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Comparative QTL analysis in peach 'Earlygold' F_2 and backcross progenies

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ABSTRACT

Based on detailed maps, DNA sequences and phenotypic data, there is a great deal of information on the genetics and genomics of 'Earlygold', a historical peach cultivar from the US. The F_2 between 'Texas' almond and 'Earlygold' peach (T × E) was used to construct the first saturated peach linkage map that later became the reference map for the *Prunus* genus. This population and the first backcross ('Texas' × 'Earlygold') × 'Earlygold' (T1E) yielded information on QTLs for a large number of agronomic traits, and T1E is being used as the basis for constructing a set of introgression lines of 'Texas' fragments into the 'Earlygold' background, currently in progress. This paper describes the construction of a high-density SNP map for 'Earlygold' using an F_2 population, and the QTL analysis of 24 traits. Results of maps and QTLs are compared with those from the 'Earlygold' parent of the T1E map, using the same set of markers and characters. Results show major differences between the two progenies in terms of numbers of markers mapped and the capability of detecting QTLs, with a large increase in the resolution of maps and QTLs when using the F_2 progeny compared to the T1E pseudo-testcross. In addition, we provide data on leaf senescence color, studied for the first time in peach, with two consistent QTLs located in the same position as other color-related genes and QTLs.

Introduction

Peach [*Prunus persica* (L.) Batsch], is an economically important stone fruit crop and one of the model species of the *Rosaceae* family (Shulaev et al., 2008). Like most cultivated members of the *Prunus* genus, such as almond, apricot, plum and cherry, peach is diploid (2n=*2x*=*16*) with a compact and sequenced genome of ~x223C 250 Mbp (Verde et al., 2013). It has a self-compatible mating system and a short intergeneration period of 3–4 years, in contrast to other rosaceous fruit tree species that are usually self-incompatible and require a longer time for fruiting. Some of the major targets in the current peach breeding programs are difficult to meet, such as extended shelf life, better fruit quality and enhanced disease resistance, mainly due to the low levels of genetic variability of the elite peach materials (Micheletti et al., 2015).

'Earlygold', an old peach cultivar bred in the US, was crossed with 'Texas' (syn. 'Texas prolific', syn. 'Mission') almond in the peach rootstock breeding program of IRTA during the 80 s and produced several hybrids. One of these, particularly fertile and prolific, was chosen to obtain an F_2 progeny to construct the first saturated linkage map of *Prunus* (Joobeur et al., 1998), which later became the reference for the genus (Aranzana et al., 2003; Dirlewanger et al., 2004). The F_2 population was useful for map construction, but only about a half of its progeny was fertile and fruited (Donoso et al., 2016), so a BC1 progeny with 'Earlygold' as recurrent parent was obtained, where most individuals produced fruit. In this cross, the 'Texas' cytoplasm resulted in male sterility in the peach nuclear genetic background unless at least one of two independent restorer factors from 'Texas' were present (Donoso et al., 2015). The BC1 progeny and further backcross and selfing generations have been used as a proof of concept for marker-assisted introgression (MAI), a fast strategy to obtain individuals with a single almond introgressed fragment in the BC2 (Serra et al., 2016). These materials are currently being used to develop an introgression line (IL) collection of almond fragments in the 'Earlygold' background.

In this paper we elaborate a high-density map of an 'Earlygold' F_2 progeny, and examine its variability for a set of characters of agronomic interest, to understand their inheritance and to find useful marker-trait associations. We compared these results with those of a QTL analysis on the BC1 ('Texas' × 'Earlygold') × 'Earlygold' studied by Donoso et al. (2016), that allowed us to examine the QTLs of 'Earlygold' in two different genetic backgrounds. These data will also provide information useful to understand the importance of the 'Earlygold' allelic variation

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in the characters that will be examined in the IL collection currently under construction.

Materials and methods

Plant materials

The 'Earlygold' F_2 population N = 75 (E × E) was used for the study. The trees are located at the IRTA experimental station of Torre Marimon (Caldes de Montbui, Spain), planted on their own roots in 2015. The spacing between the trees was 2.5 m and between the rows 4.0 m. The trees were thinned out during the second and third year of fruiting (2018 and 2019). Young leaves were collected from all the trees for DNA extraction (Doyle and Doyle, 1990), to perform genotyping. The E × E population initially consisted of 81 plants, later two plants died and four did not produce genotypic data, resulting in 75 plants being used for mapping and phenotyping.

