Mapping and Characterization QTLs for Phenological Traits in Seven Pedigree-connected Peach Families

Zena Rawandoozi (zjmansur@tamu.edu)
Texas A&M University College Station https://orcid.org/0000-0002-4909-4293
Timothy Hartmann
Texas A&M University College Station
Silvia Carpenedo
EMBRAPA Centro de Pesquisas Agropecuarias do Clima Temperado
Ksenija Gasic
Clemson University College of Agriculture Forestry and Life Sciences
Cassia da Silva Linge
Clemson University College of Agriculture Forestry and Life Sciences
Lichun Cai
Michigan State University
Eric Van de Weg
Wageningen University and Research
David Byrne
Texas A&M University College Station

Research article

Keywords: FlexQTL, Prunus persica QTL, haplotype, Pedigree-based Analysis, Bloom date, Ripening date, Fruit development period

Posted Date: September 30th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-75550/v1

Version of Record: A version of this preprint was published on March 16th, 2021. See the published version at https://doi.org/10.1186/s12864-021-07483-8.

Mapping and characterization QTLs for phenological traits in seven pedigree-connected peach families

Zena J. Rawandoozi*, zjmansur@tamu.edu, Department of Horticultural Sciences, Texas A&M University, College Station, TX 77843, USA. Phone: (979) 676-3223, Fax: (979) 845-0627

Timothy P. Hartmann, t-hartmann@tamu.edu, Department of Horticultural Sciences, Texas A&M University, College Station, TX 77843, USA.

Silvia Carpenedo, carpenedo.s@hotmail.com, Embrapa Clima Temperado, BR-392, km 78, Cx. Postal 403, 96010-971, Pelotas, Rio Grande do Sul, Brazil.

Ksenija Gasic, kgasic@clemson.edu, Department of Agricultural and Environmental Sciences, College of Agriculture, Forestry and Life Sciences, Clemson University, Clemson, SC 29634, USA.

Cassia da Silva Linge, cassia.linge@gmail.com, Department of Agricultural and Environmental Sciences, College of Agriculture, Forestry and Life Sciences, Clemson University, Clemson, SC 29634, USA.

Lichun Cai, lichun.cai725@gmail.com, Department of Horticulture, Michigan State University, East Lansing, MI 48824, USA.

Eric Van de Weg, eric.vandeweg@wur.nl, Department of Plant Breeding, Wageningen University & Research, Wageningen, Netherlands

* Corresponding author

David H. Byrne, dbyrne@tamu.edu, Department of Horticultural Sciences, Texas A&M University, College Station, TX 77843, USA.

Abstract

Background: Environmental adaptation and expanding harvest seasons are primary goals of most peach [*Prunus persica* (L.) Batsch] breeding programs. Breeding perennial crops is a challenging task due to their long breeding cycles and large tree size. Pedigree-based analysis using pedigreed families followed by haplotype construction creates a platform for QTL and marker identification, validation, and the use of marker-assisted selection in breeding programs.

Results: Phenotypic data of seven F_1 low to medium chill full-sib families were collected over two years at two locations and genotyped using the 9K SNP Illumina array. Three QTLs were discovered for bloom date (BD) and mapped on linkage group 1 (LG1) (172 – 182 cM), LG4 (48 – 54 cM), and LG7 (62 – 70 cM), explaining 17-54%, 11-55%, and 11-18% of the phenotypic variance, respectively. The QTL for ripening date (RD) and fruit development period (FDP) on LG4 was co-localized at the central part of LG4 (40 - 46 cM) and explained between 40-75% of the phenotypic variance. Haplotype analyses revealed SNP haplotypes and predictive SNP marker(s) associated with desired QTL alleles and the presence of multiple functional alleles with different effects for a single locus for RD and FDP.

Conclusions: A multiple pedigree-linked families approach validated major QTLs for the three key phenological traits which were reported in previous studies across diverse materials, geographical distributions, and QTL mapping methods. Haplotype

characterization of these genomic regions differentiates this study from the previous QTL studies. Our results will provide the peach breeder with the haplotypes for three BD QTLs and one RD/FDP QTL for the creation of predictive DNA-based molecular marker tests to select parents and/or seedlings that have desired QTL alleles and cull unwanted genotypes in early seedling stages.

Keywords: FlexQTL, *Prunus persica* QTL, haplotype, Pedigree-based Analysis, Bloom date, Ripening date, Fruit development period.

Background

Peaches and nectarines [*Prunus persica* (L.) Batsch] are deciduous fruit trees belonging to the Rosaceae family. These are native to China and grown throughout the world in a wide range of environments. The gross production value of peaches and nectarines in 2016 was $825 million in the United States and $17,107 million globally [1].

Breeding of woody perennial crops is not an easy task since their long juvenility periods and large plant size makes maintaining large populations in the field expensive [2]. The use of marker-assisted breeding (MAB) provides a tool to do an early selection of seedlings, to identify superior parents, to improve the selection of elite alleles for essential traits, and to stack desirable alleles [3, 4]. This strategy is pertinent for perennial fruit tree to reduce breeding operational costs. [3].

QTL identification in peaches conducted [5] for acidity, total sugar content, organic acids, fruit weight, bloom, and harvest dates [6, 7], and chilling injury susceptibility [8] have been limited due to the low marker density of genetic maps [9]. Recently, these issues have been overcome due to the availability of the peach genome v1.0 and v2.0 [10, 11] sequence and the development of the International Peach SNP Consortium peach 9K SNP array [11]. Moreover,

65 the Pedigree-Based Analysis (PBA) approach [12, 13] that uses multiple pedigree-linked
66 families allows the discovery of more QTL or QTL-alleles per locus across a range of genetic
67 backgrounds. This approach has facilitated the identification of QTLs for blush [14-16], ripening
68 date [15, 17, 18], soluble solids content [15-18], fruit weight, and titratable acidity [15, 17, 19].
69 Bloom date which is primarily determined by chilling requirement [20-22] is an
70 important trait determining peach adaptation for both low and high chill zones. Bloom date has
71 been reported as moderately to a highly heritable trait (0.39- 0.92) [15, 23-27]. QTLs for bloom
72 date were reported on LG1 (40-60% of phenotypic variance (PVE)), LG2 (27% PVE), LG4 (32-
73 35% PVE) and LG7 (21% PVE). Not all the QTLs were found in all the studies indicating the
74 population-specific nature of these QTLs [15, 17, 28-30].
75 Ripening date in peach trees is a crucial element for extending the production season and
76 developing cultivars that ripen throughout the harvest season. Also, the ripening process is
77 involved in the regulation of several metabolic pathways such as blush, sugar/acid balance, and
78 the flesh softening in peach fruits [31]. Narrow sense heritability (h^2) for ripening date ranges
79 from high to very high (0.79 - 0.94) [15, 32, 33]. The major QTL for controlling RD was mapped
80 on LG 4 at ~44 cM in the *Prunus* T×E reference map and a putative candidate gene was located
81 at ~10.5 Mbp on the peach genome sequence v.1 [30, 31, 34, 35]. This QTL explained ~50 to 98
82 % of the phenotypic variability. The RosBREED project has verified this locus is significant in
83 the U.S. breeding programs [18]. Likewise, a QTL for RD on chromosome 4 was detected in
84 apricot, sweet cherry [31], and almond [36].
85 Fruit development period (FDP) is the period between bloom and ripening date [37] and
86 FDP is well correlated with RD [6, 15]. This trait is highly heritable (h^2= 0.73 - 0.98) [15, 23, 26,
87 38]. QTLs for fruit development period were mapped on LGs 1, 2, 3, 4, 5, and 6 with decisive

