval bound and the *thin line* represents the whole significance interval of the QTL. The QTLs detected with the mean were not represented. The QTL codes are described in Table 2 and Supplementary Table 4

We identified a region on LG V8 with two QTLs that are potentially involved in production; the LOD score, additive effect, and proportion of phenotypic variance explained by qPDR.V-Ch8-2010 and qPDR.V-Ch8-Mean confirm the genetic control of this region on yield. Furthermore, three QTLs

for fruit weight on LG V4 (qFW.V-Ch4-2007, qFW.V-Ch4-2009, and qFW.V-Ch4-2010) identified across 3 years (Table 2, Fig. 3, Supplementary Table 4) were found in the same region as the mean, also confirmed with the multi-year analysis, which supports the presence of QTLs influencing



**Fig. 4** QTL map of ‘Big Top’. Location of putative QTLs controlling fruit quality traits analyzed by year in the ‘Big Top’ map and determined by SQM or MQM mapping. *B* refers to the ‘Big Top’ parental, the *first number* to the scaffold that it represents and *second one* to the sub-group when the scaffold is represented by more than one linkage group. The markers are listed at the *right side* of each LG, and the genetic distances

are listed at the *left side*. The QTLs are drawn at the *left* of each corresponding LG and were represented in such a way that the *thick line* represents the inner confidence interval bound and the *thin line* represents the whole significance interval of the QTL. The QTLs detected with the mean were not represented. The QTL codes are described in Table 2 and Supplementary Table 4

fruit weight in both regions (at 46 and 60 cM). In addition, on LG B8, one QTL for fruit weight (qFW.BT-Ch8-2010) was also identified in 2010 and another QTL was observed using the mean value (qFW.BT-Ch8-Mean), also identified with the multi-year analysis (qFW.BT-Ch8-MYear).

For firmness, we identified two QTLs on LG V4 and two other QTLs on LG B5. The first region (qFF.V-Ch4-2007 and qFF.V-Ch4-2009) was on LG V4 that had QTLs within an 8-cM interval (Table 2). The QTLs mapped on LG B5 (qFF.BT-Ch5-2009 and qFF.BT-Ch5-2010) were 9 cM apart, but the position was confirmed with the QTLs detected using the mean value (qFF.BT-Ch5-Mean, 12 cM) and with the multi-year analysis (qFF.BT-Ch5-MYear, 10.9 cM). Furthermore, eight QTLs were detected and mapped for SSC across different years; five QTLs (qSSC.V-Ch4-2007a, qSSC.V-Ch4-2007b, qSSC.V-Ch4-2009a, qSSC.V-Ch4-2009b, and qSSC.V-Ch4-2010) were on LG V4, one (qSSC.V-Ch2\_2-2009) on LG V2\_2, and two (qSSC.BT-Ch5-2009 and qSSC.BT-Ch5-2010) on LG B5 (Table 2, Fig. 3). On LG V4, two different regions (at 29–32 and 44 cM) were simultaneously repeated in 2007 and 2009, which indicates the presence of two different QTLs. Only the second position was confirmed with the mean value and the multi-year analysis (Table 2). On LG B5, two QTLs (qSSC.BT-Ch5-2009 and qSSC.BT-Ch5-2010) were detected, and the QTL with the mean value (qSSC.BT-Ch5-Mean) and the multi-year analysis (qSSC.BT-Ch5-MYear) were in the middle.

QTLs for pH, TA, and RI (SSC/TA) were exclusively mapped over LG B5 and around the same region. The proportions of phenotypic variances explained by these QTLs varied from 42.4 to 85.3 %. QTLs for pH (qpH.BT-Ch5-2007, qpH.BT-Ch5-2009, and qpH.BT-Ch5-2010) were located at the same genomic region across 3 years, and the same position was detected with the mean pH value (Table 2, Supplementary 4) and the multi-year analysis. TA was mapped on LG B5 at 2–3 cM across 3 years and with the mean (qTA.BT-Ch5-2007, qTA.BT-Ch5-2009, qTA.BT-Ch5-2010, and qTA.BT-Ch5-Mean). However, two positions were detected with the multi-year analysis (qTA.BT-Ch5-MYear-a and qTA.BT-Ch5-MYear-b at 2.9 and 53.7 cM). RI was not previously mapped on any peach map, and because this trait is a function of SSC and TA, it is correlated with both traits. Three QTLs were found on LG B5 during 3 years (2007, 2009, and 2010), and the QTL in 2009 (qTA.BT-Ch5-2009) was repeatedly observed using the mean value (qTA.BT-Ch5-Mean) and with the multi-year analysis (qTA.BT-Ch5-MYear).