Phenotyping

The population was evaluated for 24 traits, 18 over three seasons in the years 2017 to 2019 and six in only two seasons: chlorophyll content of the leaves and leaf dry weight (only in 2018 and 2019), leaf color at senescence and early and late leaf fall (2019 and 2020), and beginning of shooting (2020 and 2021). These characters were also analyzed by Donoso et al. (2015, 2016), except for leaf color at senescence, fruit firmness and pH that we analyze here for the first time. The phenotyping methods were essentially identical to those used by these authors. The characters scored can be classified into four main categories and their measurement is described below:

Flower: The flower shape, showy (large petals) and non-showy (small petals) is determined by a major gene (*Sh/sh*) in peach, where showy flowers are homozygous (*shsh*) for the recessive allele (Bailey and French, 1942).

Phenology: Flowering time (FT) was scored as the number of Julian days when 50% of the flowers were open. Beginning of shooting (BS) was the number of Julian days when 5% of the shoots start to appear. Maturity date (MD) was measured as the number of Julian days with 50% of the fruits mature, as determined by changes in the skin color and flesh firmness. Fruit development period (FDP) was scored as the difference in days between the flowering time and maturity date. Beginning of leaf fall (BLF) was scored as the number of Julian days when 10% of leaves had dropped, and end of leaf fall (ELF) when 90% had dropped.

Fruit: Fruit weight (FW), in grams (g), was the average weight of six mature fruits per individual using a digital balance. Fruit production (FP) was estimated on a scale of 1 to 4 (1=no fruits, 2= <10 fruits, 3 = 10–50 fruits and 4= >50 fruits). Intensity of red skin color (ISC) was visually determined by the% of red color at maturity (1 = 0–25%, 2 = 25–50%, 3 = 50–75% and 4 = 75–100%). Fruit firmness (FF) was evaluated with a hand penetrometer (Wagner, Model 53,200), taking the average value of three fruits with the measurements from both sides for each fruit. Soluble solid content (SSC), expressed in Brix degrees, was measured from the juice of three fruits using a digital refractometer (Atago, Tokyo, Japan). Titratable acidity (TA) and pH were determined using a HI-84,532–02 Titratable Acidity Mini Titrator and a pH meter (Hanna instruments, Rhode Island, USA) by diluting 5 ml of fruit juice with 45 ml of water and titrating with 0.5 M NaOH to a pH of 8.2. TA was calculated in g/l of malic acid.

Leaf: Chlorophyll content (CC) was estimated as the average from ten leaves per tree using SPAD 502 (Konica Minolta, Osaka, Japan). During the months of July-August, eleven leaves per tree were collected from the middle of the tree branches. The leaves were then scanned and their images stored as TIF files for further analysis. The leaf dimensions were measured using a Tomato Analyzer 3.0 (http://www.oardc.ohio-state.edu/vanderknaap) software. Leaf parameters analyzed (Fig. 1) were leaf length (LL), petiole length (PL), leaf blade length (LBL), leaf blade width (LBW), leaf shape (LSH) as the ratio of LBL/LBW, leaf perimeter (LP) and leaf surface (LS). All the measurements were in cm, except for LSH that is a ratio, and LS that was measured in cm^2. Later, the leaves were placed in an incubator for 3 days at 60 °C to determine the leaf dry weight (LW), in grams (g). Leaf color at senescence (LCS) was scored visually over two years, once a week, in September and October (Fig. 2) using a scale of 1 (non-purple, including green yellow and red) and 2 (purple leaf).

The phenotypic data were analyzed statistically using JMP 14.0 software (SAS Institute, Cary, NC, USA). Correlations between different traits and years were calculated using the Spearman correlation coefficient.

Construction of linkage map and QTL analysis

For linkage analysis, genotype data were obtained from the 9k International Peach SNP Consortium (IPSC) Illumina Infinium SNP array (Verde et al., 2012) in 75 plants of the E × E population. The linkage map was constructed using MapMaker/exp 3.0 (Lander et al., 1987) using the Kosambi distance function. We initially ordered the markers based on their physical position and established a set of bins (i.e. groups of markers with identical genotype for all the individuals), where each bin is separated from the adjacent bin by a single or a few recombination events. Finally, a single SNP from each bin was selected for the dataset used to construct the linkage map. References for chromosome/linkage group numbers and orientation and physical positions were those of Dirlewanger et al. (2004) and Verde et al. (2017).