88 evidence. The QTLs mapped by Hernández Mora, et al. [15] on LGs 1-6 and by Etienne, et al.
89 [6] on LG4 co-localized with ripening date QTLs.
90 Currently, DNA-based tests for a few breeding-relevant traits have been developed and
91 used in the peach marker-assisted selection application including maturity date (G4mat) [39],
92 quality traits, and fruit bacterial spot resistance. Thus, work is needed to develop DNA tests for
93 BD and FDP traits and to validate SNP-based DNA test (G4mat) for ripening date to enable their
94 use in the TX and other breeding programs [3, 40-42].
95 The objectives of this study are to identify new and/or validate the major QTLs previously
96 reported for bloom date, ripening date, and fruit development period through pedigree-based
97 analysis approach (PBA) using Texas peach/nectarine germplasm. Also, to estimate QTL
98 genotypes for important breeding parents and to identify predictive SNP marker(s) associated
99 with desired QTL alleles. Results from this research will facilitate the design of DNA tests linked
100 to these QTL(s) or genes for routine use for marker-assisted breeding

101 ## Results

102 ### Phenotypic data analysis

103 The mean BD value ranged from 42.6 ±1.9 (CA11) to 49.5 ±7.9 (TX12) and a maximum range
104 of 34, with number of observations ranged between 67 (CA11) and 143 (overall mean)
105 (Additional file 1: Table S1). In our study, the distribution of BD varied across environments and
106 overall mean (Additional file 2: Fig. S1). The CA environments were skewed towards the lower
107 values whereas the TX exhibited trimodal or multimodal profiles, with two or more separated
108 peaks in both environments. This was expected as some of the higher chill genotypes had
109 delayed bloom in the lower chill Texas site as compared to California (~540 vs. ~1,090 chilling
110 hours) [43]. Normal distribution was seen in the overall mean of BD.

RD exhibited an average between 125.9 ± 12.0 (TX12) and 157.4 ±17.7 (CA11), with a greater (75.8) and lower (55.0) RD ranges in the overall mean and TX12 data sets, respectively. CA and the overall mean data sets were slightly skewed towards the higher values while the TX data sets were skewed towards the lower values. Fruit, on average, ripened approximately 20 days later at Fowler, CA than at College Station, TX. FDP mean values ranged from 77.8 ±13.0 (TX12) and 115.3 ±16. 9 (CA11) with FDP range from ~ 56 (TX12) to 86 (overall mean) days. The minimum number of observations (59) was recorded for CA11 compared to 141 observations for the overall mean data set. Similar to RD, FDP for CA data sets were slightly skewed towards higher values compared to the TX environments which skewed towards lower values while the overall mean showed normal distribution. Fruit, on average, had development periods that were approximately 26 days longer at Fowler, CA than at College Station, TX. This was an effect of cooler temperatures during early fruit development in March and April for CA11 and CA12 (~15 and 9°C) relative to TX12 and TX13 (21 and 18°C).

Among these traits, a strong correlation was found between RD and FDP (r = 0.91) (Additional file 1: Table S2), and a moderately weak correlation was observed between FDP and BD (-0.45). The negative correlations between BD and FDP suggest that the earlier blooming genotypes experience a delay in the rate of fruit development due to cooler temperatures. A weak correlation was found between RD and BD traits (-0.14).

Genotype by environment interactions

The genotype × environment interaction (G×E) is the differential sensitivity of genotypes to different environments, if such interaction exists, the selection would be complicated and result in genetic gains reduction in a breeding program. The understanding of G×E interactions is key to increase the efficiency of marker-assisted selection for complex traits [44].

In this study, RD and FDP showed very high broad-sense heritability (H^2=0.91), strong correlations among environments (r = 0.91), and minimal G×E variance ($\sigma^2_{g\times e}/\sigma^2_g$ ratio = 0.20) (Additional file 1: Table S3 and S4) whereas BD trait, showed high broad sense heritability (H^2 = 0.74), strong correlation among environments (r = 0.83) and a moderate genotype by environment interaction ($\sigma^2_{g\times e}/\sigma^2_g = 0.70$). All traits had comparable PC2 value and ranged from 5.5 to 6.8 (Additional file 1: Table S5), implying that the environments equally discriminate the populations for these traits. Finally, the minimal G×E effect of RD and FDP is supported by the relatively similar length of the environmental vectors in the GGE biplots, especially within the same location, indicating a high correlation among them and equal discriminatory ability of the four environments (Additional file 2: Fig. S2). Also, the distance between the environmental vectors was closer between CA11 and CA12, and between TX12 and TX13 for RD and FDP, respectively, illustrate that genotypes responded similarly in these two environments. This is confirmed by the highest positive correlations between CA11 and CA12 (r = 0.87, RD and 0.84, FDP) and between TX12 and TX13 (r = 0.79, RD and 0.89, FDP) for RD and FDP) (see Additional file 1: Table S4).

For BD, the sharper angle and less distance were observed between CA12 and TX12, TX12 and TX13, and CA12 with TX13 (Additional file 2: Fig. S2), indicating a stronger correlation between these environments (r=0.73, 0.75, and 0.65) (Additional file 1: Table S4). The best discrimination of BD among genotypes was observed in the CA11 environment indicated by the longer vectors for these environments (Additional file 2: Fig. S2). Also, the environment CA11 was far from the other three environments and showed less correlation coefficient. However, the low number of observations of this environment (67) may have affected the correlation and G×E results.

Genome-wide QTL analysis

The narrow-sense heritability (h^2) varied among datasets in each trait. Minimum h^2 (0.44) for BD was observed in BD-CA11 versus maximum observed h^2 (0.82) in BD-mean (Table 1). While for RD, h^2 ranged from 0.59 (RD-TX13) to 0.83 (RD-CA12) and for FDP, the minimal h^2 was observed in FDP-CA11 (0.65) and the maximal in FDP-CA12 (0.82).

Three QTLs were mapped for BD on three linkage groups (LG1, 4, and 7) across the four environments (site × year combinations) and their overall mean. The QTL on LG1 was at the distal end and showed strong to decisive evidence in all data sets (Table 1 and Additional file 2: Fig. S3). The QTL on LG4 mapped in three environments (except CA11) and the overall analysis, showing positive and decisive evidence. Whereas the QTL on LG7 was seen in only two environments and the overall analysis with decisive evidence. FlexQTL software found one to two candidate QTLs for RD and FDP depending on the environment; however, only the QTL on the middle part of G4 passed our inclusion criteria. (Table 1 and Additional file 2: Fig. S4 and S5).

