Scientia Horticulturae 257 (2019) 108734

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

High-density genetic map and QTL analysis of soluble solid content, maturity date, and mealiness in peach using genotyping by sequencing

Gerardo Nuñez-Lillo[a], Cristóbal Balladares[a], Catalina Pavez[a], Claudio Urra[a], Dayan Sanhueza[a], Elisa Vendramin[b], Maria Teresa Dettori[b], Pere Arús[c], Ignazio Verde[b], Francisca Blanco-Herrera[a,d], Reinaldo Campos-Vargas[a], Claudio Meneses[a,e,*]

[a] Centro de Biotecnología Vegetal, Facultad de Ciencias de la Vida, Universidad Andrés Bello, Av. República 330, Santiago, Chile
[b] Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria (CREA) – Centro di Olivicoltura, Frutticoltura e Agrumicoltura (CREA-OFA), Via di Fioranello 52 00134, Rome, Italy
[c] IRTA, Centre de Recerca en Agrigenòmica CSIC-IRTA-UAB-UB, Campus UAB, Cerdanyola del Vallès (Bellaterra), 08193 Barcelona, Spain
[d] Millennium Institute for Integrative Biology (iBio), Portugal 49, Santiago, Chile
[e] FONDAP Center for Genome Regulation, Universidad Andrés Bello, Av. República 330, Santiago, Chile

ARTICLE INFO

Keywords:
Prunus persica
Genetic linkage map
Quality
Traits
Candidate
Genes

ABSTRACT

Peach (*Prunus persica*) is one of the most important temperate fruit trees in the world, based on its production and cultivated area. Consumer acceptance is the principal objective of multiple breeding programs and it is dependent on many factors. Among these factors, an important role is played by the soluble solids content (SSC) and the postharvest performance represented by mealiness (M) susceptibility as a chilling injury disorder. Additionally, a major maturity date (MD) QTL has been reported to have a pleiotropic effect on both M and SSC. The aim of this work was QTL identification of SSC, MD, and M and to identify adequate candidate genes that are linked to regulation of these traits. The analysis was performed by evaluation of fruit quality traits during three consecutive seasons in an F1 progeny of 194 siblings, which were obtained from the intraspecific cross between the yellow-flesh peach "O'Henry" and the white-flesh nectarine NR-053. The main result was the construction of a genetic linkage map with 499 markers (486 SNPs, 11 SSRs, and two morphological markers) spanning 717.6 cM, with an average distance between markers of 1.5 cM/cluster. The analysis allowed the identification of consistent QTLs for SSC and M in the linkage group LG5 and for MD in LG1, LG2, LG5, and LG6. A large number of genes were annotated in QTL intervals, which was reduced by selecting the genes with at least one SNP, which caused an amino acid variation. For SSC, the data identified four transcription factors, one gene involved directly with the sugar accumulation process, and one cell wall remodeling-related gene. For MD, 23 cell wall-related genes, three jasmonic acid-linked genes, eight transcription factors, and one ripening-related gene were identified. Finally, only one cell wall gene was identified that was associated with M. In conclusion, these results improve our understanding of the genetic control of fruit quality traits with commercial relevance in *P. persica* and specifically in the O × N mapping population.

1. Introduction

Peach (*Prunus persica*) is used as a model species for genetic and genomic studies in the *Rosaceae* family (Arús, 2005). Its genome was sequenced in 2013 (Verde et al., 2013) and a second version was released in 2015, which has a genome size of 227 Mbp and 26,873 annotated genes (Verde et al., 2017). Several peach breeding programs are currently focused on the development of new varieties. Fruit quality and postharvest performance appear to be the targeted traits (Infante et al., 2008). The conventional fruit-breeding process is time-consuming and costly. Thus, the development of genomic tools to enable marker-assisted selection is a key step to improve fruit tree breeding efficiency. Consumer acceptance of peaches and nectarines relies on many traits that are involved in determining the fruit's quality such as softening, sweetness, and acidity.

The accumulation of sugars or soluble solids content (SSC) is a crucial trait for consumer acceptance because together with acidity (the balance of sugar/acids), it determines the fruit's sweetness (Abidi, 2016). For example, in cultivars with high acidity (0.7–0.9%), consumer acceptance increases quickly as the SSC increased but it reaches a

* Corresponding author at: Centro de Biotecnología Vegetal, Facultad de Ciencias de la Vida, Universidad Andrés Bello, República 330, Santiago, Chile.
E-mail address: claudio.meneses@unab.cl (C. Meneses).

https://doi.org/10.1016/j.scienta.2019.108734
Received 18 February 2019; Received in revised form 29 July 2019; Accepted 31 July 2019
Available online 08 August 2019

plateau (11–12°Brix), while in low acidity varieties (0.30–0.5%), the acceptance increases at a higher SSC values without reaching a plateau (Crisosto and Crisosto, 2005; Cantín et al., 2009). As Cantín et al. (2009) indicated, the connection between SSC and consumer acceptance is specific for each cultivar, and no single SSC value guarantees success with consumers. Thus, an important aspect to be considered when measuring SSC is the relationship with the total sugar content based on the contribution of sucrose, fructose, sorbitol, and glucose. Previous research showed that a direct correlation between SSC and total sugar content in different peach varieties does not necessarily exist, probably because of the contribution of soluble optically active compounds other than sugars (pectins, salts, and organic acids) that can interfere and lead to an altered SSC value (Cantín et al., 2009). Even when several genetic studies have reported QTLs for SSC on linkage group LG4 of *P. persica* (Dirlewanger et al., 1999; Nuñez-Lillo et al., 2015), only a few candidate genes have been described and validated (Eduardo et al., 2013). An interesting aspect is the existing information that relates SSC to some other traits including the co-localization of a QTL in the LG5, which controls both the peach/nectarine characteristics and SSC (Quilot et al., 2004, 2005). A similar situation is described for LG1 where a QTL that controls the white/yellow flesh color and SSC are co-localized (Bliss et al., 2002; Quilot et al., 2005). The analysis of the progeny of "Venus" × "Big Top" identified QTLs for SSC and total sugars located in LG2, LG4, and LG5, as follows: sorbitol and sucrose in LG4 and LG5; glucose in LG3 and LG4; and fructose in LG1, LG2, LG4, LG5, and LG8 (Zeballos et al., 2016). Etienne et al. (2002) described the unloading of sucrose from the phloem to the cell vacuole as a key process and identified several proteins that are involved. The sucrose transporter proteins (STPs), which are located in the cellular and vacuole membranes, transfer sucrose from the phloem into the fruit flesh cell and take part in sucrose accumulation in the vacuole. Moreover, Etienne et al. (2002) described two proteins that are associated with sugar accumulation called sucrose synthase (SUS) and invertase (INV), both of which are involved in sucrose metabolism.

For producers, exporters, and consumers, the maturity date (MD) is another key trait that is targeted in breeding programs. In LG4, MD showed a pleiotropic effect on fruit quality traits such as weight, firmness, SSC, and physiological disorders (Cantín et al., 2010; Romeu et al., 2014; Nuñez-Lillo et al., 2015). Pirona et al. (2013) identified *NAC072* (Prupe.4G816800) as a candidate gene for MD using a QTL analysis. This member of the NAC family of transcription factors is located on chromosome 4 and has an InDel of 9 bp, which can be used as a molecular marker for MD. The same results were obtained by Nuñez-Lillo et al. (2015) and Romeu et al. (2014); they also identified other genes on chromosome 4 that were related to this trait, such as *ERF4* (ethylene responsive factor 4), *SPL4* (squamosa promoter binding protein-like 4), and *DFL1* (indole-3-acetic acid amido synthetase).

The physiological disorder of the fruit flesh called mealiness (M), which is characterized by dry or mealy flesh with a grainy sand-like texture (Crisosto and Labavitch, 2002), is one of the major sources of peach production losses in the Chilean fruit export industry, and is caused by the long transport time at 0 °C to distant consumers (Lurie and Crisosto, 2005). M development has been related to cell wall remodeling enzymes, and more specifically to de-esterification of the pectin matrix without its subsequent depolymerization (Manganaris et al., 2006; Jolie et al., 2010). This trait has been associated in the peach trees with chromosomic regions of LG4 using QTL strategies (Cantín et al., 2010; Martínez-García et al., 2013). Moreover, Peace et al. (2006) reported a correlation between M and cell wall remodeling enzymes and with low levels of expansins (Pegoraro et al., 2010) or an imbalance in polygalacturonase (PG) or pectin methylesterase (PME) levels (Lurie and Crisosto, 2005). However, other enzymes related to cell wall remodeling can participate in M development, such as PG inhibitors (PGIP) (Protsenko et al., 2008), Pme inhibitors (PMEI) (Giovane et al., 2004), pectate lyases (PL) (Vicente et al., 2007), and xyloglucan endotransglucosidases (XTH) (Eklöf and Brumer, 2010).

Considering the economic importance and the contribution to breeding programs, the aim of this research was to identify genomic regions (QTLs) that are involved in the control of SSC, MD, and M traits and to propose candidate genes for their regulation.

2. Materials and methods

2.1. Plant material

An F1 population with 194 individuals obtained from the cross between the cultivars "O'Henry" and NR-053 from the Chilean peach breeding program (Universidad de Chile-Andes New Varieties Administration) was used in this study. "O'Henry" produces melting yellow-fleshed peach fruit with low SSC levels and is a M-susceptible cultivar with a late harvest. NR-053 (Maillarmagie cv. Magique®), however, is an early-harvest variety that produces melting white-fleshed nectarine fruit with high SSC levels and is a M tolerant cultivar. The mapping population (O × N) consists of 5-year-old trees grown on G × N rootstock in an experimental orchard located at 34°24′S latitude and 70°50′W longitude (INIA-Rayentué, VI Region, Chile). The O × N population presented mainly an early-harvest date, showing a dominant genetic effect from the parental NR-053. The population comprises both nectarines and peaches as well as white and yellow flesh individuals (Table S1). This population segregates for peach fruit quality traits, including SSC and MD, and the physiological disorder M.

2.2. F1 population phenotyping

One hundred fruits with a green–yellow background color (commercial maturity determined using a color table) were harvested for each F1 individual (one tree per genotype). In the lab, the index of absorbance difference (I_{AD}) (Ziosi et al., 2008) was measured using a portable Vis/NIR DA-Meter (Sinteleia, Bologna, Italy). For each fruit, two measurements were taken, one per cheek. Only fruits with an average I_{AD} between 0.8 and 1.2 were selected (Lurie et al., 2013). Five fruits (five biological replicates) were used to calculate the average value for weight at harvest (g), flesh firmness (N), and SSC (ºBrix) over three consecutive seasons (2015–2017). M was measured using nine fruits that were stored for 21 days at 0 °C plus a shelf-life period (to reach consumption firmness) at room temperature using the Paper Absorbent Method (PAM) strategy (Infante et al., 2009). MD was determined as the number of days starting from September 1 (DASF) until the harvest date (November–March in the southern hemisphere) in 2015, 2016, and 2017. Flesh firmness was measured using a Fruit Pressure Tester penetrometer (Effigi, Alfonsine, Italy) on both cheeks of the fruit. SSC was recorded using a temperature-compensated refractometer (Atago, Tokyo, Japan).