QTLs for total sugar, sucrose, glucose, sorbitol, and fructose contents were mainly found in LGs V4 and B5 (Table 2, Figs. 3 and 4). These QTLs were mapped at short distance intervals for each trait. In addition, some QTLs detected with the mean value were placed at the same or very close positions as other QTLs were detected across years and with the multi-year analysis (Table 2). Remarkably, high LOD scores were

obtained for qSOR.V-Ch4-2007 and qSOR.V-Ch4-2009 (12.9 and 18.6, respectively), and the high values of phenotypic variances were explained by the nearest markers (51.2, and 57.7, respectively).

The QTLs identified for phenolic compounds were mainly on LGs B2, V2\_2, V4, and B5. QTLs for total phenolic and flavonoid contents were found in both parents and distributed on different LGs, and none of them were repeated over the years studied (Table 2). Alternatively, QTLs for anthocyanin content were identified at the same position on LG B5 for 2 years with the mean value also validated with the multi-year analysis (Table 2, Fig. 4). The QTLs detected for RAC on LGs V4 were validated with the multi-year analysis.

## Discussion

### Genetic maps

This work presents the first genetic map of the nectarine population ‘Venus’ × ‘Big Top’ with the ‘IPSC 9K peach SNP array v1’. Two preliminary studies carried out in the same population mapped 17 SSR markers on LG 4 (Cantín et al. 2010a) and six on LG 6 (Abidi et al. 2012). In the present map, LG 4 includes a total of 36 markers, with 26 and 10 markers in the ‘Venus’ and ‘Big Top’ maps, respectively. LG 6 included a total of 39 markers, with 21 markers in the ‘Venus’ map and 18 markers distributed in two LGs in the ‘Big Top’ map. SNPs allow increased saturation and genome coverage and therefore provide higher precision and accuracy for QTL dissection in this population, as was found in other *Prunus* progenies (Eduardo et al. 2013; Martínez-García et al. 2013b; Da Silva Linge et al. 2015).

Both genetic maps had more than eight LGs, which is the expected number of chromosomes in *P. persica*. Scaffolds 1 and 2 in the ‘Venus’ map and scaffolds 1 and 6 in the ‘Big Top’ map were split into two LGs. The absence of linkage between markers that belong to the same chromosome is common in genetic mapping (Chaparro et al. 1994; Dirlewanger et al. 1998; Zhebentyayeva et al. 2008; Eduardo et al. 2013).

To our knowledge, this is the sixth full map produced with the ‘IPSC 9K peach SNP array v1’ in peach, although others have been constructed with this array technology in other *Prunus*-derived progenies (Eduardo et al. 2013; Yang et al. 2013; Martínez-García et al. 2013a; Frett et al. 2014; Pacheco et al. 2014; Romeu et al. 2014; Da Silva Linge et al. 2015; Nuñez-Lillo et al. 2015). The length of genetic linkage maps mostly based on SNP markers was more saturated compared with the previously published maps, with the exception of the T × E *Prunus* reference map (Dirlewanger et al. 2006). In our population, 104 SNPs were mapped in ‘Venus’ and 122 in ‘Big Top’, which spanned 259 cM in the ‘Venus’ map and 464 cM in the ‘Big Top’ map. Other maps