For BD, the proportion of phenotypic variation explained (PVE) ranged from 17 to 54%, 11 to 55%, 11 to 18% for LG1, LG4, and LG7, respectively (Table 2). The highest posterior QTL intensity (0.94) showed in BD_CA11_G1 and the lowest intensity (0.21) was found in TX12_G4. The highest additive effect (~10 days) was in TX13_G4 and the lowest (~2 days) showed in LG1, 4, and 7 for CA12. The QTL on LG1 was co-localized across all data sets with an interval between 172 - 182 cM (peaks, 174, 176, and 178 cM) and the physical position of this chromosomal region was 43,058,300 - 45,586,061 bp on the peach genome sequence v2.0, (Table 2, Fig. 1a, and Additional file 1: Table S6). Likewise, peaks of QTL on LG4 of three data sets, except CA12, clustered at mode 50 cM, with an interval between 48 - 54 cM and physical

chromosomal position between 11,956,738 – 13,633,831 bp. Regarding LG7, the peaks co-localized at either 64 or 66 cM with an interval from 62 - 70 cM and physical chromosomal position between 15,513,277 - 17,226,623 bp on the peach genome sequence v2.0 (Table 2 and Fig. 1b, Additional file 1: Table S6).

The proportion of phenotypic variation explained (PVE) by RD QTL on LG4 ranged between 46 and 75% (Table 2). The highest posterior QTL intensity (1.80) and the highest additive effect (~19 days) were found in CA12. The observed high intensity (greater than one) in most environments implies that FlexQTL assigned two QTLs within the same QTL interval with an average distance between them of 1.0 cM across all sampled models. This distance is too short to be genetically meaningful with our current population sizes. This QTL had mode at either 44 or 45 cM, overlapping intervals from 40 to 46 cM across all data sets, and the physical chromosomal position between 10,396,616 to 11,298,736 on the peach genome sequence v2.0 (Table 2, Fig. 1c, and Additional file 1: Table S6). The proportion of phenotypic variation explained (PVE) by FDP QTL on LG4 ranged between 40 and 71% (Table 2). The highest posterior QTL intensity (1.60) was for CA12 and the lowest (0.79) for TX12. The highest additive effect (~20 days) was found in TX13. Likewise, this QTL had a mode at either 44 or 45 cM, overlapping intervals from 40 to 46 cM across all data sets, except TX12, and has a physical chromosomal position between ~10,396,616 to 11,298,736 bp of the peach genome sequence v2.0 (Table 2 and Additional file 1: Table S6). Same as RD, the high intensity that is noticed in most data sets indicates the presence of two tightly linked QTLs within the QTL interval and the gap between them averaged to 1.4 cM across all sampled models. So, the distance is also too short to be genetically dissected in these studied population sizes.

QTL associated haplotypes, number of QTL-alleles, their effect, predictive markers, and sources

On LG1, eleven SNPs in the predicted *qBDG*1 region (172.23 - 182.34 cM) (Additional file 1: Table S7) chosen for haplotyping, revealed eight SNP haplotypes across the seven parents in which H8 was a common haplotype (Table 3). The estimation of the diplotype effect identified families of two parents (Y434-40 and 'Victor') were segregating for this QTL. The results also discovered the presence of multiple *Q*-alleles of various effects associated with H1 to H7 and only one *q*-allele was linked to low phenotypic values associated with H8.

The examination of the haplotype /diplotype effects (Fig. 2a) revealed that the effect of H7 and H1could not differentiated when comparing H5H7<>H5H1 and H8H1<>H8H7. Likewise, the effects of H5 and H8 could not be differentiated when comparing H5H1 to H8H1 and H5H7 to H8H7. Also, H7 had a larger effect than H8 and H3 in the comparison H8H7<>H8H8 and H8H7<>H8H3, respectively. The effect size of H1 was greater than H2 and H3 when comparing H8H1 to H8H2 and H8H3. In general, H8 had a smaller effect than H1, H2, H3, H6, and H7, when comparing H8H8 to H8H1, H8H2, H8H3, H8H6, and H8H7. Hence, H1 and H7 had similar and the largest effects, and both coined as *Q*1, then followed by H3, H6, H2, and H8 which were represented as *Q*2, *Q*3, *Q*4, and *q*, respectively. However, the under-representation of QTL genotypes hindered the estimation of H4 and H5 effects.

All of these haplotypes could be differentiated from H8 by various pairs of adjacent SNP markers by contrasting either *AB*- or *BA*-alleles for 1) snp_1_46757382 and ss_135737 to *BB* of H8, or 2) ss_128625 and ss_128603 to *AA* of H8, and 3) ss_129512 and ss_128603 to also *AA* of H8 (Table 3 and Additional file 1: Table S7). Breeding parents 'Galaxy', Y426-371, Y435-246, Y434-40, and TX2B136 were considered as founders in this study and the sources of these SNPs

were unknown because their ancestors were not available for genotyping. On the other hand, the *Q*-allele (H5) found in F_Goldprince and the *q*-allele (H8) of both 'Victor' and TXW1490-1 was inherited from Fla3-2 through 'TropicBeauty'.

On LG4, there were 13 SNP markers in the BD QTL region (47.83 to 54.54 cM) (Additional file 1: Table S7) selected for haplotyping. This revealed five SNP haplotypes in the seven parents. H1 and H3 were the most common haplotypes (Table 3). Families of four parents (Y435-246, Y426-371, 'Galaxy', and 'Victor') were heterozygous for this QTL. H2 and H3 were associated with the *Q*-allele while H1, H4, and H5 with the *q*-allele.

The examination of the haplotype/diplotype effects in Fig. 2b revealed that H3 was not different from H5 based on H3H3<>H5H3. Also, H3 had a larger effect than H1, H2, and H4 when comparing H3H3 to H3H1, H3H2, and H3H4, respectively. Our results suggest different effects/magnitudes of some haplotypes on BD, e.g. H5H3 (*qQ*) had a larger effect than H3H2 (*QQ*). This could be explained by several reasons such as the presence of interaction with other loci, H5 *(q*) having a smaller effect on decreasing BD among the other haplotypes (H1 and H4) associated with decreasing BD, or H2 (*Q*) having less magnitude on increasing BD. The low number of diplotype observations or high variance within a diplotype class might also have caused these issues.

More than one predictive SNP marker associated with H2 and H3 (*Q*- allele) were identified (Table 3). A-allele at ss_415301 (50.09 cM) along with three more SNP markers distinguished H3, whereas the A-allele at ss_414387 (48.43 cM) and the other two SNP markers were unique for H2. In contrast, H1, H4, and H5 (*q*-allele) were distinguished by two adjacent BB-alleles at ss_414387 and ss_415301. The H3 *Q*- allele was found in TX2B136, 'Flordaprince', F_TXW1490_1, Y426-371, and F-Goldprince while the H2 *Q*- allele came from

Y435-246 and 'Galaxy'. The *q*- alleles were found in Y435-246, Y426-371, 'Galaxy', Y434-40, Fla3-2, and 'TropicBeauty'.