2.3. DNA isolation and quantification

Genomic DNA of 194 F1 O × N progeny individuals and the parents "O'Henry" and NR-053 was isolated from 50 to 100 mg of young leaves. DNA extraction was performed using a DNeasy 96 Plant Mini Kit (QIAGEN, Düsseldorf, Germany), according to the manufacturer's protocol. The DNA integrity was determined by electrophoresis on 1% agarose gels. Quantification was performed using a Qubit® 2.0 Fluorometer (Invitrogen™, Eugene, OR, USA) with a Qubit® dsDNA BR Assay Kit (Invitrogen™), according to the manufacturer's protocol.

2.4. Genotyping using SSR and SNP

Thirty SSRs distributed over the eight chromosomes in the peach trees were used to genotype the parents "O'Henry" and NR-053 and the 194 individuals in the mapping population. PCR was performed using PCR GoTaq® Master Mix (Promega, Madison, WI, USA) in a final volume of 10 μL containing 1 × reaction buffer, 1.5 mM $MgCl_2$, 0.25 μM of

forward primer, 0.25 μM of reverse primer, and 40 ng of DNA. The temperature profile was as follows: 94 °C for 4 min, then 35 cycles of 94 °C for 40 s, Tm for 30 s and 72 °C for 30 s, and finally 72 °C for 5 min in a thermocycler PCR system 9700 (Applied Biosystems, Foster City, CA, USA). The PCR products were detected by capillary electrophoresis using the Fragment Analyzer™ Automated CE System (Advanced Analytical Technologies, Ames, IA, USA), and then separated using a dsDNA Reagent Kit 35–500 bp (Analytical Advanced Technologies), according to the manufacturer's recommendations. To determine the number and size of the alleles in the electropherograms the software ProSize 2.0 (Advanced Analytical Technologies, Ames, IA, USA) was used.

SNP genotyping was performed using a Genotyping By Sequencing (GBS) strategy in the Sequencing Facilities at Cornell University (New York, USA) using 188 siblings (this number of samples was used for technical reasons, and the samples were randomly selected) from the mapping population plus the parents "O'Henry" and NR-053 in a HiSeq2500. The samples were aligned against the reference genome of *Prunus persica* v2.0.a1 (Verde et al., 2017) with the BWA alignment software v0.7.8-r455 (Li and Durbin, 2010). The SNP calling pipeline used was Tassel 3.0 (Glaubitz et al., 2014) and the results were analyzed using the software VCFtools v0.1.11 (Danecek et al., 2011) and Plink v1.07 (Purcell et al., 2007).

2.5. Map construction and QTL analysis

Map construction of the F1 O × N population was performed using the software JoinMap®4.1 (Van Ooijen and Voorrips, 2001; Van Ooijen, 2011). Marker selection for map construction was performed by marker segregating distortion using a chi-square test with a p-value < 0.05. The O × N map was obtained using the two-way pseudo-testcross strategy (Grattapaglia and Sederoff, 1994). Two maps were constructed separately for each of the two parents, and the *map integration tool* was used to obtain the consensus O × N map, using the heterozygous loci in both maps as anchors.

Linkage analysis was performed using a minimum Logarithm of Odds (LOD) value of 3.0 and a maximal recombination fraction of 0.45 (Ogundiwin et al., 2009). The genetic distance in centimorgans (cM) was calculated using the Kosambi's mapping function (Kosambi, 1943).

QTL analysis was performed with the MapQTL®6.0 software (Van Ooijen and Maliepaard, 1996) using the phenotypic data of three evaluation seasons in the consensus O × N-map, as well as each parental map independently. The Interval Mapping method was used for parametric traits, considering a significant QTL with a LOD value > 3.0, while the Kruskal–Wallis method was used for non-parametric traits. A QTL with p-value < 0.01 was considered to be significant.

2.6. Sequencing and variant identification in population parents

DNAs from the parents "O'Henry" and NR-053 were sequenced and used for variant identification analysis. Construction of DNA libraries was performed using 100 mg of tissue from each parent with the TruSeq Nano DNA library prep kit (Illumina, San Diego, CA, USA), according to the manufacturer's protocol. The quality, size, and concentration of the DNA libraries were determined using the Fragment Analyzer™ Automated CE System (Advanced Analytical Technologies, Ames, IA, USA) with the DNF-474 High Sensitivity NGS Fragment Analysis Kit (Advanced Analytical Technologies, Ames, IA, USA), according to the manufacturer's protocol. Finally, two libraries were sequenced on HiSeq2500 by the Macrogen Sequencing Service (Seoul, Korea). The sequence analysis was undertaken using the CLC Genomic Workbench v10.0 software (CLC Bio, Aarhus, Denmark). The *Trim Sequences* function of the software was used to clean the paired sequences using the default parameters. The *Map Reads to Reference* function was used to assemble the trimmed reads to the *Prunus persica* v2.0.a1 (Verde et al., 2017) reference genome, while the non-specific reads were ignored. Variant identification was determined using the *Fixed Ploidy Variant Detection* function using the base quality filter with the following parameters: neighborhood radius, 5.0; minimum central quality, 20.0; and minimum neighborhood quality, 15.0. Using the default parameters, the *Compare Sample Variants Track* function was used to identify variants that were present in only one of the parents, and finally, the functional annotation of the variants was made using the *Amino Acid Changes* function to filter out the synonymous variants. Variants were filtered for minimum depth equal to half of the coverage, and a maximum depth equal to double the coverage.

3. Results

3.1. Phenotyping

One hundred and ninety-four siblings of an F1 "O'Henry" (O) × NR-053 (N) population were phenotyped for quality traits during three evaluation seasons (2015, 2016, and 2017). The average value for flesh firmness considering the three evaluation seasons at the harvest stage was 45.8 N. The average fruit weight was 169.4 g, ranging from 92.8 g (minimum) to 318.5 g (maximum; Table S1).

The SSC averaged 12.4, 13.3, and 13.8 ºBrix in the 2015, 2016, and 2017 seasons, respectively, with a transgressive segregation pattern of distribution. The minimum and maximum observed values were 7.3 and 22.2 ºBrix (Fig. 1A) with a Pearson correlation between seasons of 0.79 (p-value < 0.01). For MD during the three seasons, average values of 139, 144, and 130 DASF were recorded, with a minimum and maximum of 105 and 168 DASF. Pearson correlation for this trait was 0.87 (p-value < 0.01; Fig. 1B). Finally, the average values for M, expressed as percentage of juice in the flesh were 27.9% (2015), 31.2% (2016), and 44.8% (2017) of juice after cold storage plus the shelf-life period. The minimum and maximum values were 13.4% and 56.1% of juice and the Pearson correlation between seasons was 0.48 (p-value < 0.01; Fig. 1C).

The normality of the phenotypic data was estimated using a Shapiro–Wilk test (SSC, MD, and M). The results showed a normal distribution for SSC (p-values < 0.98) and MD (p-values < 0.81) for all evaluated seasons. For M, p-values of 0.018, 0.013, and 0.016, were estimated for the three consecutive seasons. Thus, the data for M did not have a normal distribution and non-parametric testing was used to detect QTLs for this trait.

In the 2017 season, the segregation distribution of MD showed an earlier ripening for the entire F1 population (105–144 DASF) compared with the other seasons (2015 and 2016). In 2017, the segregation distribution showed that fruit had a higher percentage of juice compared to the other seasons, and only two mealy siblings (with less than 30% of juice) were found. The juice percentage exhibited minimum and maximum values of 27.2% and 56.1%, respectively (Fig. 1C). However, the Pearson correlation for each trait between the seasons was maintained (SSC, 0.79; MD, 0.87; and M, 0.48).

3.2. SNPs detected by GBS in the O × N population and linkage map construction

The F1 O × N population was genotyped using SSR, SNP, and fruit morphological markers (peach/nectarine and white/yellow). Among the 30 SSRs tested, 11 were polymorphic and 19 were monomorphic in the mapping population. No off-type siblings were found (data not shown). One hundred and eighty-eight siblings plus "O'Henry" and NR-053 parents were genotyped using a Genotyping By Sequencing strategy (GBS) with a coverage of 12.1X, obtaining a total of 29,484 high quality SNPs (Table S2). These markers were filtered for SNPs with less than two missing values (1.65%) and chi-square test results with p-value < 0.05, obtaining 486 SNPs, were useful for map construction.

For linkage map construction, 486 SNPs, 11 SSRs and two morphological markers (fruit pubescence (peach/nectarine), and flesh color

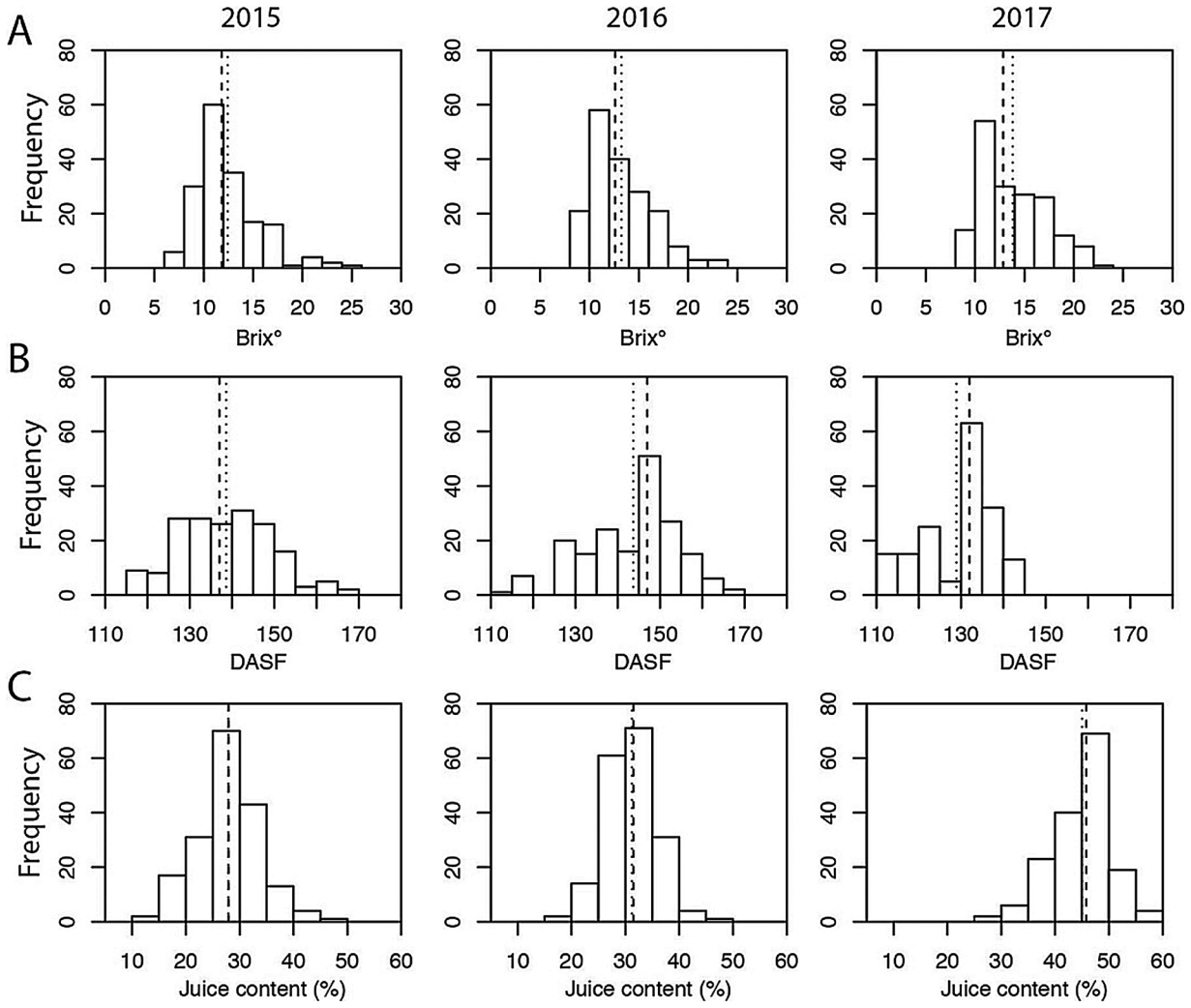

Fig. 1. Phenotyping results. Histograms representing the measured data in three evaluation seasons for soluble solids content (SSC), maturity date (MD), and mealiness (M). The dashed lines represent the average, and the dotted lines represent the median value for each trait. (A) SSC phenotype; (B) MD phenotype; (C) M phenotype.