have been developed using the ‘IPSC 9K peach SNP array v1’ in different F1 populations. Eduardo et al. (2013) analyzed ‘Bolero’ × ‘OroA’ progeny and obtained two maps with 231 and 87 markers in nine and five LGs, which span 405 and 228.5 cM, for the ‘Bolero’ and ‘OroA’ maps, respectively. Romeu et al. (2014) found less saturation on ‘V6’ and ‘Granada’ maps (178 SNPs and 76 SNPs that span 480 and 276 cM, representing 2.94 and 3.87 cM/marker, respectively). Furthermore, two dense maps constructed with other SNP markers in peach- and peach-almond-derived progenies spanned 422 and 369 cM, respectively (Martínez-García et al. 2013b). The average marker density in the previously mentioned maps was similar and comparable to our results, although in some cases, the genome was not entirely covered (Romeu et al. 2014; Sánchez et al. 2014).

Scaffolds 1, 2, and 6 with unsaturated regions explain the absence of linkage (Fig. 1) and why two LGs were found on one chromosome. Scaffold 1 was split into two LGs in both parents, scaffold 2 in the ‘Venus’ map, and scaffold 6 in the ‘Big Top’ map. Different authors revealed unsaturated regions in scaffolds 1, 2, 4, and 5 (Frett et al. 2014) and scaffolds 4, 5, 7, and 8 (Sánchez et al. 2014). Moreover, Sánchez et al. (2014) found that chromosomes 2, 1, and 3 were missing in a pseudotest cross population between the cultivars ‘MxR\_01’ and ‘Granada’. The lack of polymorphic SNPs in certain chromosomes is caused by homozygosity in the peach genome and is probably due to germplasm background (Romeu et al. 2014; Sánchez et al. 2014; Nuñez-Lillo et al. 2015). Verde et al. (2012) evaluated and validated the SNP array and reported common gaps in chromosomes 1, 2, and 5. These unsaturated sections may represent putative centromeric regions that would explain these events. Anchoring to the reference genome sequence (Verde et al. 2013), the putative order of the SNPs in our map was initially established in Mbp by comparison with the physical map v1.0. Although assembly and orientation mistakes have been somehow accumulated in the sequence genome, in general, marker order in our maps was in agreement with peach genome sequence v1.0 (Supplementary Table 3). In this work, three markers unexpectedly occurred on different LGs compared with the putative order established in the array: SNP\_IGA\_536394 on LG V6, SNP\_IGA\_430365 on LG B7, and SNP\_IGA\_913769 on LG B3. Nevertheless, by comparison with the physical map v2.0, all markers were reassigned to their correct chromosome except SNP\_IGA\_430365 that was positioned on chromosome Pp03 (Supplementary Table 3). The new position on LG B3 for SNP\_IGA\_913769, which was physically located in scaffold 13 on the peach genome v1.0, was confirmed with genome assembly v2.0. As a conclusion, the refinements included in the updated peach genome v2.0 have confirmed chromosome positions determined in our genetic maps.

Other changes related to the putative order of the SNPs were corrected by comparison with the peach physical map

v2.0 (one inversion on LG B1\_1 and order for B7 and B8) (see Supplementary Table 3). Moreover, we confirmed a new orientation for chromosomes 2 and 7 by comparing mapped markers on B2 and B7 chromosomes with their positions in the peach physical map v2.0. It is expected to correct the changes described in peach populations mapped with the same SNP array and methodology (Eduardo et al. 2013; Romeu et al. 2014; Sánchez et al. 2014; Da Silva Linge et al. 2015). As it was previously mentioned, pseudomolecule 2 was wrongly mapped in T × E (Verde et al. 2013) and inversions and translocations have been commonly described on chromosomes LGs 1, 2, 4, 7, and 8 (Eduardo et al. 2013; Martínez-García et al. 2013b; Da Silva Linge et al. 2015). Finally, lack of markers on V5 may be due to identity by descent or ascertainment biases in the SNP markers represented in the array (Nielsen et al. 2004; Albrechtsen et al. 2010), as discussed by Eduardo et al. (2013). However, other specific characteristic of the population, such as size, genetic background, or any other unknown particularity, may be affected.