On LG7, the 13 SNPs (62.05- 68.91cM) in the BD QTL region (Additional file 1: Table S7) were chosen for haplotyping. Seven SNP haplotypes were discovered across the seven parents (Table 3). Estimation of the diplotype effect found families of five parents (Y426-371, Y434-40, 'Victor', 'Galaxy', and TX2B136) were segregating for this QTL. H1, H2, H3, and H6 were assigned to the *Q*-allele and H4, H5, and H7 to *q*-allele (Table 3). The analysis of the haplotype/effects showed that the effects of H2 and H4 could not be differentiated based on H6H2<>H6H4 and the same was observed between H2 and H3 when comparing H7H2 <>H7H3 (Fig. 2c). H6 had a greater effect than H7 in the comparison H6H2 to H7H2. While H5 showed a smaller effect than H2 and H3 when comparing H7H5 to both H7H2 and H7H3, respectively. Likewise, the different effects of haplotypes were noticed in this QTL for the same reasons mentioned earlier. The A-allele at the SNP marker ss_778808 (15.6 Mb, 62.48 cM) (Table 3) was associated with *Q*-alleles. This SNP allele inherited from the parents 'Galaxy', Y426-371, Y435-246, Y434-40, and TX2B136. The sources of *q*- allele came from F_TXW1490_1, 'Galaxy', Y426-371, Y434-40, TX2B136, and from 'Flordaprince' through 'TropicBeauty'.

15 SNP markers in the predictive QTL region for both RD and FDP traits (42.33 to 45.19 cM) (Additional file 1: Table S7) on the middle part of LG4 were picked for haplotype analyses. FlexQTL implies this genomic region had more than one QTL within the same interval.

Results discovered four SNP haplotypes associated with RD and FDP across the seven parents of which H1 was common (Table 3). Families of five parents (Y426-371, Y434-40, 'Galaxy', 'Victor', and TXW1490-1) were segregating in this region.

The diplotype analysis revealed the presence of four statistically distinct phenotypic classes (Fig. 3 a and b). H3 had a larger effect than H1 and H4 when comparing H4H3<>H4H1 and H1H3 <> H4H1, respectively. Likewise, H2 showed a smaller effect than H1 on both RD and FDP when comparing H1H1<>H1H2 and from H4H1<>H4H2 just in FDP not RD as their effects could not be differentiated (Fig. 3 a and b). Thus, the effect size of haplotypes can be ordered as H3 > H4 > H1 > H2 that is differentiated by *Q*1, *Q2*, *q1*, and *q2*, respectively. The major finding in this study was the presence of multiple QTL alleles of different effects for a single locus. This may explain why the Bayes Factor values and high intensities of most data sets of this study suggested the presence of two QTLs.

Seven SNP markers were identified, each of which distinguished H3 and H4 from the other two haplotypes (Table 3). In this study, 'Galaxy', TX2B136, Y426-371, Y435-246, and Y434-40, were considered as founders as their direct parents and earlier generations do not exist or were not available for genotyping. *Q1* (H3) was found in parent Y426-371, Y434-40, and 'Galaxy' (Table 3) while *Q2* (H4) inherited from Fla3-2 through 'TropicBeauty'. *q1* (H1) was from Y434-40, Y435-246, 'Galaxy', TX2B136, F_Goldprince, and F_TXW1490-1. Y426-371 parent was the only source of *q2* (H2). Thus, RD and FDP shared the same specific haplotypes and favorable SNP alleles associated with increasing/decreasing phenotypic values.

Discussion

In this study, the bloom date was moderate to highly heritable (0.44 – 0.82) as has been previously reported [15, 24-27] in a range of germplasm, indicating that expression of bloom date is not heavily influenced by environmental effects which were supported by G×E results. Narrow sense heritability was moderate to high for RD (0.59 to 0.83) as was found in previous studies [15, 18, 26, 45-47]. FDP also has an important additive genetic component as indicated

293 by a high to very high (0.65 to 0.82) estimated narrow-sense heritability reported in this and
294 previous studies [15, 24-27].
295 Our QTL for BD on LG1 was flanked by snp_1_46757382 and ss_128603, spanned the
296 region from 43.1 – 45.6 Mb with PVE from ~17 to 54 %. This QTL was previously described in
297 different germplasm, by Romeu, et al. [30] in the 'V6' × 'Granada' progeny (low- medium chill)
298 (41.2 Mb) at the end of LG1, PVE ~60 %) and by Fan, et al. [29] using 'Contender' (high chill)
299 and 'Fla.92-2C' (low chill) population (at 45.6 Mb, PVE ~40%).
300 The QTL at the middle region of LG4 for BD mapped between ss_413934 and
301 ss_419614, in the interval between 12 – 13.6 Mb, and PVE ranged from 11 to 55 %. This QTL
302 overlaps with the BD QTL on LG4 (qFD4.2) at nearest markers ss_417840 and ss_440116 (13.1
303 to 16.0 Mb) reported by Hernández Mora, et al. [15].
304 Lastly, the QTL at the distal end of LG7 was flanked by ss778568 and snp_7_17628094,
305 spanned from 15.5 to 17.2 Mb and explained ~11 to 18 % of BD phenotypic variation. This
306 finding agreed with Romeu, et al. [30] who found a QTL for BD on LG7 at the nearest marker
307 ss_779224 (15.7 Mb), which was close to our QTL peaks (ss_780816 (16.3 Mb) and ss_779362
308 (15.7 Mb)). Moreover, this region overlapped with the QTL (15.4 to 19.4 Mb; PVE ~60%) that
309 was reported by Fan, et al. [29].
310 The only one of the three QTLs was detected in CA11 is probably due to that this environment
311 had a low number of phenotypic data (82 records). The G×E for BD in the studied populations
312 may result from the response of the high-chill seedlings to the lack of chill hours that delayed the
313 blooming period.
314 In summary, this study provides more evidence that three mapped QTLs for BD on LG1,
315 4, and 7 are major loci for controlling BD and were supported by other studies using low- and

316 medium-chill germplasm and bi-parental family mapping. It was also supported by the polygenic
317 nature of BD inheritance. Additional QTLs for BD were also reported on LG2 [15, 48], LG3 [17,
318 30], LG6 [15, 30, 49], and LG8 [15, 17]. Thus, further studies using more diverse germplasm
319 will be important to continue to characterize additional QTLs and candidate genes to identify the
320 genetic pathway regulating the BD in peach.

321 The examination of haplotype/diplotype effects uncovered the high prevalence of a few
322 haplotypes, e.g. H8 (*q*-allele), H3 (*Q*-allele), and H7 (*q*-allele) on LG1, 4, and 7, respectively,
323 reflecting the relatively narrow genetic base of peach germplasm. Also, the results revealed the
324 presence of multiple *Q*-alleles of different effects for the QTL on LG1 (*Q1, Q2, Q3,* and*, Q4*)
325 along with only one *q*-allele. In general, the small family sizes and consequently the low/lack
326 representation of various compound diplotypes (e.g. 6 to 9 observations in some diplotypes of
327 LG1) hindered the ability to make conclusions on the haplotype effects (H4 and H5) or the
328 interplay among the three mapped QTLs for BD**.**

329 One QTL associated with RD and FDP was mapped at the middle part of LG4 (10.4 -
330 11.3 Mb) with PVE 46 - 75 % and 40 - 71 %, respectively. This specific genomic region was
331 reported as associated with RD trait previously by Nuñez-Lillo, et al. [35] (~10.9 Mb), Romeu, et
332 al. [30] (~10.7 Mb), Frett [18] (10.7 - 11.3 Mb), Eduardo et al. (2011; 2013) (~11.0 – 11.2 Mb)
333 with candidate gene ppa008301m for maturity, and Hernández Mora, et al. [15] (~11.2 – 14.1
334 Mb). This held true using early-, mid-, and late-maturing populations. The co-localization
335 between QTLs for RD and FDP was supported by the strong correlation (r=0.87) (data not
336 shown) between these traits in this study as well as previous work [6, 15].