(white/yellow)) were used. Of these 499 markers, 143 (denoted as < hkxhk > or < abxcd >) were heterozygous for both parents and were used as anchor markers. One hundred and forty-five were heterozygous only for the "O'Henry" parent (denoted as < lmxll >) and 211 only for the parental line, NR-053 (denoted as < nnxnp >). Each group of markers, plus the anchor markers, were used to construct the "O'Henry" and the NR-053 parental map, independently (O-map and N-map, respectively).

For the O-map, 282 markers were mapped spanning 472.04 cM in eight LGs with an average distance between markers of 1.77 cM/marker (Fig. S1). In the N-map, 347 markers were mapped spanning 732.998 cM with an average distance between markers of 2.11 cM/marker (Fig. S2). Finally, a consensus map was constructed to generate the O × N-map (Fig. 2). For the morphological markers on the constructed map, the flesh color marker was mapped on the NR-053 parental map (N-LG1), and located on Pp01:13,914,855..29,174,501 (flanked by the markers CBV_G1_N13914855 and CBV_G1_N29174501; Fig. 2), which is consistent with the reported flesh color candidate gene *PpCCD4* located on Pp01:26,613,248..26,616,233 (Falchi et al., 2013). However, Vendramin et al. (2014) reported a peach/nectarine candidate gene that was located on Pp05:15,892,316..15,894,063, which is described as the transcription factor *PpeMYB25* in the same location that is described in the "O'Henry" parental map (O-LG5) between the markers CBV_G5_O14929750 and CBV_G5_O16747980 (Pp05:14,929,750..16,747,980; Fig. 2). Despite the efforts to genotype other types of markers in LG5 to identify one anchor marker, no useful markers were identified because it was impossible to obtain a consensus O × N-LG5. Therefore, the two parental linkage groups were used independently. The final map contains 499 markers (486 SNP, 11 SSR, and two morphological markers) in 477 unique positions or clusters distributed in nine LGs spanning a total of 717.6 cM, with an average distance of 1.50 cM between clusters. Finally, the consensus O × N-map represents 91.0% of the total Peach v2.0 pseudomolecules, with a minimum LG representativity of 75.9% (LG8). However, some important gaps (larger than 5 Mb) were observed in several cases on LG1, LG2, and LG6. The average physical-to-genetic distance ratio was 3.46 cM/Mb.

3.3. QTL analysis for SSC, MD, and M

The QTL analysis was performed using the saturated O × N genetic linkage map that was described above, and the phenotypic data that were obtained from three evaluation seasons (2015, 2016, and 2017) for SSC, MD, and M. One consistent QTL was identified for SSC on chromosome 5 (LG5) of the O-map ($QTL_{SSC\text{-}LG5}$) between the 0.0 and 29.4 cM markers of O-LG5 (Fig. 3). This region represents approximately 6.1 Mbp containing 1,211 genes (Table 1).

For MD, four QTLs were observed in LG1, LG2, LG5, and LG6

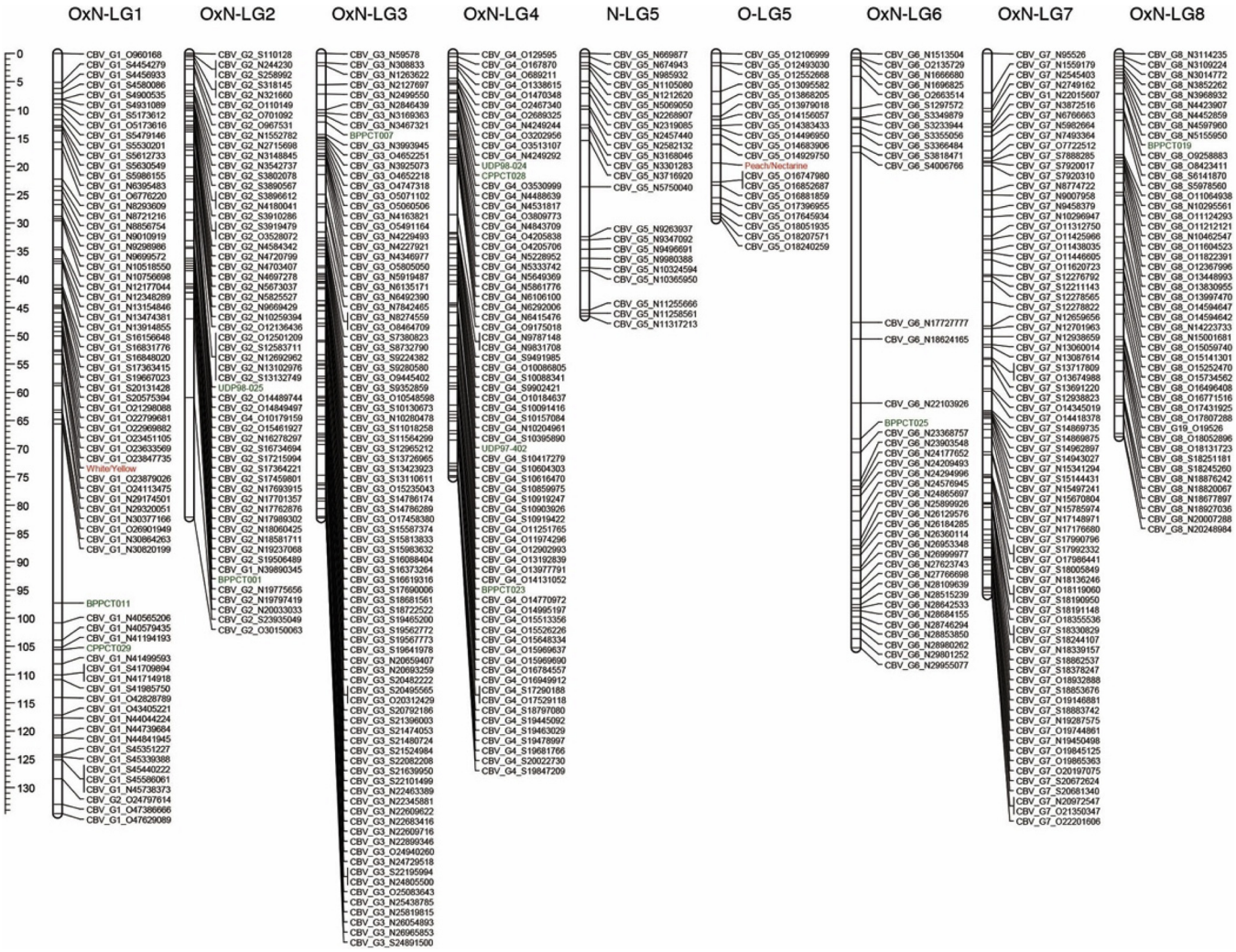

Fig. 2. Genetic linkage map of the O × N population. The nine linkage groups (LGs) of the consensus map (O × N-map) that was constructed are represented by vertical bars. Genetic marker names are listed to the right side of each LG. The morphological markers are shown in red, SSR markers in green and SNP markers in black. The anchor SNP markers, SNP markers heterozygous only for "O'Henry" and SNP markers heterozygous only for NR-053 are denoted with an "S", "O", or "N", respectively. The assembly coordinate of each SNP marker in peach reference v2.0 is specified for all SNP marker names. The ruler on the left side refers to genetic distance in centimorgans (cM).

(Fig. 3). In O × N-LG1, one QTL was detected ($QTL_{MD\text{-}LG1}$) spanning approximately 7.1 Mbp between the 103.9 and 134.6 cM markers, and it contained 997 genes. $QTL_{MD\text{-}LG2}$ was located between the 26.3 and 82.3 cM markers on O × N-LG2, which was equivalent to approximately 14.7 Mbp spanning 2,282 genes. For O-LG5, a reliable QTL containing 657 genes was identified between the 12.8 and 26.6 cM markers, and this corresponded to approximately 3.3 Mbp ($QTL_{MD\text{-}LG5}$). Only one QTL ($QTL_{MD\text{-}LG6}$) was identified in O × N-LG6, between the 0.0 and 81.1 cM markers (23 Mbp) containing 2,311 genes (Table 1) for this trait. A total of 6,247 genes were associated with at least one QTL for MD. Finally, a single QTL for M (Fig. 3) was identified on N-LG5 between the 0.0 and 4.9 cM markers ($QTL_{M\text{-}LG5}$), corresponding to 542.7 kbp containing 60 genes (Table 1).

3.4. SNP/InDel identification by whole genome sequencing of population parents

To identify the DNA variants between the parents of the F1 population (OxN), which could be associated with candidate genes on chromosomic regions containing QTLs for SSC, MD, and M, whole genome re-sequencing of both parents was performed. A total of 104,835,112 reads for "O'Henry" and 88,346,396 reads for NR-053 (SRA experiments SRR8173852 and SRR8173853) were obtained. Additionally, 83.2% of the reads were mapped against the reference genome obtaining a coverage of 38.5X for "O'Henry", and 81.4% of the reads for NR-053, with 46.5X coverage (data not shown).

Variant detection was performed for each parent against the reference genome, revealing 648,302 and 614,008 polymorphisms (SNP and InDel) for "O'Henry" and NR-053, respectively. The variants between the parents were selected, obtaining 519,764 polymorphisms. Finally, identified polymorphisms were filtered by coverage and base calling quality (> Q30), obtaining a final list of 278,984 variants.

In $QTL_{SSC\text{-}LG5}$, 11,434 variants were identified, of which 268 were non-synonymous amino acid changes in 128 genes. For MD, 22,363 variants were detected, of which 202 showed aminoacidic changes in 95 genes on $QTL_{MD\text{-}LG1}$. In $QTL_{MD\text{-}LG2}$, 44,076 variants were found and 234 led to amino acid changes in the CDS region of 88 genes. In $QTL_{MD\text{-}LG5}$, 5,216 variants were identified, of which 123 resulted in changes in the amino acids of 60 different CDS regions. In $QTL_{MD\text{-}LG6}$, 18,734 variants were detected, of which 413 were related to amino acid changes in 190 genes. Finally, for $QTL_{M\text{-}LG5}$, 36 of the 3,026 variants detected were associated with non-synonymous amino acid changes in 18 genes.