QTLs for all the traits were analyzed using the interval mapping

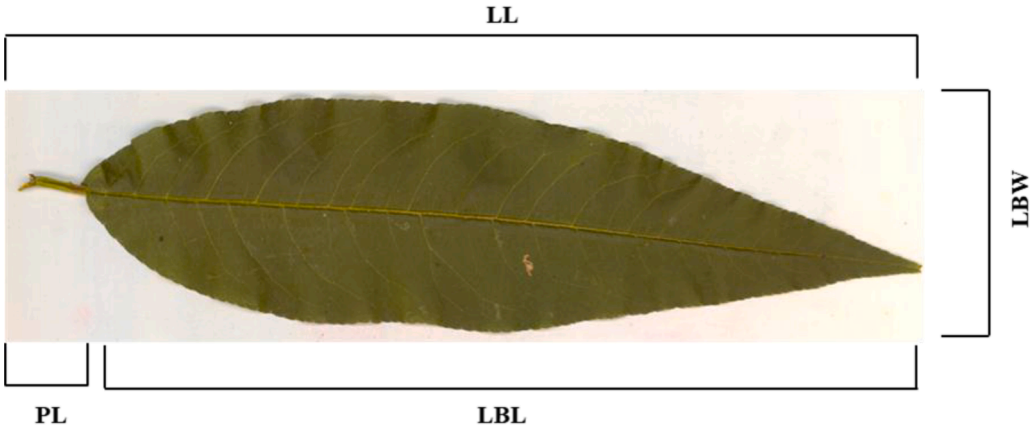

Fig. 1.. Schematic representation of leaf dimensions (PL petiole length, LBL leaf blade length, LL leaf length, LBW leaf blade width).

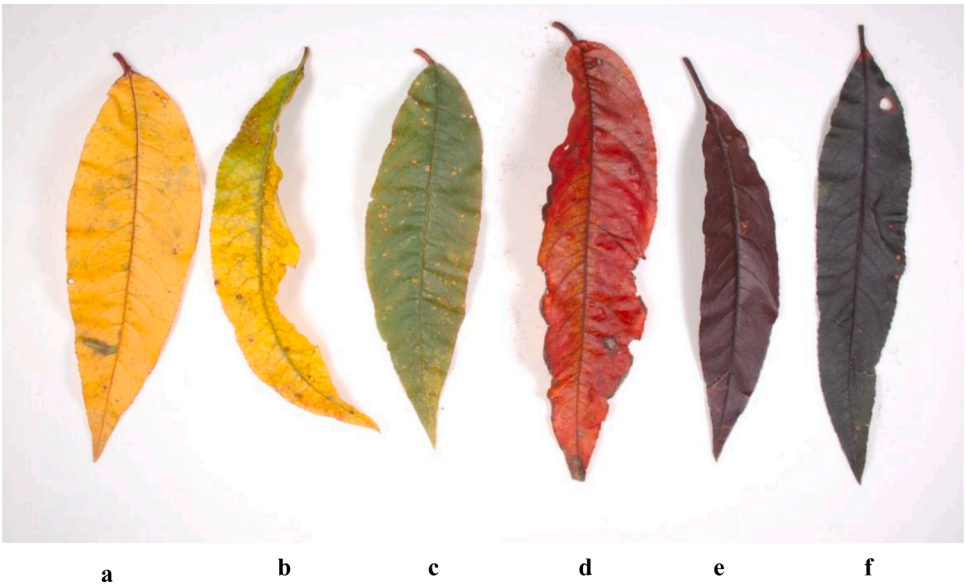

Fig. 2.. Leaf color at senescence a, b-yellow, c-green d-red and e,f-purple.

method with the MapQTL 6.0 software (Van Ooijen et al. 2002). All the QTLs with a LOD $\geq$ 3.0 were considered as significant, as were those QTLs with a LOD $\geq$ 2.5 in one year and a LOD $\geq$ 3.0 in the rest. The QTLs were considered consistent if they were detected every year. The maps and positions of the QTLs were drawn using the MapChart 2.3 software (Voorrips, 2002).

Gene action (GA) was established following the guidelines of Tanksley (1993), based on the values of additivity, a=(A-B)/2, and dominance d = H-[(A + B)/2], where A and B are the average values of the trait in the homozygous individuals for a given marker in the female and male parent, respectively, and H that of the heterozygotes. We considered gene action additive (A) if the quotient $|d/a| \leq 0.5$, dominant (D) if $0.5 < |d/a| \leq 1.5$, and overdominant (O) if $|d/a| > 1.5$.

Results

Linkage maps and map comparisons

From the SNPs of the 9k chip, 1640 were polymorphic in the E × E population and distributed in 269 bins. The E × E map covered a total genetic distance of 439.1 cM, detecting the expected eight linkage groups with a higher end of 76.2 cM for linkage group 2 (G2) to a lower end of 37.1 cM for G4. The overall physical length covered by this map was 186.7 Mbp, 82.7% of the total physical distance (225.6 Mbp) of the peach genome v2.0a1 (Verde et al., 2017). There were 24 gaps of >2Mbp, the largest at the distal end of G4 (15.3 Mbp), overall accounting for 116.4 Mbp, equivalent to 52% of the total physical distance of the sequenced peach genome (Supplementary Table 1).