### QTL analysis

Based on the results in our population, even though the progeny size is limited, we identified important regions in the peach genome that control fruit quality traits. Many of the QTLs detected in our progeny were previously found in other peach mapping populations. However, we described 16 QTLs that control peach fruit quality traits for the first time (one for production, fruit weight, firmness, and total sugar and sucrose contents; two for total phenolic and anthocyanin contents, and RAC; and five for flavonoid content). Five of them were validated with the multi-year approach.

The precision of phenotypic evaluation is very important for accurate QTL mapping. A reliable QTL map can only be produced from reliable phenotypic data. Replicated phenotypic evaluations during different years improve the accuracy of QTL mapping by reducing experimental error and background noise (Salazar et al. 2013). Most of the QTLs found in our study were consistent for at least 2 years and were detected also with the multi-year analysis, although others were not repeated across all 4 years of study. The multi-year approach allowed the detection of QTLs that were not considered significant by doing single-year analysis (Dirlewanger et al. 2012). The characteristics of these QTLs are included in Table 2 and Supplementary Table 4. Variation in QTL position over time is commonly found in QTL analysis, and similar performance, including non-repetitiveness of QTLs across different years, different locations found in yearly analyses, and detection with the mean value, was reported by other authors for peach and other *Prunus* species (Dirlewanger et al. 1999;

Etienne et al. 2002; Verde et al. 2002; Quilot et al. 2004; Eduardo et al. 2011; Dirlwanger et al. 2012; Salazar et al. 2013).

The QTL that controls peach production found on LG V8 is reported for the first time. No other authors have evaluated this trait as yield in terms of kg/tree, although Dirlwanger et al. (1999) reported a QTL on LG 6 for productivity (number of fruits per tree), which is a somewhat related trait in terms of overall productivity. However, from an agronomical point of view, these are separate traits. A remote possibility exists that these QTLs could be related to a translocation between LGs 6 and 8, as was reported in the  $F_2$  'Garfi'  $\times$  'Nemared' population (Jáuregui et al. 2001). For fruit weight, as we found in the 'Venus'  $\times$  'Big Top' population, some authors have identified QTLs on LGs 1, 2, 4, and 6 in other mapping populations in peach (Quilot et al. 2004; Eduardo et al. 2011; Fresnedo-Ramírez et al. 2015). However, newly discovered QTLs that control peach fruit weight were reported for the first time on LG 7 (Da Silva Linge et al. 2015), LG 5 (Fresnedo-Ramírez et al. 2015), and LG 8 (qFW.BT-Ch8-2010). The QTLs qFW.V-Ch4-2009 and qFW.V-Ch4-2010 for fruit weight found on LG V4 were located on the same genomic region across multiple years, with the nearest marker at 45 cM. This indicates the presence of a single QTL on LG V4 for this trait also confirmed with the mean and the multi-year analysis. The QTL qFW.V-Ch4-2007 (60 cM) was also confirmed with the multi-year analysis (62.1 cM). Another QTL, located on B8, had a high LOD score and explained an important part of the variation, which was only detected in 2010 (25 cM) but partially confirmed with the mean (9 cM) and the multi-year analysis (16 cM). The low saturation in this region of the chromosome is one possible explanation for these results.

The QTLs for firmness on LG V4 (qFF.V-Ch4-2007 and qFF.V-Ch4-2009) and LG B5 (qFF.BT-Ch5-2009 and qFF.BT-Ch5-2010) potentially represent two single QTLs that were validated with the mean (qFF.V-Ch4-Mean) and the multi-year analysis (qFF.V-Ch4-MYear; qFF.BT-Ch5-MYear). The QTLs found for firmness on LG V4 (qFF.V-Ch4-2009 and qFF.V-Ch4-Mean) were previously reported by Cantín et al. (2010a) in the same population. Furthermore, for SSC, we found different genomic regions on LG 4, as other authors have previously identified in peach (Abbott et al. 1998; Dirlwanger et al. 1999; Quarta et al. 2000; Etienne et al. 2002; Quilot et al. 2004; Cantín et al. 2010a; Eduardo et al. 2011; Sánchez et al. 2014). Cantín et al. (2010a) found a QTL for SSC on LG 4 that explained more than 80 % of the total variation using SSR markers and composite interval mapping for QTL mapping in this population. Two QTLs on LG 4 could explain the variation in total SSC. The first region (qSSC.V-Ch4-2007a and qSSC.V-Ch4-2009a) explained less than 19 % of phenotypic variance, and the second (qSSC.V-Ch4-2007b, qSSC.V-Ch4-2009b, and qSSC.V-Ch4-2010) explained 28.0, 27.6, and 21.4 % of the total variance, respectively. The second region was confirmed with the mean value at 44 cM