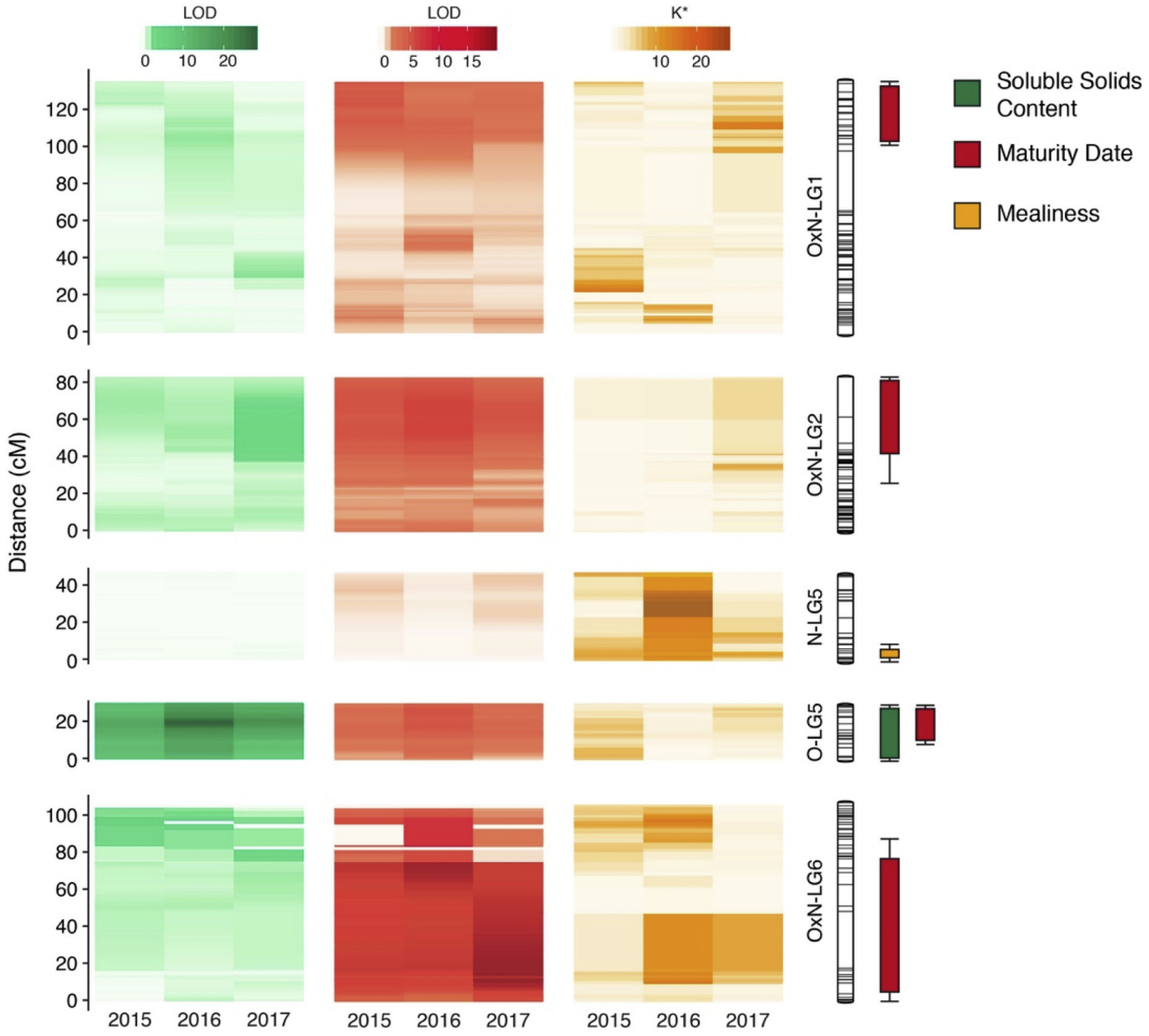

Fig. 3. QTL analysis for soluble solids content, maturity date, and mealiness in the O × N population. On the right side, the linkage groups in the consensus O × N-map are represented by vertical bars and the boxes represent the consistent QTL regions that are defined for SSC (green), MD (red), and M (yellow) in the three evaluation seasons that were tested for each trait. On the left side, the genetic distance of each linkage group in centimorgans (cM) is represented, and the significance value for each QTL analysis is detailed in colored heatmaps. Each evaluation season is represented by an independent heatmap bar. The most saturated color represents a major correlation (LOD score and K) between each phenotype and the respectively genetic linkage position for SSC (green), MD (red), and M (yellow).

3.5. Candidate genes

In summary, 128 annotated genes with at least one variant were identified in a CDS region that produces a non-synonymous amino acid change for SSC. In the four chromosomal regions for MD, 433 annotated genes were selected. Eighteen genes were associated with at least one non-synonymous SNP or InDel for M.

Comparing this list of genes with previously-reported gene information and putative gene functions, we selected candidate genes for SSC, MD, and M.

Of the 128 genes that were related to SSC, four transcription factors were identified, along with one cell wall remodeling enzyme, and one sucrose-related gene. The latter participated directly in the regulation of the SSC because the differences in the amino acid sequences could produce changes in the protein conformation and/or enzymatic activity (Table 2). For MD, 433 genes we redetected, eight of which were transcription factors, 23 were associated with cell wall remodeling functions, three were related to the jasmonate biosynthetic pathway, and one was related to ripening (Table 2). Finally, three of 18 genes that were identified for M were selected, two of which encode integral components of the plasma membrane and one that is described as having a cell wall remodeling function (Table 2).

4. Discussion

4.1. Fruit quality traits phenotype data

Three evaluation seasons were used to phenotype the SSC, MD, and M traits in the O × N mapping population (Fig. 1). Fruit harvested in 2017 displayed consistent data for SSC compared to previous seasons. However, a shift in the MD was recorded, with it being earlier in 2017 than in 2015 or 2016; this was consistent with an improvement in the performance during postharvest. In the 2017 season, the population almost did not exhibit M development. These differences can probably be explained by changes in the environmental conditions between seasons (Campos-Vargas et al., 2005). This suggests that a delay in the harvest time of melting flesh cultivars may produce an increase in chilling injury susceptibility. Additionally, it is necessary to consider the association between the MD and M reported by Lurie and Crisosto (2005), in which early season varieties are tolerant to chilling injury

Table 1
Summary of the consistently detected QTLs for soluble solids content, maturity date, and mealiness over three evaluated seasons.

Quality trait	QTL	Season	LG[+]	Physical position[++]	Peak (cM)	LOD	% Exp	Nearest marker
Soluble solids content	$QTL_{SSC\text{-}LG5}$	2015	O-LG5	Pp05:12,106,999..18,240,259	17.07	14.54	32.70	CBV_G5_O14929750
	$QTL_{SSC\text{-}LG5}$	2016	O-LG5	Pp05:12,106,999..18,240,259	22.78	24.84	48.40	CBV_G5_O16747980
	$QTL_{SSC\text{-}LG5}$	2017	O-LG5	Pp05:12,106,999..18,240,259	17.07	17.67	38.40	CBV_G5_O14929750
Maturity date	$QTL_{MD\text{-}LG1}$	2015	OxN-LG1	Pp01:40,579,435..47,629,089	123.40	4.92	12.10	CBV_G1_S45339388
	$QTL_{MD\text{-}LG1}$	2016	OxN-LG1	Pp01:40,579,435..47,629,089	108.10	3.63	9.10	CBV_G1_N41499593
	$QTL_{MD\text{-}LG1}$	2017	OxN-LG1	Pp01:40,579,435..47,629,089	123.40	3.12	7.50	CBV_G1_S45351227
	$QTL_{MD\text{-}LG2}$	2015	OxN-LG2	Pp02:18,581,711..30,150,063	60.75	4.44	11.00	CBV_G2_S23935049
	$QTL_{MD\text{-}LG2}$	2016	OxN-LG2	Pp02:17,459,801..30,150,063	60.75	5.72	14.00	CBV_G2_S23935049
	$QTL_{MD\text{-}LG2}$	2017	OxN-LG2	Pp02:19,797,419..30,150,063	60.75	4.88	12.50	CBV_G2_S23935049
	$QTL_{MD\text{-}LG5}$	2015	O-LG5	Pp05:14,383,433..17,645,934	17.07	3.34	9.20	CBV_G5_O14929750
	$QTL_{MD\text{-}LG5}$	2016	O-LG5	Pp05:12,552,668..17,645,934	25.53	5.07	12.50	CBV_G5_S17396955
	$QTL_{MD\text{-}LG5}$	2017	O-LG5	Pp05:14,383,433..17,645,934	17.07	3.17	8.30	CBV_G5_S14929750
	$QTL_{MD\text{-}LG6}$	2015	OxN-LG6	Pp06:1,513,504..23,903,548	70.65	16.14	34.50	CBV_G6_N23368757
	$QTL_{MD\text{-}LG6}$	2016	OxN-LG6	Pp06:1,513,504..29,955,077	70.65	18.24	38.10	CBV_G6_N23368757
	$QTL_{MD\text{-}LG6}$	2017	OxN-LG6	Pp06:1,513,504..23,903,548	14.17	19.68	41.70	CBV_G6_S3366484
Mealiness[+++]	$QTL_{M\text{-}LG5}$	2015	N-LG5	Pp05:669,877..2,268,907	2.18	8.60	NA	CBV_G5_N1105080
	$QTL_{M\text{-}LG5}$	2016	N-LG5	Pp05:669,877..11,317,213	23.6	27.48	NA	CBV_G5_N5750040
	$QTL_{M\text{-}LG5}$	2017	N-LG5	Pp05:669,877..1,212,620	2.72	6.39	NA	CBV_G5_N1105080

()[+], O × N linkage groups; ()[++], peach genome v2.0.a1; ()[+++], phenotype measured by KW method using significance values of K*.

Table 2
Candidate genes (CG) for SSC, MD and M on QTL regions. These CGs were selected with at least one single nucleotide variant (amino acid change) between the parents of the O × N population.