The map of 'Earlygold' (the E map) constructed with the ('Texas' × 'Earlygold') × 'Earlygold' BC1 population (Donoso et al., 2015) using the same SNP chip plus 41 SSR markers, is similar in many respects to the one we present here (see comparisons in Supplementary Table 1 and Supplementary Figure 1), particularly with respect to the physical coverage (189.7 Mbp), number of bins (214), number of gaps >2 Mbp (23; the same as in the E × E map) and physical distance covered by these gaps (109.0 Mbp). The genetic distance of the E map (521.2 cM) was 16% higher than that of the E × E map, and some linkage groups were clearly longer than in the E × E map (Supplementary Table 1), although not significantly longer (the paired t-test of the differences of the genetic distances between the common markers in the extremes of each chromosome was 1.21). The most striking difference between the E and E × E maps was the number of SNP markers that could be mapped: 1640 in E × E and only 1050 in E, resulting in a marker density of 0.27 cM/marker in E × E compared to 0.47 in the E map. When examining the mapped markers in detail, we observed that their distribution was similar in both maps, and that most of the 1050 SNPs of E (97%) were mapped in E × E, but of the SNPs mapped only in E × E (623) the majority (411; 66%) had a dominant pattern of inheritance, with only two genotypes from the three expected (3:1 ratio), instead of the usually codominant 1:2:1 SNP inheritance expected in an F_2 progeny.

Trait phenotypic data and QTL analysis

All of the 24 traits scored were quantitative, except for flower shape (*Sh*), which was scored as qualitative and placed on G8 at the position 23.8 cM with the nearest marker (SNP_IGA_862,006), with physical position 13,825,065 bp, cosegregating with the gene. Trait distributions and main features are described in Supplementary Table 2 and Supplementary Figure 2. Those that significantly departed from normal in more than one year were phenology-related (FT, BS, MD, FDP, FP, BLF and ELF), leaf color at senescence (LCS) and three fruit characters (ISC, FF and pH).

Correlation analysis was performed between all quantitative traits and the years in which they were measured (Supplementary Table 3). The correlations between years for a particular trait were high and positive for FT, BS, MD, FDP, FW, SSC, CC, and LCS, and intermediate-to-low for the rest. For correlations between traits, there was a clear positive correlation between MD, FDP, FW and SSC, and between BLF and ELF. Most leaf traits (LP, LS, LBW, LL and LB) of the same year were strongly correlated, but the correlation decreased when comparing data from different years. A negative correlation was observed for MD and characters related to anthocyanin coloration of senescent leaves (LCS) and intensity of the red skin fruit color (ISC).

QTLs were detected for 19 traits, between one to four QTLs per trait, and none were identified for four traits (ELF, FP, LBW and LW), giving a total of 33 QTLs (Supplementary Table 4 and Supplementary Figure 3). Twelve QTLs of those detected (36%) were consistent in all years studied (Table 1, Supplementary Table 4 and Fig. 3). Most of the consistent QTLs detected explained the variability of phenology (FT, BS, MD and FDP) and fruit (FW, SSC, TA) traits, whereas of the QTLs for the leaf traits, only CC and LCS were consistent.

Considering only consistent QTLs (Table 1; Fig. 1), two were found for flowering time, one on each of G7 and G8 with R^2 = 28.3–42.8% and R^2 = 16.5–20.9% respectively. Also, two QTLs for beginning of shooting,

Table 1
Summary of consistent QTLs detected in the E × E map ('Earlygold' F2 population) and E map ['Earlygold' map from the ('Texas' × 'Earlygold') × 'Earlygold' population]. Trait category, map type, QTL names, LOD score of the maximum peak, linkage group, map position of the maximum peak, percentage of explained phenotypic variance (R^2), additivity (a), dominance (d), d/a, gene action (GA).