337 Also, all data sets, except TX12, showed decisive evidence (BF ≥ 10) with high intensity
338 for the presence of a second QTL on LG4. This could be explained by that TX12 had higher

339 temperatures during the critical fruit development months (March and April) [50] compared to
340 other sites. The higher temperatures accelerated RD and shortened FDP in this environment
341 which minimized the phenotypic variation as mentioned earlier (Additional file 1: Table S1)
342 Furthermore, the haplotype analysis of this chromosomal region revealed multiple predictive loci
343 (ss_410398, ss_410794, and ss_412662) for decreasing and increasing for either RD or FDP.
344 Likewise, the examination of the relative effects of haplotypes and estimated QTL genotypes
345 revealed a series of QTL alleles of different effect at this locus that we coined *Q*1, *Q*2, *q*1, and
346 *q*2. The use of multi-parent populations for finding multiple functional alleles of different effect
347 was also reported for two acidity QTLs/genes in apple by Verma, et al. [51] and for the blush
348 QTL in peach using the current germplasm by Rawandoozi, et al. [16]. In our germplasm the RD
349 QTL on LG4 co-localized with a QTL for soluble solids concentration (SSC) and blush reported
350 by Rawandoozi, et al. [16]. These co-localizations had also been reported by other studies [15,
351 34, 52]. A pleiotropic effect of the RD has been reported on several quality traits [15, 34, 35, 39].
352 Co-factor analysis could be useful in future studies to account for one trait when analyzing
353 another, e.g. accounting for RD for analyzing SSC or blush traits.
354 Overall, additional QTL mapping through pedigree-based analysis across a wider range of
355 breeding germplasm is needed to identify and characterize additional QTLs to understand the
356 whole genetic pathway controlling RD and FDP traits. Moreover, larger family sizes would
357 ensure better representation of QTL genotype classes for estimating QTL effects and allow
358 improved downstream analysis in case of multiple QTL alleles of different effects at a single
359 locus and/or gene by gene interaction.
360 At the genomic region of the detected QTLs for these traits, candidate genes have been
361 reported. For BD, the QTL interval (43,058,300-45,586,061bp) of LG 1, the most promising

candidate genes for the major QTL affecting blooming time and chilling requirement in LG1 were the Dormancy-associated MADS-box (DAM) genes within the evergrowing (*evg*) locus in peach, apricot, and almond [29, 53, 54].

Prupe.1G531600 (DAM5) and Prupe.1G531700 (DAM6) genes were identified as potential candidate genes of lateral bud endodormancy release in peach [29, 55, 56]. Prupe.1G531500 gene is described as MADS-box protein short vegetative phase (SVP) and it plays a role in controlling meristem development during the vegetative phase and flower development as well as in floral meristem determination [57]. Prupe.1G549600 and Prupe.1G548000 genes are described as agamous-like MADS-box proteins AGL11 and AGL12, respectively. AGL11 is a vital gene to control ovule identity and associated placental tissues in Arabidopsis [58]. While a MADS-box gene AGL12 regulates root development and flowering transition in Arabidopsis [59]. Prupe.1G554100 (AGL80) is also a member of the MADS-box family of genes. In Arabidopsis, AGL80 was found to be involved in female gametophyte development [60].

Likewise, many candidate genes have been reported within the interval (11,956,738-13,633,831bp) of LG4. Prupe.4G208000 is described as a Forkhead-associated (FHA) domain-containing protein (DDL) that plays an important role in plant growth and development [61]. Prupe.4G197000 gene was proposed to link to auxin synthesis and response which is known to be involved in fruit set and ripening [62]. Prupe.4G202200, Fertilization Independent Endosperm (FIE) polycomb group protein, in Arabidopsis thaliana FIE regulates endosperm and embryo development and suppresses flowering during embryo and seedling development [63]. Prupe.4G207300 (uclacyanin) is associated with pollen grain development in rice [64]. Prupe.4G205500 (early nodulin-like protein 1) gene is reported to be engaged in determining the reproductive potential in *Arabidopsis* [65]. In the QTL region (15,513,277-17,226,623 bp) of

LG7, Prupe.7G130900, CURLY LEAF (CLF) gene, is associated with the repression of FLOWERING LOCUS T (FT) gene and other flowering-time genes during the vegetative growth of the plant [66]. Prupe.7G153400 gene is described as a ATP-dependent DNA helicase (DDM1), the importance of this gene was previously reported for DNA methylation in genes and transposable elements [67]. Prupe.7G133100 (Zeaxanthin epoxidase) gene has been identified to play an important role in resistance to stresses, seed development, and dormancy in *Arabidopsis* [68].

Within the RD/FDP locus on LG4 (10,582,092 to 11,298,736), a list of candidate genes has been previously reported in this region. NAC072 (Prupe.4G816800) is the candidate gene for controlling the ripening date in peach [39]. Also, there are three other genes proposed to be involved in the determination of RD/FDP in peach. Prupe.4G79900 gene is needed for normal embryo development in *Arabidopsis* and maize [69, 70]. Prupe.4G179800 gene is described as Early nodulin- like protein 1 and PtNIP1in *Arabidopsis* and loblolly pine, respectively [71]. It is expressed in immature zygotic and somatic embryos of developing seeds. Prupe.4G179200 gene with functional annotation Purine permease 10 in *Arabidopsis* and OsPUP7 in rice [72], and showed a flowering delay in rice. Finally, Prupe.4G185800 [73] and Prupe.4G187100 [74] genes that were reported to be associated with the regulation of the anthocyanin biosynthetic pathway in peach. Hence, these results confirming the pleiotropic effect of the RD on several quality traits including blush that was previously reported [15, 16, 34, 35, 39].

Conclusions

Pedigree-based analysis was successfully used as a statistical method for discovering and validating QTLs. Four QTLs associated with three important phenological traits were validated using low- medium-chill peach/nectarine germplasm. Two minor QTLs were also identified.

This approach increases the genetic background explored, improves statistical power, and allows the simultaneous detection and validation of QTLs.

QTLs for BD on LG1, 4, and 7 were verified and the SNP haplotypes associated with increasing or decreasing BD were identified. A single QTL with multiple QTL alleles of different effects was detected on the central part of LG4 for both RD and FDP. These mapped QTLs are optimal targets for developing new predictive, DNA-based molecular marker tests to enable MAB. Our findings would help breeding programs make crossing decisions to pick the combination of parents that have SNP haplotypes associated with lowering BD to produce progeny with better adaptation to subtropical environments like Texas or raising BD to ensure better adaptation to temperate environments whereas the results of RD and FDP will facilitate better targeting for specific ripening periods.

Methods

Plant materials

This study included 162 seedlings from seven related F_1 families derived from seven parents descending from 12 founders (Fig. 4). Parents were medium to low chill selections from the Texas A&M University breeding program, and high chill selections from the USDA Stone Fruit Breeding Program in Parlier, CA. The number of seedlings in each family ranged from 8 to 36 with an average of 20. These seedlings, along with parental genotypes, were budded onto 'Nemaguard' peach rootstocks and planted in College Station, TX, and Fowler, CA. Each site included one replicate of each seedling and three (Fowler) to four (College Station) replicates of each parent. Phenotypic and fruit quality characteristics of the eight parents used in the study are shown in Additional file 1: Table S8.