QTL name	Gene ID	Gene description	Amino acid changes description
$QTL_{SSC\text{-}LG5}$	Prupe.5G130400	Transcription factor bHLH14	G419S
	Prupe.5G172400	Transcription factor MYB98	S101Y
	Prupe.5G175100	Probable polygalacturonase	S31fs; G318D; K466 T; W567R; W567C
	Prupe.5G208500	MADS-box protein MADS6	N239S
	Prupe.5G241700	Sucrose synthase 6	K64 T; E67 G; S189 L; F233S; N560S
$QTL_{MD\text{-}LG1}$	Prupe.1G519800	UDP-glycosyltransferase 85A2	G443E
	Prupe.1G520200	UDP-glycosyltransferase 85A2	Q475E
	Prupe.1G529500	Pectinesterase/pectinesterase inhibitor 7	N544fs
	Prupe.1G530800	Putative transcription factor bHLH107	H89N
	Prupe.1G536600	Rhamnogalacturonate lyase	G482D;G583D;G599D
	Prupe.1G548400	12-oxophytodienoate reductase OPR2	Y284fs;M152 T;N34L
	Prupe.1G548600	12-oxophytodienoate reductase OPR2	T153I
	Prupe.1G548900	12-oxophytodienoate reductase OPR2	E288K
$QTL_{MD\text{-}LG2}$	Prupe.2G100500	Pectinesterase/pectinesterase inhibitor 51	Q43 K;T34P
	Prupe.2G166800	Putative ripening-related protein 2	K9Q
	Prupe.2G243600	UDP-glycosyltransferase 76F1	R118 G; Q297 H; F307 L; F314 L; R316D
$QTL_{MD\text{-}LG5}$	Prupe.5G172400	Transcription factor MYB98	S101Y
	Prupe.5G175100	Probable polygalacturonase	S31fsI;K466 T;W567R;C41fs
	Prupe.5G208500	MADS-box protein MADS6	N239S
$QTL_{MD\text{-}LG6}$	Prupe.6G019600	Beta-glucosidase 12	P48S
	Prupe.6G019700	Beta-glucosidase 12	N532S;N533S
	Prupe.6G020100	Beta-glucosidase 12	P48S
	Prupe.6G024900	Cellulose synthase-like protein E1	R82fs;Y234 H;T253 P;K259E;L385fs;P485 L;K578E
	Prupe.6G025000	Cellulose synthase-like protein E1	Y477 H;H412Y;P84 T;F38I;Y682 H;H617Y
	Prupe.6G036300	Probable WRKY transcription factor 2	G659R;G628R
	Prupe.6G041400	Transcription factor RF2b	A95_E96insAAA;G192del;L391H
	Prupe.6G050000	UDP-glycosyltransferase 87A1	E254 V;K235T
	Prupe.6G050100	UDP-glycosyltransferase 87A1	W390*;L283 P;R261H
	Prupe.6G050200	UDP-glycosyltransferase 87A2	H448Y
	Prupe.6G128200	Pectinesterase PPME1	Q181H
	Prupe.6G134900	Beta-glucosidase 24	F47V
	Prupe.6G136400	Beta-glucosidase 12	L452F
	Prupe.6G139100	Transcription factor E2FA	N67K
	Prupe.6G150400	Beta-glucosidase BoGH3B	R25Q;M23I
	Prupe.6G150500	Beta-glucosidase BoGH3B	F9V
	Prupe.6G214300	Transcription factor RAX3	E157G
	Prupe.6G233300	UDP-glycosyltransferase 86A1	F290L
	Prupe.6G233400	UDP-glycosyltransferase 86A1	L385P
	Prupe.6G233600	UDP-glycosyltransferase 86A1	H160L
	Prupe.6G244300	WRKY transcription factor 44	N116 T;Q137 H;T166I;L422P
$QTL_{M\text{-}LG5}$	Prupe.5G008200	Glucan endo-1,3-beta-glucosidase 12	I86L

(mealiness) and late season varieties are very susceptible to this injury.

4.2. Linkage map evaluation and validation

The consensus genetic linkage map constructed for the O × N population contained a total of 499 markers that were distributed in nine linkage groups. Bielenberg et al. (2015) used the same methodology to construct a genetic linkage map for peach, obtaining a map with similar characteristics but with half the number of markers and a density of 2.85 cM/marker. Linkage maps that were built using other strategies were obtained in peach with map densities that were similar to the results reported for the O × N-map; for example, Eduardo et al. (2013); Yang et al. (2013); Nuñez-Lillo et al. (2015), and Verde et al. (2017) using the IPSC 9 K SNP array v1 (Verde et al., 2012), obtained genetic maps with marker densities of 1.62, 1.15, 1.64, and 1.07 cM/marker, respectively. Two major gaps were found in the LG1 and LG6, and even when less exigent parameters were used, no markers were identified. The genetic physical ratio (cM/Mb) was 3.46 higher than those reported by Verde et al. (2017) for seven different linkage maps, highlighting the higher recombination rate in the O × N cross.

The O × N map has a genome coverage of 91%, which is comparable with other linkage maps that were constructed using different strategies. Da Silva et al. (2015) generated a linkage map in a "NJ Weeping" × "Bounty" population with a genome coverage of 93.6%, while Verde et al. (2017) used three genetic maps, "Texas" × "Earlygold" (T × E), IF7310828 × "Ferganensis" (P × F), and "Contender" × "Ambra" (CxA) with genome coverage of 97.5%, 96.9%, and 88.1%, respectively.

The positions of microsatellites and morphological markers mapped on the O × N-map were evaluated. The physical positions of the microsatellites on the O × N-map were consistent with those described in the *P. persica* genome v2.0 (Verde et al., 2017), which was obtained from the Rosaceae.org database (data not shown) with few order discrepancies.

Considering the high marker density compared with previously-constructed linkage maps, the high percentage of the peach genome represented (91%), and the correct positions of the microsatellites and morphological markers on the O × N-map, the O × N-map is considered to be useful for performing QTL analysis and to identify the genes involved in SSC, MD, and M inheritance.

4.3. Chromosomic regions related to SSC, MD, and M

In previous peach studies, the QTLs for fruit quality traits were usually linked to chromosome 4 (Dirlewanger et al., 1999; Etienne et al., 2002; Quilot et al., 2004; Cantín et al., 2010; Eduardo et al., 2013; Socquet-Juglard et al., 2013; Nuñez-Lillo et al., 2015; Font i Forcada et al., 2019). However, the latest research has identified candidate genes for different quality traits. Font i Forcada et al. (2019) presented 347 SNPs that were associated with fruit quality in peach (sorbitol and total sugars and MD) that were distributed in all of the eight LGs. A pleiotropic effect has been reported in LG4 for the MD phenotype on other traits such as SSC, firmness, fruit weight, and M (Eduardo et al., 2011; Pirona et al., 2013; Nuñez-Lillo et al., 2015). Pirona et al. (2013) identified a NAC transcription factor (*NAC072*) as a candidate gene for MD on chromosome 4, detecting an InDel of 9 bp in the last exon of this gene; Nuñez-Lillo et al. (2015) detected a deletion of 26 kbp in the same region of *NAC072*. The deletion was homozygous (absence of *NAC072*) and was associated with a slow ripening trait, suggesting a strong correlation between *NAC072* function and MD control.

The results obtained with the O × N population showed a correlation between SSC, MD, and M in LG1, LG2, LG5, and LG6, but not in LG4, suggesting that their control is independent of *NAC072*. This regulation could be provided by a complementary *NAC072* pathway or by downstream factors in the same pathway. The nucleotide variations observed by Pirona et al. (2013) and Nuñez-Lillo et al. (2015) on LG4 (i.e. the deletions in *NAC072*), were not detected in our O × N population.

4.4. Candidate genes related to SSC

In peach, QTLs for SSC have previously been found to be associated with chr1 (Quilot et al., 2004), chr2 (Verde et al., 2002; Quilot et al., 2004; Zeballos et al., 2016; Hernández Mora et al., 2017), chr4 (Dirlewanger et al., 1999; Etienne et al., 2002; Quilot et al., 2004; Nuñez-Lillo et al., 2015; Zeballos et al., 2016; Hernández Mora et al., 2017), chr5 (Quilot et al., 2004; Zeballos et al., 2016; Hernández Mora et al., 2017), and chr6 (Dirlewanger et al., 1999; Etienne et al., 2002; Verde et al., 2002; Quilot et al., 2004; Hernández Mora et al., 2017),the most powerful of which are those on chromosome 4. The genetic results obtained from the O × N population show a strong correlation between SSC and a 12–18 Mbp region of chromosome 5. Using a multiprogeny approach, Quilot et al. (2004); Zeballos et al. (2016) and Hernández-Mora et al. (2017) identified similar QTLs for SSC on chr5 in populations containing "Big Top" (yellow nectarine tree) and "Belbinette" **(white** peach tree) as parents with transgressive distributions. In the study presented by Font i Forcada et al. (2019) were described two SNPs related to the sugar content and sorbitol in the promoter of the candidate gene ppa025636 m located in the LG4.

In O × N, a candidate gene was identified for SSC, and it is described as sucrose synthase 6 (*SUS6*), which is directly-associated with the sugar accumulation process (Table 2). Sucrose is the most important individual sugar that is present in peach fruit at maturity and it is also an energy source and fruit flavor preservative (Cantín et al., 2009). Sucrose synthase proteins have been reported to be the main enzymes that are responsible in the fruit sugar accumulation process, catalyzing the conversion of glucose and fructose to sucrose inside the cell (Moriguchi et al., 1990). This enzyme has a low activity during the initial stages of fruit growth, followed by a rapid increase in activity during fruit maturation, concomitant with sucrose accumulation (Moriguchi et al., 1992). It, thus, plays a crucial role in the sucrose accumulation process, which could directly explain the distribution of the SSC phenotype. This behavior agrees with previous information where the progeny from "O'Henry" presented higher levels of SSC compared with the progeny from different parents (Cantín et al., 2009).

However, only one gene that is related to the softening process with a cell wall remodeling function was identified. It was described as a probable polygalacturonase (PG) associated with the SSC phenotype. Etienne et al. (2002) described the relationship between sugar accumulation and softening processes in fruit development. The accumulation of sugars in the vacuole produces an influx of water into the cell by osmosis, concomitant with cell expansion. Pressey et al. (1971) reported a positive correlation between PG activity and softening in peach fruit development, as determined by fruit firmness and the levels of water soluble pectins.

Finally, three transcription factors were identified with amino acid variations related to SSC: bHLH14, MYB98, and MADS6 (Table 2). The transcription factor bHLH14 belongs to the subgroup III d + e of the *Arabidopsis thaliana* bHLH family, together with At4g00870, which is a known jasmonic acid (JA)-responsive transcription factor (Zhang et al., 2018). Song et al. (2013) reported that this bHLH subgroup of transcription factors acts as repressors and function redundantly to negatively regulate JA responses. The transcription factor MYB98 has a reported function associated with the regulation of pollen tube guidance genes and filiform apparatus formation in *A. thaliana* (Punwani et al., 2008), while the MADS-box protein identified as MADS6 or transcription factor CAULIFLOWER A has been associated with specifying floral organ identities and meristem fates (Li et al., 2011). Although no functions of these transcription factors have been reported in fruit, it is possible that they participate in the regulation of sucrose accumulation genes, thus explaining the SSC phenotype distribution. Expression

analysis is required to complement this information to identify their possible role in fruit development.