Trait	Map	QTL name	LOD	G	Position (cM)	R^2	a	d	d/a	GA
Flowering time	E × E	qFT7	5.28–8.98	7	30.1–36.9	28.3–42.8	−2.07 to −2.75	−0.11 to −1.90	0.04 to 0.92	A-D
	E × E	qFT8	2.86–3.78	8	2.8–23.5	16.5–20.9	−0.12 to 1.91	−0.85 to 2.50	−0.45 to −18.66	A-O
End of flowering	E[1]	qFT7	2.50–4.65	7	36.8–40.8	8.9–14.3	3.07 to −4.24	–	–	–
Beginning of shooting	E × E	qBS7	5.70–9.27	7	34.9–35.9	30.9–45.2	−2.84 to −3.46	−0.62 to 0.15	−0.04 to 0.22	A
	E × E	qBS4	3.25–4.42	4	4.3	19.0–24.9	2.06 to 2.30	1.05 to 1.09	0.45 to 0.52	A-D
	E[1]	qBS7	4.24–8.13	7	41.4	12.1–23.5	3.72 to 7.03	–	–	–
Maturity date	E × E	qMD4	24.91–27.14	4	34.3–37.1	80.6–82.0	9.62 to 10.93	−1.23 to −5.18	−0.11 to −0.51	A-D
Fruit development period	E × E	qFDP4	23.84–30.12	4	37.1	80.1–85.0	9.27 to 11.32	−1.22 to −5.26	−0.10 to −0.49	A
Fruit weight	E × E	qFW4	4.85–7.73	4	33.6–37.1	28.0–38.6	12.18 to 18.32	−0.87 to −6.40	−0.35 to 0.21	A
Soluble solid content	E × E	qSSC4	8.77–9.51	4	36.4–37.1	44.3–47.4	1.23 to 1.62	−0.45 to 0.20	−0.27 to 0.14	A
Titratable acidity	E × E	qTA6	3.04–6.43	6	48.1–55.6	17.4–34.9	−0.67 to −0.90	0.09 to 0.24	−0.13 to −0.26	A
Chlorophyll content	E × E	qCC3	2.87–3.39	3	46.3–49.0	16.3–19.0	−0.47 to 0.12	1.15 to 3.28	−2.45 to 27.33	O
Leaf color at senescence	E × E	qLCS3	5.01–5.19	3	35.3	26.8–28.6	0.30 to 0.33	0.35 to 0.42	1.06 to 1.40	D
	E × E	qLCS4	7.88–8.48	4	36.4–37.1	40.0–41.0	−0.40 to −0.41	−0.006 to 0.01	−0.02 to 0.02	A

[1] Data from the E map are obtained from Donoso et al. (2016).

one on each of G7 and G4 with R^2 = 30.9–45.2% and R^2 = 19.0–24.9%, respectively. A QTL with a large effect for maturity date (R^2 = 80.6–82.0%) and fruit development period (R^2 = 80.1–85.0%) was found at the end of G4, and, at the same position, two other QTLs were identified for fruit weight and soluble solid content explaining 28.0–38.6% and 44.3–47.4% of the phenotypic variance, respectively. For titratable acidity, a QTL was detected at the end of G6, with R^2 =17.3–34.9%. Three QTLs were identified for leaf-related traits, one for chlorophyll content on G3 (R^2 =16.3–19.0%) and two for leaf color at senescence on G3 and G4 explaining 26.8–28.6% and 40.0–41.0% of phenotypic variance, respectively.

Comparison with the QTLs detected in the E map of the T1E population

Only six QTLs were detected on the map of 'Earlygold' obtained from the T1E population by Donoso et al. (2016), all of which we found using the E × E population. Two were consistent QTLs in E, one for end of flowering time, and the other for beginning of shooting, both located on G7 at the position of the consistent qFT7 for flowering time in the E × E map (Table 1). Two additional QTLs, for maturity date and fruit development period, both on G4 in the E map, correspond to the consistent qMD4 and qFDP4, respectively, identified here (Table 1). In the E map, these two latter QTLs were found only in one of the three years studied. Similarly, beginning of flowering time (BFT) that was mapped to the same position as qFT7, was detected in only two of the three years studied in E, and a QTL for beginning of shooting in G4 identified in only one of the three years in E probably corresponds to the consistent qBS4 in E × E. In all, for a set of 20 common characters, we found six QTLs in E and 26 in E × E (see Table 1 and Supplementary Table 4).

Discussion

Due to the self-compatibility system of peach, it is possible to obtain F_2 progenies as well as F_1 or backcross progenies for genetic analysis. F_1 segregating progenies are frequently used for QTL analysis as they are the usual progeny type used in peach breeding. Since the parents used are partly heterozygous lines or cultivars, they segregate in the F_1 progeny. These progenies, also termed pseudo-backcrosses (Grattapaglia and Sederoff 1994), are generally analyzed as two different backcross one (BC1) populations, each corresponding to one of the parents of the cross, and linked by markers that are heterozygous in both parents and then segregate 1:2:1 (or 1:1:1:1 when using multi-allelic markers). F_2 populations are also used, especially to better understand the inheritance of specific characters as they recover the three possible genotypes, unlike BC1 populations that recover only two, making them suitable for the analysis of gene action (dominance, additivity, overdominance). F_2 progenies are also more appropriate for constructing linkage maps, as they have more recombination events per meiosis (those of both parents), so more accurate maps can be constructed with the same number of progeny (Allard 1956).