Plot establishment and design

The College Station plot was randomized, whereas the Fowler plot was organized by progeny. Trees at College Station were planted in 2010 in staggered double-rows, with 1.7 meters between rows, 0.67 meters within rows, and 5 meters between double rows. All trees were trained as a central leader. Trees at Fowler were planted in 2010, with 4 meters between rows, and one meter within rows and trained as a two-scaffold 'Y'. At each location, irrigation, fertilization, pest and weed control, pruning, and fruit thinning were done according to typical commercial practice.

College Station is located in east-central Texas with a sub-humid and warm temperate climate with mild winters and warm to hot, humid summers. Fowler is located in the San Joaquin Valley in central California and is ideal for peach production with a semi-arid Mediterranean climate. The temperature ranged between 4.0 to 36.5 °C (Fowler) and between 7.0 to 35.0 °C (College Station) for Min Ave. Jan. Temp. and Max Ave Jul. Temp., respectively. College Station has greater rainfall than Fowler (1022 versus 248 mm), higher humidity (67.5% versus 55.1%), warmer night temperatures during fruit development (15.8 °C versus 12.4 °C), and more cloudy days (College Station receives 27% less sunlight per year) [50]. Besides, College Station is more subject to late spring freezes, low chill accumulation, and has a heavy textured soil. These environmental factors make College Station much less suitable for stone fruit production as compared to Fowler.

Phenotypic Evaluations

Phenotypic data was taken at both locations across two years (2011-2012 in CA, and 2012-2013 in TX) on individual trees for three phenological traits, bloom date (BD), ripening date (RD), and fruit development period (FDP). The date of first (10% blossoms open) and full bloom (60% to 80% of the blossoms open) were visually assessed in the field and recorded for each tree. Ripening date was determined when 20% of fruits are pickable by visually inspecting the

presence of a few soft fruits in the field for maturity two times per week. Both full bloom and ripening dates were converted to Julian days (0-365). FDP was calculated as the number of days between the date of full bloom and ripening date.

Broad sense heritability and Genotype by Environment interaction

A linear mixed model with the residual maximum likelihood (REML) procedure used to estimate the additive (σ_A^2), non-additive (σ_d^2), and G×E ($\sigma_{g\times e}^2$) variances for all traits. In the linear mixed model, the genotypes and G×E were considered as random effects.

GGE biplots R package version 0.1.1 was used to explain the variation due to genotypes and G×E. The sum of parental [female parent (FP), pollen/male parent (PP)] variances was treated as additive variance (σ_A^2), progeny variance was regarded as non-additive variance (σ_d^2), and the sum of the parental and progeny variances was regarded as the genotypic variance (σ_g^2). The interaction of genotype (FP, PP, or Progeny) by environment was treated as the genetic-environmental variance ($\sigma_{g\times e}^2$).

Broad sense heritability across the environments was calculated as:

$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_{g\times e}^2}{E}}$ Where E is representing the number of environments [75-77].

Pearson correlations coefficient was calculated between phenotypic traits per environment and across environments using R software.

SNP genotyping and genetic linkage map

Individuals were previously genotyped as part of the US Peach Crop Reference Set and Breeding Pedigree Set [2] using the IPSC 9K SNP Array for Peach [11]. The raw iScan data was initially processed into the GenomeStudio software v2010.3 [78] using the Genotyping Module

with a Gen Call threshold of 0.15. Parentage records and SNP data curation was performed as described before [79].

After filtering null alleles and non-Mendelian error conflicts across our germplasm 1,487 informative SNPs were retained. Their physical position on the peach genome v2.0 [80] was assessed and scaled to an approximate genetic map by using a conversion factor where every 1 Mb corresponded to 4 cM [79]. The markers were evenly distributed over the eight chromosomes.

QTL detection

Genotypic and phenotypic data for the seedlings were combined for QTL mapping. The pedigree-based QTL analysis approach was implemented through FlexQTL software to increase the accuracy of QTL mapping. It allows for a QTL to be evaluated across diverse genetic backgrounds while, simultaneously, increases the chances of recombination events nearby the QTL of the trait of interest [13, 81]. FlexQTL analyses were conducted on data from each location and the overall mean (of both locations) three times with different chain length, and prior and maximum QTL number to reach an effective chain size (ECS) [82] of at least 100 for phenotypic mean, residual variance and number of QTLs as needed to make sound inferences and conclusions. The length of Markov Chain Monte Carlo (MCMC) simulations varied between 100,000 and 3,600,000 iterations, from which one thousand simulations were sampled for statistical inference, thus sampling every 100 to 2,500 iterations. ECS values and trace and intensity plots were evaluated for convergence [13]. Traits were first tested with a mixed model (allowing QTLs with additive and dominant effects). Since none of the traits showed dominance, they were reanalyzed with an additive model. The statistical evidence for QTLs was evaluated by twice the natural logarithm of the obtained Bayes Factors (BF) [$2ln(BF)$] [83]; values greater

than 2, 5 and 10, can be interpreted as indicating positive, strong, and decisive evidence, respectively. For inferences on the number of QTLs, we considered loci that had a 2lnBF ≥ 5, or that 2 ≤ 2lnBF < 5 for at least two data sets. Also, the QTLs with overlapping intervals of at least 2 cM and explained at least 10% of the phenotypic variation were considered for haplotyping. QTL intervals were defined as a series of successive 2-cM bins with intensities corresponding to 2lnBF > 2.

Additive variance ($\sigma^2_{A(trt)}$) for each trait was calculated by subtracting the residual variance (σ^2_e) from the phenotypic variance (σ^2_P) (both are obtained from FlexQTL). And the narrow-sense heritability (h^2) was calculated as follows:

$h^2 = \frac{\sigma^2_{A(trt)}}{\sigma^2_P} \times 100$ where: $\sigma^2_{A(trt)}$ is the variance of the trait

The proportion of phenotypic variance explained (PVE) by each QTL was estimated from FlexQTL output for the additive model (pure additive effect) through the following equation:

$PVE = \frac{\sigma^2_{A(qtl)}}{\sigma^2_P} \times 100$ where: $\sigma^2_{A(qtl)}$ is the variance of QTL

Our QTL nomenclature is a modification of that of Fan et al. [29]. Thus, in the name *qTTGa*, 'TT' stands for the trait, 'G' the linkage group number, 'a' or 'b' letter to distinguish different QTLs for the same trait in one linkage group. Next, an identifier 'LLYY' may be added whenever useful to specify the environment where the QTL underlying phenotypic data came from where 'LL' specifies the location (State, CA or TX) and 'YY' the year in which the trait was evaluated. The QTL name is in italics, while the identifier is not.

SNP haplotypes and QTL genotypes of important breeding parents

Considering the 1,487 informative SNP markers, SNPs within the interval of a significant QTL were chosen for haplotyping. Haplotypes were constructed across the germplasm using FlexQTL and PediHaplotyper [19].