4.5. Candidate genes related to MD

Twenty-three genes associated with cell wall remodeling enzymes were related to MD. The cell wall varies in composition and in the enzymes involved in the remodeling process within and between individual cells, tissues, and species. The basic cell wall structure is a single complex polysaccharide network featuring interactions between cellulose, pectin, and hemicellulose (Dick-Pérez et al., 2011). Nine candidate genes involved in cellulose formation were identified (Table 2): two were described as cellulose synthase-like proteins E1 (Richmond and Somerville, 2000), and seven were described as beta-glucosidases (Table 2). These candidate genes were reported as enzymes that are involved in the degradation of cellulose to glucose (Esen, 1993). Fourteen genes related to the pectin structure were also identified. Nine UDP-glycosyltransferases, which are proteins that mediate the transfer of glycosyl residues regulating properties of the acceptors such as their bioactivity, solubility, and transport within the cell (Ross et al., 2001); one rhamnogalacturonate lyase, which directly degrades rhamnogalacturonans (Naran et al., 2007); one pectinesterase (PPME1); and two pectinesterase/pectinesterase inhibitors (PEi7 and PEi51) were also identified. These enzymes regulate the de-esterification of pectin and conversion of protopectin to soluble pectin and pectate (Jiang et al., 2002); the role of these proteins was reported by Brummel and Harpster (2001) as being associated with fruit softening during tomato fruit ripening. Finally, one polygalacturonase (PG) was found. These cell wall remodeling enzymes can participate in fruit softening, explaining the differences in the harvesting time observed in the O × N population.

However, three candidate genes, described as 12-oxophytodienoate reductases (OPR2), were identified as being associated with MD. All three participated in the JA biosynthetic pathway (Table 2). The JA biosynthetic pathway starts by converting linoleic acid to 12-oxophytodienoic acid (OPDA) through the participation of proteins such as lipoxygenases, allene oxide synthases, and allene oxide cyclases; OPDA is subsequently reduced by OPR proteins to produce JA (Dave and Graham, 2012). Dave and Graham (2012) described an OPDA signaling pathway that is independent of JA signaling in seeds, which is associated with the regulation of germination potential. Ribot et al. (2008) demonstrated an OPDA-independent signaling route of JA and ABA in response to root wounding. The results obtained here suggest a possible OPDA- or JA-signaling imbalance, which could explain the distribution of the MD phenotype, but complementary analysis is required to determine the real participation of these signals in the regulation of fruit development.

Finally, eight transcriptional regulators were identified in the MD analysis (Table 2). The transcription factor bHLH107 was mentioned by Zhang et al. (2018) as a member of the subgroup Vb of the *A. thaliana* bHLH family proteins that are associated with genes such as At1g68810 and At3g25710, which are related to the upwardly curly leaf phenotype (An et al., 2014) and ABA-mediated signaling transduction (Kim and Kim, 2006). The transcription factor WRKY2 has been reported to be related to seed germination (Jiang and Yu, 2009), xylem development (Guillaumie et al., 2010), and osmotic stress (Wen-Bo and Di-Qiu, 2009). However, WRKY44 is associated with functions such as the drought response (Han et al., 2015) and the defense response against herbivores and microbes (Bonaventure et al., 2011). Moreover, RF2b is a transcription factor that is strongly related to virus resistance in rice (Dai et al., 2004; Liu et al., 2007; Dai et al., 2008). The transcription factor E2FA in *A. thaliana* has been reported to control proliferation, endoreduplication, and differentiation (De Veylder et al., 2002; Boudolf et al., 2004), and the transcription factor RAX3 has been described as a regulator of axillary meristem formation (Dubos et al., 2010) and plant shoot branching (Guo, 2015).

The transcription factors MYB98 and MADS6 were described-previously in the "Candidate genes for SSC" section, and together with the probable PG, they are candidates for both SSC and MD phenotypes, suggesting a possible partial pleiotropic effect between MD and SSC. Similar situation was described by Font i Forcada et al. (2019), they found a SNP very close to the position of SNP_IGA_700469 in the LG6 close to marker CPPCT030 and the gene ppa006828, associated with sorbitol and harvest date; this gene is related to peroxisome biogenesis. Associated to MD was described the UDP96-001 marker close to the SNP_IGA_630302, candidate gene ppa024155 described as the core histone H2B. Finally, the same group associated to MD and flavonoids the candidate gene ppa021329 on chromosome 6, this gene is similar to lycopene cyclase.

4.6. Candidate genes related to M

A QTL for M in the LG5 from the O × N population was obtained, and this phenotype has been reported consistently with a QTL on the LG4 of peach in several studies (Peace et al., 2006; Eklöf and Brumer, 2010; Martínez-García et al., 2013; Socquet-Juglard et al., 2013). The correlation between MD and M reported by Lurie and Crisosto (2005), together with the QTL for MD and M that was obtained consistently in the LG4 of peach suggests a pleiotropic effect of MD in other phenotypes, including M. It has been postulated that genetic variations in the transcription factor NAC072 are responsible for this pleiotropic effect (Pirona et al., 2013; Nuñez-Lillo et al., 2015). In the O × N population, non-nucleotidic variations in this transcription factor (data not shown) in the parents were detected, and no QTL for M in the LG4 of peach was obtained. This suggests that the absence of genomic variations in the NAC072 region in the parents significantly reduces the pleiotropic effect of MD in the O × N population. Thus, a QTL for M in LG5 was obtained, allowing us to study the M phenotype without interference from MD.

For M, one gene was identified, which has been related to cell wall remodeling functions, and is described as a glucan endo-1,3-beta-glucosidase 12 (Table 2).

However, it is possible that the M phenotype is not determined by variation in the CDS region of a gene. It is necessary to explore this possibility by analyzing the promoter region of candidate genes because variations may produce differences in expression levels that could explain the phenotype variation. Thus, it is necessary to complement or to integrate the genetic/genome data with transcriptome analysis to identify the genes that are involved in the M trait.

5. Conclusions

The results obtained in this work are the basis for understanding the genetic control of fruit quality traits in the O × N mapping population. The analysis allowed the identification of candidate genes for MD, SSC, and M, which could be validated by functional strategies. In the future, this work will need to be complemented by metabolomic and transcriptomic data using contrasting siblings for each trait to correlate the genetic variations with gene expression levels to find the regulatory gene network that is responsible for the SSC, MD, and M traits.

Funding

This work was supported by Fondo Nacional de Desarrollo Científico y Tecnológico FONDECYT-CHILE1160584, Fondo de Fomento al Desarrollo Científico y Tecnológico FONDEF-CHILED13i10005 and Corporación de Fomento de la Producción CORFO-CHILE Biofrutales13CTI21520-SP04.

Acknowledgments

We thank Michael Handford (Universidad de Chile) for language

support.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:10.1016/j.scienta.2019.108734.

References

Abidi, W., 2016. Quality attributes and chilling injury susceptibility of new peach [*Prunus persica* (L.) Batsch] cultivars grown under Tunisian conditions. JMEST 3, 4223–4227.

An, R., Liu, X., Wang, R., Wu, H., Liang, S., Shao, J., Qi, Y., An, L., Yu, F., 2014. The over-expression of two transcription factors, ABS5/bHLH30 and ABS7/MYB101, leads to upwardly curly leaves. PLoS One 9, e107637 https://doi.org/10.1371/journal.-pone.0107637.

Arús, P., 2005. Integrating genomics into rosaceae fruit breeding. Acta Hortic. 738, 29–35 https://doi.org/10.17660/ActaHortic.2007.738.1.

Bielenberg, D.G., Rauh, B., Fan, S., Gasic, K., Abbott, A.G., Reighard, G.L., Okie, W.R., Wells, C.E., 2015. Genotyping by sequencing for SNP-based linkage map construction and QTL analysis of chilling requirement and bloom date in peach (*Prunus persica* (L.) Batsch). PLoS One 10, e0139406 https://doi.org/10.1371/journal.pone.0139406.

Bliss, F.A., Arulsekar, S., Foolad, M.R., Becerra, V., Gillen, A.M., Warburton, M.L., Dandekar, A.M., Kocsisne, G.M., Mydin, K.K., 2002. An expanded genetic linkage map of *Prunus* based on an interspecific cross between almond and peach. Genome 45, 520–529 https://doi.org/10.1139/g02-011.

Bonaventure, G., VanDoorn, A., Baldwin, I.T., 2011. Herbivore-associated elicitors: FAC signaling and metabolism. Trends Plant Sci. 16, 294–299 https://doi.org/10.1016/j.tplants.2011.01.006.

Boudolf, V., Vlieghe, K., Beemster, G.T., Magyar, Z., Torres Acosta, J.A., Maes, S., Van Der Schueren, E., Inzé, D., De Veylder, L., 2004. The plant-specific cyclin-dependent kinase CDKB1;1 and transcription factor E2Fa-DPa control the balance of mitotically dividing and endoreduplication cells in Arabidopsis. Plant Cell 16, 2683–2692 https://doi.org/10.1105/tpc.104.024398.

Brummel, D.A., Harpster, M.H., 2001. Cell wall metabolism in fruit softening and quality and its manipulation in transgenic plants. Plant Mol. Biol. 47, 311–339 https://doi.org/10.1023/A:1010656104304.

Campos-Vargas, R., Becerra, O., Baeza-Yates, R., Cambiazo, V., González, M., Meisel, L., Orellana, A., Retamales, J., Silva, H., Defilippi, B.G., 2005. Seasonal variation in the development of chilling injury in 'O`Henry' peaches. Sci. Hort. 110, 79–83 https://doi.org/10.1016/j.scienta.2006.06.019.

Cantín, C.M., Gogorcena, Y., Moreno, M.A., 2009. Analysis of phenotypic variation of sugar profile in different peach and nectarine *Prunus persica* (L.) Bastch] breeding progenies. J. Sci. Food Agric. 89, 1909–1917 https://doi.org/10.1002/jsfa.

Cantín, C.M., Crisosto, C.H., Ogundiwin, E.A., Gradziel, T., Torrents, J., Moreno, M.A., Gogorcena, Y., 2010. Chilling injury susceptibility in an intra-specific peach [*Prunus persica* (L.) Bastch] progeny. Postharvest Biol. Tec. 58, 79–87 https://doi.org/10.1016/j.postharvbio.2010.06.002.

Crisosto, C.H., Labavitch, J.M., 2002. Developing a quantitative method to evaluate peach (*Prunus persica*) flesh mealiness. Postharvest Biol. Tec. 25, 151–158 https://doi.org/10.1016/S0925-5214(01)00183-1.

Crisosto, C.H., Crisosto, G.M., 2005. Relationship between ripe soluble solids concentration (RSSC) and consumer acceptance of high and low acid melting flesh peach and nectarine (*Prunus persica* (L.) Batsch) cultivars. Postharvest Biol. Tec. 38, 239–246 https://doi.org/10.1016/j.postharvbio.2005.07.007.

Da Silva, C., Bassi, D., Bianco, L., Pacheco, I., Pirona, R., Rossini, L., 2015. Genetic dissection of fruit weight and size in a F2 peach (*Prunus persica* (L.) Batsch) progeny. Mol. Breeding 35, 71–89 https://doi.org/10.1007/s11032-015-0271-z.

Dai, S., Zhang, Z., Chen, S., Beachy, R.N., 2004. RF2b, a rice bZIP transcription activator, interacts with RF2a and is involved in symptom development of rice tungro disease. PNAS 101, 687–692 https://doi.org/ 10.1073/pnas.0307687100.

Dai, S., Wei, X., Alfonso, A.A., Pei, L., Duque, U.G., Zhang, Z., Babb, G.M., Beachy, R.N., 2008. Transgenic rice plants that overexpress transcription factors RF2a and RF2b are tolerant to rice tungro virus replication and disease. PNAS 105, 21012–21016 https://doi.org/ 10.1073/pnas.0810303105.