Here we constructed a linkage map with the 9k peach IPGI SNP chip in the selfed progeny of 'Earlygold', which resulted in a high-density map with coverage of all eight peach chromosomes, with large fragments of DNA without segregating markers, approximately half of the 'Earlygold' genome, suggesting as in previous studies (Eduardo et al., 2013; Martínez-García et al., 2013; Donoso et al., 2015; Serra et al., 2017) that there are large regions of the peach genome identical by descent owing to the high level of coancestry of the cultivated gene pool. This map was similar in most respects (covered distance, chromosome length, gaps without markers) to the one obtained for 'Earlygold' in the interspecific backcross ('Texas' × 'Earlygold') × 'Earlygold' that was previously analyzed by Donoso et al. (2015). There was an important difference between these two maps, concerning the number of markers that could be integrated on the map: 1640 SNPs in E × E, 56% more than the 1050 in E. The fact that about two-thirds of the additional markers in E × E had dominant segregations suggests that a major cause for this difference is that these SNPs could have been discarded in the E map because they did not segregate (AA x Aa situations) or that segregations could have been strongly skewed compared to the two expected (1:1 and 1:2:1), resulting a loss of information compared to what can be obtained in an F_2 population. While the quality of dominant markers is lower than codominant ones for mapping and QTL analysis, the results indicate the importance of considering all possible segregation types before the initial marker filtering steps to maximize the available information for genetic analysis when using large sets of data.

The gene for flower type, *Sh*, was found as expected in G8 and at a similar map position as in Ogundiwin et al. (2009), who mapped this gene for the first time, and as in Donoso et al. (2016). Its physical position on chromosome 8 is also compatible with the results of Micheletti et al. (2015) and Cao et al. (2016), both using genome-wide association analysis.

A major QTL (qMD4) explaining >80% of the phenotypic variance for maturity date was found on G4. A QTL in this position has been detected by other authors in a very broad transect of peach materials as well as in other *Prunus* species (Dirlewanger et al., 2012; Hernández Mora et al. 2017; Serra et al., 2017; Aranzana et al., 2019; Rawandoozi et al., 2021; Chen et al., 2021). In certain cases, this trait has been integrated in the maps as a major gene *MD/md* (Eduardo et al., 2011; Pirona et al., 2013). A strong candidate gene for this character is an NAC transcription factor (*Prupe.4G186800.1*), orthologous to the *Nor* gene in