and validated at the same position with the multi-year analysis. Other genomic regions that control SSC on LG V2\_2 (qSSC.V-Ch2\_2-2009) and LG B5 (qSSC.BT-Ch5-2009 and qSSC.BT-Ch5-2010) were also reported in other populations and were found on LG 2 in peach (Verde et al. 2002; Quilot et al. 2004; Eduardo et al. 2011), LG 5 in peach (Quilot et al. 2004) and apricot (Salazar et al. 2013), and both LGs in peach and *Prunus*-related progenies (Fresnedo-Ramírez et al. 2015). Many QTLs have been previously described for SSC because it is one of the most widely studied traits; SSC is used as a standard universal method to define quality in fruits, and it is a quick and simple evaluation method.

Other major QTLs that control pH and TA were found only on the 'Big Top' map on LG 5. The additive effect and proportion of total variance explained by these QTLs (up to 85 %) revealed which regions control these traits. Moreover, these QTLs had a consistent position relative to the *D* gene, which controls lack of acidity in fruit (Abbott et al. 1998; Dirlwanger et al. 1998, 2004). For TA, other authors mapped QTLs in the proximal part of LG 5 (Dirlwanger et al. 1999; Etienne et al. 2002), as was found on the 'Big Top' map (qTA.BT-Ch5-2007, qTA.BT-Ch5-2009 and qTA.BT-Ch5-2010). Another position at 53.7 cM was also detected in our population with the multi-year analysis (qTA.BT-Ch5-MYear). Quilot et al. (2004) identified a QTL associated with TA at 52 cM on LG 5 when mapping two interspecific populations. These positions are also consistent with markers developed for sub-acidic traits in peach (Eduardo et al. 2014) and with the co-localization of QTLs for TA and pH on LG 5 (Fresnedo-Ramírez et al. 2015). On the contrary, any equivalent QTL on LG 5 was found in apricot for malic acid (which is synonymous with TA) and pH (Salazar et al. 2013), even in peach (Eduardo et al. 2011). The position of the QTLs found for RI (SSC/TA) indicates that TA has more influence than SSC in the genetic control of this trait, because the QTLs were in the same position as acidity (TA) (Table 2). This fact was also confirmed with the significant correlations found between these traits (Supplementary Table 2).

Some of the QTLs that control total sugar content, which explain more than 15 % of phenotypic variance, were detected across 2009 and 2010 and were mapped on LGs 2, 4, and 5. QTLs for this trait were previously described on LG 2 (Quarta et al. 2000; Quilot et al. 2004) and LG 5 (Quilot et al. 2004) but never on LG 4. However, in this study, only the region that controls total sugar content on LG 5 was repeated with the mean and validated with the multi-year approach.

For sucrose content, the QTL detected on LG B5 (qSUC.BT-Ch5-2007) was consistent with the QTLs found with the mean and the multi-year analysis (Table 2, Supplementary Table 4, Fig. 4), which indicates the presence of a major QTL that controls this trait. QTLs for sucrose were previously described on LGs 3, 5, 6, and 7 (Dirlwanger et al. 1999; Etienne et al. 2002; Quilot et al. 2004). LG 4 was not

previously reported to control sucrose content in peach. In addition, the QTLs that control glucose and fructose contents found on LG V4 at 48 cM were consistent with the QTLs found at the same position with the multi-year analysis. The LOD scores and percent of total variance found also indicate the existence of a major QTL that controls glucose and fructose contents on LG 4 as previously detected in other peach mapping populations (Abbott et al. 1998; Dirlewanger et al. 1999; Etienne et al. 2002; Quilot et al. 2004). The QTLs found for fructose content were also previously reported by various authors on LG 5 (Abbott et al. 1998; Dirlewanger et al. 1999) and on LGs 1 and 2 (Quilot et al. 2004).