Danecek, P., Auton, A., Abecasis, G., Albers, C.A., Banks, E., DePristo, M.A., Handsaker, R.E., Lunter, G., Marth, G.T., Sherry, S.T., McVean, G., Durbin, R., 2011. The variant call format and VCFtools. Bioinformatics 27, 2156–2158 https://doi.org/ 10.1093/bioinformatics/btr330.

Dave, A., Graham, I.A., 2012. Oxylipin signaling: a distinct role for the jasmonic acid precursor cis-(+)-12-oxo-phytodienoic acid (cis-OPDA). Front. Plant Sci. 3 https://doi.org/10.3389/fpls.2012.00042.

De Veylder, L., Beeckman, T., Beemster, G.T., de Almeida Engler, J., Ormenese, S., Maes, S., Naudts, M., Van Der Schueren, E., Jacqmard, A., Engler, G., Inzé, D., 2002. Control of proliferation, endoreduplication and differentiation by the *Arabidopsis* E2Fa-DPa transcription factor. EMBO J. 21, 1360–1368 https://doi.org/ 10.1093/emboj/21.6.1360.

Dick-Pérez, M., Zhang, Y., Hayes, J., Salazar, A., Zebatina, O.A., Hong, M., 2011. Structure and interactions of plant cell-wall polysaccharides by two- and three-dimensional magic-angle-spinning solid-state NMR. Biochem. 50, 989–1000 https://doi.org/ 10.1021/bi101795q.

Dirlewanger, E., Moing, A., Rothan, C., Svanella, L., Pronier, V., Guye, A., Plomion, C., Monet, R., 1999. Mapping QTL controlling fruit quality in peach (*Prunus persica* (L.) Batsch). Theor. Appl. Genet. 98, 18–31 https://doi.org/10.1007/s001220051.

Dubos, C., Stracke, R., Grotewold, E., Weisshaar, B., Martin, C., Lepiniec, L., 2010. MYB transcription factors in Arabidopsis. Trends Plant Sci. 15, 573–581 https://doi.org/10.1016/j.tplants.2010.06.005.

Eduardo, I., Chietera, G., Pirona, R., Pacheco, I., Troggio, M., Banchi, E., Bassi, D., Rossini, L., Vecchietti, A., Pozzi, C., 2013. Genetic dissection of aroma volatile compounds from the essential oil of peach fruit: QTL analysis and identification of candidate genes using dense SNP maps. Tree Genet. Genomes 9, 189–204 https://doi.org/ 10.1007/s11295-012-0546-z.

Eduardo, I., Pacheco, I., Chietera, G., Bassi, D., Pozzi, C., Vecchietti, A., Rossini, L., 2011. QTL analysis of fruit quality traits in two peach intraspecific populations and importance of maturity date pleiotropic effect. Tree Genet. Genomes 7, 323–335 http://doi.org/ 10.1007/s11295-010-0334-6.

Eklöf, E.M., Brumer, H., 2010. The XTH gene family: an update on enzyme structure, function, and phylogeny in xyloglucan remodeling. Plant Physiol. 153, 456–466 https://doi.org/10.1104/pp.110.156844.

Esen, A., 1993. B-Glucosidases: Overview, Biochemistry and Molecular Biology. pp. 1-14. American Chemical Society, Washington DC, USA.

Etienne, C., Rothan, C., Moing, A., Plomion, C., Bodénes, C., Svanella-Dumas, L., Cosson, P., Pronier, V., Monet, R., Dirlewanger, E., 2002. Candidate genes and QTL for sugar and organic acid content in peach [*Prunus persica* (L.) Batsch]. Theor. Appl. Genet. 105, 145–159 https://doi.org/ 10.1007/s00122-001-0841-9.

Falchi, R., Vendramin, E., Zanon, L., Scalabrin, S., Cipriani, G., Verde, I., Vizzotto, G., Morgante, M., 2013. Three distinct mutational mechanisms acting on a single gene underpin the origin of yellow flesh in peach. Plant J. 76, 175–187 https://doi.org/10.1111/tpj.12283.

Font i Forcada, C., Guajardo, V., Chin-Wo, S.R., Moreno, M.A., 2019. Association mapping analysis for fruit quality traits in *Prunus persica* using SNP markers. Front. Plant Sci. 9, 2005. https://doi.org/10.3389/fpls.2018.02005.

Giovane, A., Servillo, L., Balestrieri, C., Raiola, A., D'Avino, R., Tamburrini, M., Ciardiello, M.A., Camardella, L., 2004. Pectin methylesterase inhibitor. Biochim. Biophys. Acta 1696, 245–252. https://doi.org/10.1016/j.bbapap.2003.08.011.

Glaubitz, J.C., Casstevens, T.M., Lu, F., Harriman, J., Elshire, R.J., Sun, Q., Buckler, E.S., 2014. TASSEL-GBS: a high capacity genotyping by sequencing analysis pipeline. PLoS One 9. https://doi.org/10.1371/journal.pone.0090346.

Grattapaglia, D., Sederoff, R., 1994. Genetic linkage maps of *Eucalyptus grandis* and *Eucalyptus urophylla* using a pseudo-testcross: mapping strategy and RAPD markers. Genetics 137, 1121–1137.

Guillaumie, S., Mzid, R., Méchin, V., Léon, C., Hichri, I., Destrac-Irvine, A., Trossat-Magnin, C., Delrot, S., Lauvergeat, V., 2010. The grapevine transcription factor WRKY2 influences the lignin pathway and xylem development in tobacco. Plant Mol. Biol. 72, 215–234. https://doi.org/10.1007/s11103-009-9563-1.

Guo, D., 2015. The WRKY transcription factor WRKY71/EXB1 controls shoot branching by transcriptionally regulating RAX genes in Arabidopsis. Plant Cell 27, 3112–3127. https://doi.org/10.1105/tpc.15.00829.

Han, Y., Zhang, X., Wang, W., Wang, Y., Ming, F., 2015. The suppression of WRKY44 by GIGANTEA-miR172 pathway is involved in drought response in *Arabidopsis thaliana*. PLoS One 10. https://doi.org/10.1371/journal.pone.0073541.

Hernández Mora, J.R., Micheletti, D., Bink, M., Van de Weg, E., Cantín, C., Nazzicari, N., Caprera, A., Dettori, M.T., Micali, S., Banchi, E., Campoy, J.A., Dirlewanger, E., Lambert, P., Pascal, T., Troggio, M., Bassi, D., Rossini, L., Verde, I., Quilot-Turion, B., Laurens, F., Arús, P., Aranzana, M.J., 2017. Integrated QTL detection for key breeding traits in multiple peach progenies. BMC Genomics 18, 404. https://doi.org/10.1186/s12864-017-3783-6.

Infante, R., Meneses, C., Rubio, P., Seibert, E., 2008. Quantitative determination of flesh mealiness in peach [*Prunus persica* L. (Batch.)] through paper absorption of free juice. Postharvest Biol. Tec. 51, 118–121 https://doi.org/ 10.1016/j.post-harvbio.2008.06.006.

Infante, R., Meneses, C., Crisosto, C.H., 2009. Preconditioning treatment maintains taste characteristic perception of ripe 'September Sun' peach following cold storage. Int. J. Food. Sci. Tech. 44, 1011–1016 https://doi.org/ 10.1111/j.1365-2621.2008.01864.x.

Jiang, C.M., Li, C.P., Chang, H.M., 2002. Characterization of pectinesterase inhibitor in jelly fig (*Ficus awkeotsang* Makino) achenes. J. Agr. Food. Chem. 50, 4890–4894 https://doi.org/ 10.1021/jf011568.

Jiang, W., Yu, D., 2009. *Arabidopsis* WRKY2 transcription factor mediates seed germination and postgermination arrest of development by abscisic acid. BMC Plant Biol. 9, 96–99. https://doi.org/10.1186/1471-2229-9-96.

Jolie, R.P., Duvetter, T., Vandevenne, E., Van Vuggenhout, S., Van Loey, A.M., Hendrickx, M.E., 2010. A pectin-methylesterase-inhibitor-based molecular probe for in situ detection of plant pectin methylesterase activity. J. Agr. Food. Chem. 58, 5449–5456. https://doi.org/10.1021/jf100248u.

Kim, J., Kim, H.Y., 2006. Molecular characterization of a bHLH transcription factor involved in *Arabidopsis* abscisic acid-mediated response. Biochim. Biophys. Acta 1759, 191–194. https://doi.org/10.1016/j.bbaexp.2006.03.002.

Kosambi, D.D., 1943. The estimation of map distances from recombination values. Ann. Hum. Genet. 12, 172–175.

Li, H., Liang, W., Hu, Y., Zhu, L., Yin, C., Xu, J., Dreni, L., Kater, M.M., Zhang, D., 2011. Rice MADS6 interacts with the floral homeotic genes SUPERWOMAN1, MADS3, MADS58, MADS13 and DROOPING LEAF in specifying floral organ identities and meristem fate. Plant Cell 23, 2536–2552 https://doi.org/ 10.1105/tpc.111.087262.

Li, H., Durbin, R., 2010. Fast and accurate long-read alignment with burrows-wheeler transform. Bioinformatics 26, 589–595 http://doi.org/ 10.1093/bioinformatics/btp698.

Liu, Y., Dai, S., Beachy, R.N., 2007. Role of the C-terminal domains of rice (*Oryza sativa* L.) bZIP proteins RF2a and RF2b in regulating transcription. Biochem. J. 205, 243–249. https://doi.org/10.1042/BJ20061375.

Lurie, S., Friedman, H., Weksler, A., Dagar, A., Zerbini, P.E., 2013. Maturity assessment at harvest and prediction of softening in an early and late season melting peach. Postharvest Biol. Tec. 76, 10–16. https://doi.org/10.1016/j.postharvbio.2012.08.007.

Lurie, S., Crisosto, C.H., 2005. Chilling injury in peach and nectarine. Postharvest Biol. Tec. 37, 195–208. https://doi.org/10.1016/j.postharvbio.2005.04.012.

Manganaris, G.A., Vasilakakis, M., Diamantidis, G., Mignani, I., 2006. Diverse metabolism of cell wall components of melting and non-melting peach genotypes during ripening after harvest or cold storage. J. Environ. Sci. Health B 86, 243–250. https://doi.org/10.1002/jsfa.2310.

Martínez-García, P.J., Parfitt, D.E., Ogundiwin, E.A., Fass, J., Chan, H.M., Ahmad, R., Lurie, S., Dandekar, A., Gradziel, T.M., Crisosto, C.H., 2013. High density SNP mapping and QTL analysis for fruit quality characteristics in peach (*Prunus persica* L.). Tree Genet. Genomes 9, 19–36. https://doi.org/10.1007/s11295-012-0522-7.

Moriguchi, T., Abe, K., Sanada, T., Yamaki, S., 1992. Levels and role of sucrose synthase, sucrose-phosphate synthase, and acid invertase in sucrose accumulation in fruit of Asian pear. J. Am. Soc. Hortic. Sci. 112, 274–278. https://doi.org/10.21273/JASHS.117.2.274.

Moriguchi, T., Sanada, T., Yamaki, S., 1990. Seasonal fluctuations of some enzymes relating to sucrose and sorbitol metabolism in peach fruit. J. Am. Soc. Hortic. Sci. 115, 278–281. https://doi.org/10.21273/JASHS.115.2.278.