To examine for the presence of multi-allelic QTLs, haplotype effects were analyzed manually. Haplotype effects were deduced from combinations of diplotypes. For instance, the effects of haplotypes H1 and H2 could be estimated by comparing the effects of the H3|H1 and H3|H2 diplotypes. Statistical significance of differences was evaluated using the Steele–Dwass nonparametric multiple comparison test ($P < 0.05$) using JMP Pro Version 13.2 (SAS Institute Inc., Cary, NC, 2016). Then, haplotypes were assigned to QTL allele categories (Q or q) based on the direction of their effects by increasing or decreasing phenotypic values of each trait. In case of multi-allelic series, Q and q alleles were differentiated by an index number. Lastly, QTL genotypes were assigned to each individual. The SNP allele sequences of haplotypes along with pedigree records allowed tracing back of favorable alleles to their original sources.

Abbreviations

BF: Bayes factor; CG: Candidate gene; cM: Centimorgan; DNA: Deoxyribonucleic acid; ECS: Effective chain size; F_1: First filial generation; FS: full-sib; h^2: Narrow-sense heritability; H^2: Broad-sense heritability; LG: Linkage group; Mb: Megabase pair; MCMC: Markov Chain Monte Carlo; PVE: Phenotypic variance explained; QTL: Quantitative trait loci; SNP: Single nucleotide polymorphism; BD: Bloom date; RD: Ripening date; FDP: Fruit development period; MAB: Marker-assisted breeding.

Declarations

Ethics approval and consent to participate

539 Not applicable

540 **Consent for publication**

541 Not applicable

542 **Availability of data and materials**

543 The genotypic and phenotypic datasets of seven full-sib peach families used in this
544 study can be found in the Dryad Repository, https://doi.org/10.5061/dryad.tmpg4f4vp
545 (https://datadryad.org/stash/share/oWBiP7isZFdQbY8zS0nTubqrhrT0RntovILSNJp9Xxc
546)

547 **Competing interests**

548 The authors declare that they have no competing interest.

549 **Funding**

550 This work was supported by the USDA National Institute of Food and Agriculture (NIFA)
551 Specialty Crop Research Initiative (SCRI) project, “RosBREED: Enabling marker-
552 assisted breeding in Rosaceae” (2009-51181-05808). The project supported
553 phenotyping, genotyping, map construction, annual meetings for training in the use of
554 FlexQTL software on the national project level, and travel to annual meetings.

555 **Authors’ contributions**

556 D.H.B. conceived this study, Z.R. carried out the analysis, T.H., D.H.B., and S.C.
557 provided phenotypic data, K.G., C.L., L.C. developed the SNP genotyping and produced
558 the linkage map, and E.V.W provided support for performing the pedigree-based

analysis and interpretation of the results. Z.R., D.H.B., and E.V.W drafted the manuscript.

All authors read and approved the final and reviewed manuscript.

Acknowledgments

The authors wish to thank Dr. Nahla Bassil and her lab team for conducting DNA extraction, Dr. David Ramming of the USDA for supplying germplasm used in this study, the Burchell Nursery, Inc., and Fruit Dynamics, Inc. for facilitating the development and maintenance of the research plot and assistance with the fruit evaluations at Fowler, CA, to Ricky Garcia and Natalie Anderson and the Department of Horticultural Sciences, Texas A&M University for their support in the field and lab, to Dr. Sujeet Verma (University of Florida) for his advice during data analysis.

References

1. The Statistics Division of the Food and Agriculture Organization of the United Nations http://www.fao.org/faostat/en/#data/QC. Accessed 20 Jan 2020
2. Peace CP, Luby JJ, van de Weg WE, Bink MCAM, Iezzoni AF. A strategy for developing representative germplasm sets for systematic QTL validation, demonstrated for apple, peach, and sweet cherry. Tree Genet & Genomes. 2014;10(6):1679-1694. https://doi.org/10.1007/s11295-014-0788-z.
3. Bliss FA. Marker-assisted breeding in horticultural crops. Acta Hort. 2010;859:339-350. https://doi.org/10.17660/ActaHortic.2010.859.40.

4. Testolin R, Cipriani G. Molecular markers for germplasm identification and characterization. Acta Hort. 2010;859:59-72. https://doi.org/10.17660/ActaHortic.2010.859.5.

5. Zeballos J: Identification of genomic region related to fruit quality traits in peach. Zaragoza, Spain: Universidad de Lleida; 2012.

6. Etienne C, Rothan C, Moing A, Plomion C, Bodénès C, Svanella-Dumas L *et al*. Candidate genes and QTLs for sugar and organic acid content in peach [*Prunus persica* (L.) Batsch]. Theor Appl Genet. 2002;105(1):145-159. https://doi.org/10.1007/s00122-001-0841-9.

7. Quilot B, Wu BH, Kervella J, Génard M, Foulongne M, Moreau K. QTL analysis of quality traits in an advanced backcross between *Prunus persica* cultivars and the wild relative species *P. davidiana*. Theor Appl Genet. 2004;109(4):884-897. https://doi.org/10.1007/s00122-004-1703-z.

8. Cantín CM, Crisosto CH, Ogundiwin EA, Gradziel T, Torrents J, Moreno MA *et al*. Chilling injury susceptibility in an intra-specific peach [*Prunus persica* (L.) Batsch] progeny. Postharvest Biol Technol 2010;58(2):79-87. https://doi.org/10.1016/j.postharvbio.2010.06.002.

9. Eduardo I, Chietera G, Pirona R, Pacheco I, Troggio M, Banchi E *et al*. 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. 2013;9(1):189-204. https://doi.org/10.1007/s11295-012-0546-z.

10. Arús P, Verde I, Sosinski B, Zhebentyayeva T, Abbott AG. The peach genome. Tree Genet & Genomes. 2012;8(3):531-547. https://doi.org/10.1007/s11295-012-0493-8.

11. Verde I, Bassil N, Scalabrin S, Gilmore B, Lawley CT, Gasic K *et al*. Development and Evaluation of a 9K SNP Array for Peach by Internationally Coordinated SNP Detection and Validation in Breeding Germplasm. PLOS ONE. 2012;7(4):e35668. https://doi.org/10.1371/journal.pone.0035668.

12. Bink MCAM, Totir LR, Braak CJF, Winkler CR, Boer MP, Smith OS. QTL linkage analysis of connected populations using ancestral marker and pedigree information. Theor Appl Genet. 2012;124(6):1097-1113. https://doi.org/10.1007/s00122-011-1772-8.

13. Bink MCAM, Jansen J, Madduri M, Voorrips RE, Durel CE, Kouassi AB *et al*. Bayesian QTL analyses using pedigreed families of an outcrossing species, with application to fruit firmness in apple. Theor Appl Genet. 2014;127(5):1073-1090. https://doi.org/10.1007/s00122-014-2281-3.

14. Frett TJ, Reighard GL, Okie WR, Gasic K. Mapping quantitative trait loci associated with blush in peach [*Prunus persica* (L.) Batsch]. Tree Genet & Genomes. 2014;10(2):367-381. https://doi.org/10.1007/s11295-013-0692-y.

15. Hernández Mora JR, Micheletti D, Bink MCAM, Van de Weg E, Cantín C, Nazzicari N *et al*. Integrated QTL detection for key breeding traits in multiple peach progenies. BMC Genomics. 2017;18:404. https://doi.org/10.1186/s12864-017-3783-6.