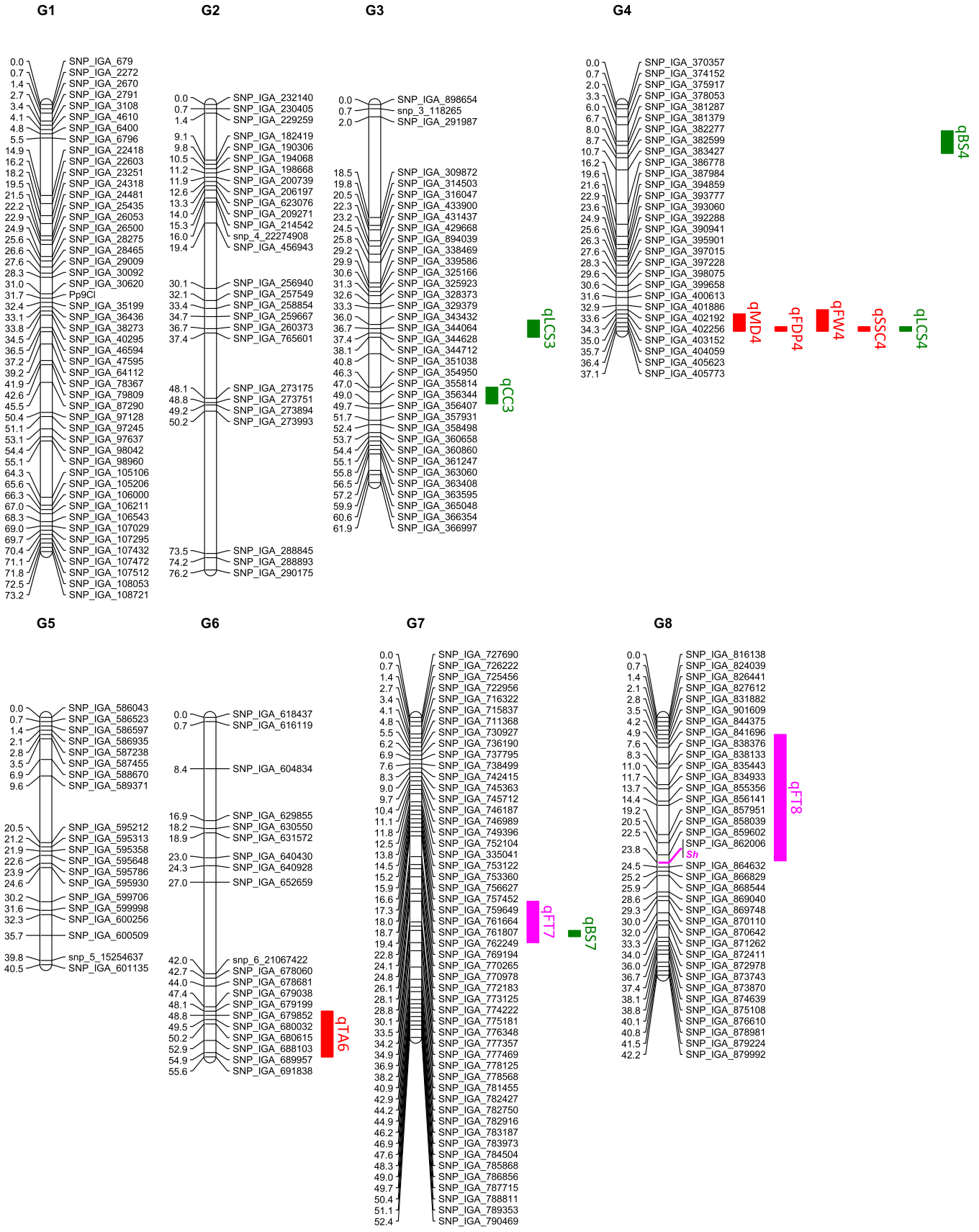

Fig. 3.. Map of the 'Earlygold' F2 progeny with the positions of a major gene (*Sh*) and 12 consistent QTLs over the years. Colors of the bars of QTLs are as follows: pink flower, red fruit and green leaf traits.

tomato (Pirona et al., 2013). The same region includes a gene (*Sr/sr*) responsible for another character, slow ripening, that determines the presence of individuals producing fruit that do not ripen, remaining immature on the tree for a long time (Eduardo et al., 2015; Núñez-Lillo et al., 2015). A 26.6 kb deletion containing the *Prupe.4G186800.1* gene was associated with the *sr* allele (responsible for the slow ripening phenotype) suggesting that *MD* and *Sr* may be the same gene (Eduardo et al., 2015; Meneses et al., 2016). Another major gene mapped in this region is *Alf/alf* that determines the formation of the thick mesocarp characteristic of the peach fruit in almond × peach progenies (Donoso et al., 2016). Additionally, this region of chromosome 4 contains several QTLs involved in the inheritance of other fruit traits, including FDP, which can be considered as an alternative measurement of MD (Hernández Mora et al. 2017), and many other characters including SSC, fruit weight, acidity, pH and red skin color (Eduardo e al. 2011, Hernández Mora et al. 2017; Rawandoozi et al., 2021). It is unknown whether the concurrence of genes involved in the inheritance of so many characters in this region is due to the action of a highly polymorphic single gene with a broad set of pleiotropic effects, or to a cluster of genes that would constitute a hotspot for a diverse set of characters in peach and other *Prunus*. In this work, we identified consistent QTLs for four characters in this region, including FDP, SSC, FW and LCS: in all cases, late maturity was associated with an increase of these traits except for leaf color at senescence where late maturity and presence of anthocyanin color were negatively correlated. The QTLs of all traits at this region were essentially additive (Table 1) and with a generally large effect (R^2=28.0–85.0%). Overall, this region appears to have a major impact on the phenotype and is usually the determinant of critical aspects of agronomic characters that are affected by phenology and fruit-related traits. It deserves detailed exploration at the genetic level to understand its nature and how different haplotypes may shape expression of the traits involved in this highly polymorphic region.

Two consistent QTLs found for flowering time (qFT7) and beginning of shooting (qBS7) mapped at the same location, suggesting that they could correspond to the same locus. These two characters differed in two QTLs, qBS4, located at a different position to the MD-related QTLs, and qFT8, indicating that these characters may be encoded by a partly overlapping set of genes. Their positions also coincide with those found in peach and other *Prunus* species (Fan et al., 2010; Silva et al., 2005; Dirlewanger et al., 2012; Hernández Mora et al. 2017) where they have been studied along with a cohort of other QTLs that depend on the specific population studied.

We identified a consistent QTL for fruit juice titratable acidity (TA) on G6 (qTA6), at a different position of the major gene determining subacid vs. acid fruit (*D/d*) that maps on G5 (Dirlewanger et al., 2006) and is homozygous for the allele (*d*) that confers fruit acidity in 'Earlygold'. This QTL was already detected with strong evidence in the multiple progeny analysis performed by Hernández Mora et al. (2017), confirming its value as a factor that may be used to modulate fruit acidity in the absence of the subacid allele.