Naran, R., Pierce, M.L., Mort, A.J., 2007. Detection and identification of rhamnogalacturonan lyase activity in intercellular spaces of expanding cotton cotyledons. Plant J. 50, 95–107 https://doi.org/ 10.1111/j.1365-313X.2007.03033.x.

Nuñez-Lillo, G., Cifuentes-Esquivel, A., Troggio, M., Micheletti, D., Infante, R., Campos-Vargas, R., Orellana, A., Blanco-Herrera, F., Meneses, C., 2015. Identification of candidate genes associated with mealiness and maturity date in peach [*Prunus persica* (L.) Batsch] using QTL analysis and deep sequencing. Tree Genet. Genomes 11, 86–98 https://doi.org/ 10.1007/s11295-015-0911-9.

Ogundiwin, E.A., Peace, C.P., Gradziel, T.M., Parfitt, D.E., Bliss, F.A., Crisosto, C.H., 2009. A fruit quality gene map of Prunus. BMC Genomics 10, 587–599. https://doi.org/10.1186/1471-2164-10-587.

Peace, C.P., Crisosto, C.H., Garner, D.T., Dandekar, A.M., Gradziel, T.M., Bliss, F.A., 2006. Genetic control of internal breakdown in peach. Acta Hortic. 713, 489–496. https://doi.org/10.17660/ActaHortic.2006.713.73.

Pegoraro, C., Chaves, F.C., Manica-Berto, R., Casaril, J., Schreinert dos Santos, R., Silva, J.A., Rombaldi, C.V., 2010. Transcript accumulation of cell wall metabolism and endomembrane transport genes in woolly and non-woolly peach. Sci. Hortic. 126, 366–370. https://doi.org/10.1016/j.scienta.2010.07.033.

Pirona, R., Eduardo, I., Pacheco, I., Da Silva Linge, C., Mara, M., Verde, I., Tartarini, S., Dondini, L., Pea, G., Bassi, D., Rossini, L., 2013. Fine mapping and identification of a candidate gene for a major locus controlling maturity date in peach. BMC Plant Biol. 13, 166–178. https://doi.org/10.1186/1471-2229-13-166.

Pressey, R., Hinton, D.M., Avants, J.K., 1971. Development of polygalacturonase activity and solubilization of pectin in peaches during ripening. J. Food Sci. 36, 1070–1073. https://doi.org/10.1111/j.1365-2621.1971.tb03348.x.

Protsenko, M.A., Buza, N.L., Krinitsyna, A.A., Bulantseva, E.A., Korableva, N.P., 2008. Polygalacturonase inhibiting protein is a structural component of plant cell wall. Biochem. Mosc. Suppl. Ser. A Membr. Cell Biol. (Mosc) 73, 1053–1062. https://doi.org/10.1134/S000629790.

Punwani, J.A., Rabiger, D.S., Lloyd, A., Drews, G.N., 2008. The MYB98 subcircuit of the synergid gene regulatory network includes genes directly and indirectly regulated by MYB98. Plant J. 55, 406–414 https://doi.org/ 10.1111/j.1365-313X.2008.03514.x.

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A., Bender, D., Maller, J., Sklar, P., de Bakker, P.I., Daly, M.J., Sham, P.C., 2007. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am. J. Hum. Genet. 81, 559–575. https://doi.org/10.1086/519795.

Quilot, B., Wu, B.H., Kervella, J., Génard, M., Foulongne, M., Moreau, K., 2004. QTL analysis of quality traits in an advanced backcross between *Prunus persica* cultivars and the wild relative species *P. Davidiana*. Theor. Appl. Genet. 109, 884–897. https://doi.org/10.1007/s00122-004-1703-z.

Quilot, B., Kervella, J., Genard, M., Lescourret, F., 2005. Analysing the genetic control of peach fruit quality through an ecophysiological model combined with a QTL approach. J. Exp. Bot. 56, 3083–3092. https://doi.org/10.1093/jxb/eri305.

Ribot, C., Zimmerli, C., Farmer, E.E., Reymond, P., Poirier, Y., 2008. Induction of the *Arabidopsis* PHO1;H10 gene by 12-oxo-phytodienoic acid but not jasmonic acid via a CORONATINE INSENSITIVE1-dependent pathway. Plant Physiol. 147, 696–706 https://doi.org/ 10.1104/pp.108.119321.

Richmond, T.A., Somerville, C.R., 2000. The cellulose synthase superfamily. Plant Physiol. 124, 495–498. https://doi.org/10.1104/pp.124.2.495.

Romeu, J.F., Monforte, A.J., Sánchez, G., Granell, A., García-Brunton, J., Badenes, M.L., Ríos, G., 2014. Quantitative trait loci affecting reproductive phenology in peach. BMC Plant Biol. 14, 52–67. https://doi.org/10.1186/1471-2229-14-52.

Ross, J., Li, Y., Lim, E., Bowles, D.J., 2001. Higher plant glycosyltransferases. Genome Biol. 2 reviews 3004.1-3004.6.

Socquet-Juglard, D., Kamber, T., Pothier, J.F., Christen, D., Gessler, C., Duffy, B., Patocchi, A., 2013. Comparative RNA-Seq analysis of early-infected peach leaves by the invasive phytophatogen *Xanthomonas arboricola* pv. Pruni. PLoS One 8. https://doi.org/10.1371/journal.pone.0054196.

Song, S., Qi, T., Fan, M., Zhang, X., Gao, H., Huang, H., Wu, D., Guo, H., Xie, D., 2013. The bHLH subgroup IIId factors negatively regulate jasmonate-mediated plant defense and development. PLoS Genet. 9. https://doi.org/10.1371/journal.pgen.1003653.

Van Ooijen, J.W., 2011. Multipoint maximum likelihood mapping in a full-sib family of an outbreeding species. Genet. Res. 93, 343–349. https://doi.org/10.1017/S0016672311000279.

Van Ooijen, J.W., Maliepaard, C., 1996. MapQTL Version 3.0, Software for the Calculation of QTL Positions on Genetic Map. Plant Res. Int, Wageningen. The Netherlands.

Van Ooijen, J.W., Voorrips, R.E., 2001. JoinMap Version 3.0: Software for the Calculation of Genetic Linkage Maps. Plant Res. Int. Wagening, The Netherlands.

Vendramin, E., Pea, G., Dondini, L., Pacheco, I., Dettori, M.T., Gazza, L., Scalabrini, S., Strozzi, F., Tartarini, S., Bassi, D., Verde, I., Rossini, L., 2014. A unique mutation in a MYB gene cosegregates with the nectarine phenotype in peach. PLoS One 9, e112032. https://doi.org/10.1371/journal.pone.0090574.

Verde, I., Jenkins, J., Dondini, L., Micali, S., Pagliarani, G., Vendramin, E., Paris, R., Aramini, V., Gazza, L., Rossini, L., Bassi, D., Troggio, M., Shu, S., Grimwood, J., Tartarini, S., Dettori, M.T., Schmutz, J., 2017. The peach v2.0 release: high-resolution linkage mapping and deep resequencing improve chromosome-scale assembly and contiguity. BMC Genomics 18, 225. https://doi.org/10.1186/s12864-017-3606-9.

Verde, I., Abbott, A.G., Scalabrini, S., Jung, S., Shu, S., Marroni, F., Zhebentyayeva, T., Dettori, M.T., Grimwood, J., Cattonaro, F., Zuccolo, A., Rossini, L., Jenkins, J., Vendramin, E., Meisel, L.A., Decroocq, V., Sosinski, B., Prochnik, S., Mitros, T., Policriti, A., Cipriani, G., Dondini, L., Ficklin, S., Goodstein, D.M., Xuan, P., Del Fabbro, C., Aramini, V., Copetti, D., Gonzalez, S., Horner, D.S., Falchi, R., Lucas, S., Mica, E., Maldonado, J., Lazzari, B., Bielenberg, D., Pirona, R., Miculan, M., Barakat, A., Testolin, R., Stella, A., Tartarini, S., Tonutti, P., Arús, P., Orellana, A., Wells, C., Main, D., Vizzotto, G., Silva, H., Salamini, F., Schmutz, J., Morgante, M., Rokhsar, D.S., 2013. The high-quality draft genome of peach (*Prunus persica*) identifies unique patterns of genetic diversity, domestication and genome evolution. Nat. Genet. 45, 487–494. https://doi.org/10.1038/ng.2586.

Verde, I., Bassil, N., Scalabrini, S., Gilmore, B., Lawley, C.T., Gasic, K., Micheletti, D., Rosyara, U.R., Cattonaro, F., Vendramin, E., Main, D., Aramini, V., Blas, A.L., Mockler, T.C., Bryant, D.W., Wilheim, L., Troggio, M., Sosinki, B., Aranzana, M.J., Arús, P., Lezzoni, A., Morgante, M., Peace, C., 2012. Development and evaluation of a 9K SNP array for peach by internationally coordinated SNP detection and validation in breeding germplasm. PLoS One 7, e35668. https://doi.org/10.1371/journal.pone.0035668.

Verde, I., Quarta, R., Cedrola, C., Dettori, M.T., 2002. QTL analysis of agronomic traits in a BC1 peach population. Acta Hort. 592, 291–297 https://doi.org/ 10.17660/ActaHortic.2002.592.41.

Vicente, A.R., Saladié, M., Rose, J.K.C., Labavitch, J.M., 2007. The linkage between cell wall metabolism and fruit softening: looking to the future. J. Environ. Sci. Health B 87, 1435–1448. https://doi.org/10.1002/jsfa.2837.

Wen-Bo, J., Di-Qiu, Y., 2009. *Arabidopsis* WRKY2 transcription factor may be involved in osmotic stress response. Acta Bot. Yunnan. 31, 427–432. https://doi.org/10.3724/SP.J.1143.2009.09046.

Yang, Y., Foulquié-Moreno, M.R., Clement, L., Erdei, É, Tanghe, A., Schaerlaekens, K., Dumortier, F., Thevelein, J.M., 2013. QTL analysis of high thermotolerance with superior and downgraded parental yeast strains reveals new minor QTLs and converges on novel causative alleles involved in RNA processing. PLoS Genet. 9, e1003693. https://doi.org/10.1371/journal.pgen.1003693.

Zeballos, J.L., Abidi, W., Giménez, R., Monforte, A.J., Moreno, M.A., Gogorcena, Y., 2016. Mapping QTLs associated with fruit quality traits in peach [*Prunus persica* (L.) Batsch] using SNP maps. Tree Genet. Genomes 12, 37. https://doi.org/10.1007/s11295-016-0996-9.

Zhang, C., Feng, R., Ma, R., Shen, Z., Cai, Z., Song, Z., Peng, B., Yu, M., 2018. Genome-wide analysis of basic helix-loop-helix superfamily members in peach. PLoS One 13, e0195974. https://doi.org/10.1371/journal.pone.0195974.

Ziosi, V., Noferini, M., Fiori, G., Tadiello, A., Trainotti, L., Casadoro, G., Costa, G., 2008. A new index based on vis spectroscopy to characterize the progression of ripening in peach fruit. Postharvest Biol. Tec. 49, 319–329. https://doi.org/10.1016/j.postharvbio.2008.01.017.