The four QTLs that control sorbitol content were in a cluster located on LG 4 between 45 and 54 cM in the map. The high LOD scores and total phenotypic variance explained by these QTLs across the 4 years indicate the presence of a major QTL on LG 4 that controls sorbitol content which is also consistent with the mean and the multi-year approach. QTLs for sorbitol were previously described on LGs 2, 4, and 5 (Dirlewanger et al. 1999; Quilot et al. 2004); however, any QTL on LG 2 was found in this study.

Finally, QTLs for total phenolic and flavonoid contents were mapped on LG 2 in both parental maps. The proximity of the nearest assigned markers indicates that a single QTL on LG 2 controls phenolic content. It is very well known that flavonoids are highly correlated with phenolic content ( $r=0.828$ ; Supplementary Table 2) because flavonoids belong to this family (Cantín et al. 2009b; Abidi et al. 2011; Font i Forcada et al. 2013). The observation that QTLs of RAC, total phenolic, and flavonoid contents reside at the same position on LG 4 indicates that most RAC activity is related to flavonoid content ( $r=0.761$ ; Supplementary Table 2), as was previously reported (Cantín et al. 2009b; Abidi et al. 2011; Font i Forcada et al. 2013). Other QTLs for antioxidant compounds were found on other LGs (V3, V7, B5, and B8). Only QTLs that control anthocyanin content were consistent over 2 years on LG B5 and were confirmed with the mean and validated with the multi-year approach.

Considering the synteny between *Prunus* and *Malus* (Dirlewanger et al. 2004; Arús et al. 2012), in our progeny, the QTLs detected that control phenolic content were not located in the same genomic regions as in apple (Chagné et al. 2012; Verdu et al. 2014). Chagné et al. (2012) found QTLs for flavonoid and anthocyanin contents on LG 16 (syntenic with LG 6 and part of LG 1 in *Prunus*), anthocyanin content on LG 9, and phenolic content on LG 17 (both syntenic with LG 3) and LG 15 (syntenic with part of LG 1). Other positions have been found in cider apples for anthocyanin content on LG 5 (syntenic with LG 4) and flavonoid content on LGs 15 and 17 (part of LGs 1 and 3 in *Prunus*, respectively). All of these data indicate that the genomic regions that control polyphenols in the Rosaceae family are not entirely conserved.

Our results provide the first insights into the genetic control of total phenolic content in peach. Mapping of QTLs for polyphenolic content provides important knowledge for future studies to develop new cultivars with increased antioxidant properties.

## Conclusions

We report, for the first time, the identification of QTLs for fruit quality traits in the ‘Venus’ × ‘Big Top’ progeny using the ‘IPSC 9K peach SNP array v1’, which was developed by Illumina. We detected 54 QTLs that represent 34 genomic regions across 4 years of evaluation using the SQM and MQM mapping strategies. We found new and stable QTLs for fruit weight, firmness, total phenolic and anthocyanin contents, and relative antioxidant capacity in peach. LG V4 in ‘Venus’ and LG B5 in ‘Big Top’ contained the most important genomic regions that control fruit quality traits in peach. The colocalization and clustering of the majority of the detected QTLs might indicate that these genes are tightly linked. In some cases, pleiotropic effects may occur. Furthermore, the multi-year approach helped to confirm and detect minor QTLs or QTLs from traits potentially affected by climatic conditions.

The results presented in this work enhance the existing maps developed with the same SNP array and open the possibility of using marker-assisted selection to improve fruit quality in peach. Further studies must be carried out to validate the QTLs revealed here to identify new candidate genes in peach. Moreover, these data will facilitate the development of new peach cultivars that bear fruit with increased concentrations of polyphenolic compounds that benefit human health.

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**Data Archiving Statement** V x BT linkage maps and QTL positions are available on Genome Database for Rosaceae at tfGDR1025 accession number.

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