The leaf color at senescence was analyzed here for the first time in peach and we found two loci, both with strong effects (R^2=28.6–40.5%), one on G4 (qLCS4) and the other on G3 (qLCS3). A major gene for red vs. green leaf color (*Gr/gr*) has been described in peach and mapped to G6 (Lambert and Pascal, 2011), which discards its involvement in the variability observed in the 'Earlygold' progenies. The position of qLCS3 corresponds to that of the gene that determines yellow vs. anthocyanin anther color (*Ag/ag*; Arús et al., 1994), the "highlighter" gene that controls peach fruit skin red blush (*H/h*; Bretó et al., 2017), and to various QTLs determining anthocyanin color in fruit skin and flesh of several stone fruit crops (Sooriyapathirana et al., 2010; Socquet-Juglard et al., 2013; Donoso et al., 2016; Calle et al., 2021; Fiol et al., 2021). The phenotypic variability of these genes in *Prunus* has been attributed to the variation of one or several tandemly-duplicated transcription factor genes (*PpMYB10.1, PpMYB10.2* and *PpMYB10.3*) involved in the anthocyanin metabolic pathway (Tuan et al., 2015, Fiol et al.2021). While the QTL on G3 has been generally detected as the main determinant in the color-related traits studied in *Prunus*, it is often accompanied by a QTL on G4, corresponding to the position of qLCS4 found here, such as for skin color in peach, almond × peach and Japanese plum crosses (Frett et al., 2014; Donoso et al., 2016; Salazar et al., 2017; Hernández Mora et al. 2017) and flesh color in cherry (Calle et al., 2021). The inheritance of non-anthocyanic vs. anthocyanic senescing leaves in E × E appears to have an oligogenic inheritance, based on the combination of genotypes of these two QTLs and their interaction. qLCS3 had a dominant gene action and qLCS4 was additive (see Table 1) with alleles B and A, respectively, being responsible for absence of anthocyanin color. Individuals having one of these alleles or both in homozygosis, selected using the SNPs closest to the LOD peaks of the QTLs (SNP_IGA_344,086 for qLCS3 and SNP_IGA_405,773 for qLCS4), were usually (90.2% of the cases) non-colored or anthocyanic, as predicted by the markers.

One remarkable finding of this research is that using an F_1 population, i.e. with the 'Earlygold' QTLs studied using a backcross type segregation, we only found a subset (6) of the 26 QTLs that were detected using an F_2 progeny. There are two reasons for this important difference. First, trait segregation between peach and almond, corresponding to QTLs heterozygous in the 'Texas' × 'Earlygold' hybrid parent in the F_1 progeny, produced often by alleles with greater relative effects than those segregating within 'Earlygold', may have interfered with the identification of QTLs at the same or different genomic locations, resulting in a loss of efficiency in the detection of 'Earlygold' QTLs. And second, heterozygous QTLs in both 'Earlygold' and the 'Texas' × 'Earlygold' hybrid parent would segregate 3:1 or 1:2:1 in the progeny, but they were analyzed with markers that segregated 1:1, resulting in a reduction of power to identify QTLs; heterozygous individuals for the QTL cannot be used for genetic analysis in this case, halving the effective population size. While F_1 progenies between partly heterozygous parents are often used for QTL analysis in clonally propagated species, a more efficient QTL analysis can be done with other population types (particularly F_2 progenies). In addition, F_2 populations allow for the analysis of QTL action (dominance, additivity and overdominance), which is not possible with backcross populations. One possible way to rescue as much information as possible from F_1 segregating progenies is analyzing QTLs in two steps, the first one as a backcross, followed by a second analysis using only 1:2:1 segregating markers. The latter analysis would detect F_2 segregating QTLs with more precision, as the information from both parents will be used, resulting in an increase of their LOD values in addition to the possible identification of new QTLs not significantly detectable when using a BC1 progeny for the analysis.

One of the reasons for the analysis of the segregation of 'Earlygold' is that this cultivar has been used as the recurrent parent for the introgression line collection peach/almond currently under construction. An inbred line is typically used for that in the IL collections available, but in the absence of peach inbred lines of the major commercial gene pool with sufficient vigor, we opted for 'Earlygold' which, as we have shown here and in previous studies (Donoso et al., 2015), has large homozygous regions adding up to approximately half of its genome. Knowing the segregating QTLs of this genotype is important as the IL collection will be segregating for them, and their variability may interfere with the analysis of the characters segregating between peach and almond, which are the relevant ones in this case. Our results indicate that there is essentially one region of concern, that of chromosome 4 that contains the QTLs related with maturity date and affects many other fruit traits. Having this region with a genetic composition that results in a similar expected phenotype for all plants of the IL collection is a clear conclusion from the data presented here.

CRediT authorship contribution statement

Naveen Kalluri: Methodology, Investigation, Formal analysis, Writing – original draft. **Iban Eduardo:** Funding acquisition, Resources,

Writing – review & editing. **Pere Arús:** Conceptualization, Funding acquisition, Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

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