16. Rawandoozi ZJ, Hartmann TP, Carpenedo S, Gasic K, da Silva Linge C, Cai L *et al*. Identification and characterization of QTLs for fruit quality traits in peach through a multi-family approach. BMC Genomics. 2020;21(1):522. https://doi.org/10.1186/s12864-020-06927-x.

17. Fresnedo-Ramírez J, Bink MCAM, van de Weg E, Famula TR, Crisosto CH, Frett TJ *et al*. QTL mapping of pomological traits in peach and related species breeding germplasm. Mol Breeding. 2015;35:166. https://doi.org/10.1007/s11032-015-0357-7.

18. Frett TJ: Genetic determinism of *Xanthomonas arboricola* pv. *pruni* (Xap) resistance, fruit quality, and phenological traits in peach and incorporation of marker-assisted selection (MAS) in the University of Arkansas peach and nectarine breeding program. Diss. Fayetteville, AK: Univ. Ark.; 2016.

19. Voorrips RE, Bink MCAM, Kruisselbrink JW, Koehorst-van Putten HJJ, van de Weg WE. PediHaplotyper: software for consistent assignment of marker haplotypes in pedigrees. Mol Breeding. 2016;36:119. https://doi.org/10.1007/s11032-016-0539-y.

20. Byrne DH, Sherman WB, Bacon TA: Stone fruit genetic pool and its exploitation for growing under warm winter conditions. In: Temperate Fruit Crops in Warm Climates. Edited by Erez A. Dordrecht: Springer Netherlands; 2000: 157-230. https://doi.org/10.1007/978-94-017-3215-4_8.

21. Ruiz D, Campoy JA, Egea J. Chilling and heat requirements of apricot cultivars for flowering. Environ Exp Bot. 2007;61(3):254-263. https://doi.org/10.1016/j.envexpbot.2007.06.008.

22. Alburquerque N, Garcia-Montiel F, Carrillo A, Burgos L. Chilling and heat requirements of sweet cherry cultivars and the relationship between altitude and the probability of satisfying the chill requirements. Environ Exp Bot. 2008;64(2):162-170. https://doi.org/10.1016/j.envexpbot.2008.01.003.

23. Monet R, Bastard Y. Estimation du coefficient de régression enfant/parent de quelques caractères du pêcher dans le cas de familles issues d'autofécondations. Agronomie. 1982;2(4):347-358.

24. Mowrey BD, Sherman WB. Flower bud set and relationship to vigor in 18-month peach seedlings. Proc Fla State Hort Soc. 1986;99:209-210.

25. Hansche PE. Heritability of spring bloom and fall leaf abscission dates in *Prunus persica*. HortScience. 1990;25(12):1639-1641. https://doi.org/10.21273/HORTSCI.25.12.1639.

26. de Souza VAB, Byrne DH, Taylor JF. Heritability, genetic and phenotypic correlations, and predicted selection response of quantitative traits in peach: I. An analysis of several reproductive traits. J Amer Soc Hort Sci. 1998;123(4):598-603. https://doi.org/10.21273/JASHS.123.4.598.

27. de Souza VAB, Byrne DH, Taylor JF. Predicted breeding values for nine plant and fruit characteristics of 28 peach genotypes. J Amer Soc Hort Sci. 2000;125(4):460-465. https://doi.org/10.21273/JASHS.125.4.460.

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

29. Fan S, Bielenberg DG, Zhebentyayeva TN, Reighard GL, Okie WR, Holland D *et al*. Mapping quantitative trait loci associated with chilling requirement, heat requirement and bloom date in peach (*Prunus persica*). New Phytologist. 2010;185(4):917-930. https://doi.org/10.1111/j.1469-8137.2009.03119.x.

30. Romeu JF, Monforte AJ, Sánchez G, Granell A, García-Brunton J, Badenes ML *et al*. Quantitative trait loci affecting reproductive phenology in peach. BMC Plant Biol. 2014;14:52. https://doi.org/10.1186/1471-2229-14-52.

31. Dirlewanger E, Quero-García J, Le Dantec L, Lambert P, Ruiz D, Dondini L *et al*. Comparison of the genetic determinism of two key phenological traits, flowering and maturity dates, in three *Prunus* species: peach, apricot and sweet cherry. Hered. 2012;109(5):280-292. https://doi.org/10.1038/hdy.2012.38.

32. Hansche PE. Heritability of fruit quality traits in peach and nectarine breeding stocks dwarfed by the dw gene. HortScience. 1986;21(5):1193-1195.

33. de Souza VAB, Byrne DH, Taylor JF. Heritability, genetic and phenotypic correlations, and predicted selection response of quantitative traits in peach: II. An analysis of several fruit traits. J Amer Soc Hort Sci. 1998;123(4):604-601. https://doi.org/10.21273/JASHS.123.4.604.

34. Eduardo I, Pacheco I, Chietera G, Bassi D, Pozzi C, Vecchietti A *et al*. QTL analysis of fruit quality traits in two peach intraspecific populations and importance of maturity date pleiotropic effect. Tree Genet & Genomes. 2011;7(2):323-335. https://doi.org/10.1007/s11295-010-0334-6.

35. Nuñez-Lillo G, Cifuentes-Esquivel A, Troggio M, Micheletti D, Infante R, Campos-Vargas R *et al*. 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. 2015;11:86. https://doi.org/10.1007/s11295-015-0911-9.

36. Sánchez□Pérez R, Howad W, Dicenta F, Arús P, Martínez□Gómez P. Mapping major genes and quantitative trait loci controlling agronomic traits in almond. Plant Breeding. 2007;126(3):310-318. https://doi.org/10.1111/j.1439-0523.2007.01329.x.

37. Blake MA. The J.H. Hale as a Parent in Peach Crosses. Proc Amer Soc Hort Sci. 1932;29:131-136.

38. Vileila-Morales EA, Sherman WB, Wilcox CJ, Andrews CP. Inheritance of short fruit development period in peach. J Amer Soc Hort Sci. 1981;106:399-401.

39. Pirona R, Eduardo I, Pacheco I, Da Silva Linge C, Miculan M, Verde I *et al*. Fine mapping and identification of a candidate gene for a major locus controlling maturity date in peach. BMC Plant Biol. 2013;13(1):166. https://doi.org/10.1186/1471-2229-13-166.

40. Byrne DH. Trends in stone fruit cultivar development. HortTechnol 2005;15(3):494-500. https://doi.org/10.21273/HORTTECH.15.3.0494.

41. Edge-Garza DA, Luby JJ, Peace C. Decision support for cost-efficient and logistically feasible marker-assisted seedling selection in fruit breeding. Mol Breeding. 2015;35(12):223. https://doi.org/10.1007/s11032-015-0409-z.

42. Ru S, Main D, Evans K, Peace C. Current applications, challenges, and perspectives of marker-assisted seedling selection in Rosaceae tree fruit breeding. Tree Genet Genomes. 2015;11. https://doi.org/10.1007/s11295-015-0834-5.

43. Average High/Low Temperatures for KCLL. Seasonal Averages http://www.wunderground.com/NORMS/DisplayNORMS.asp?AirportCode=KCLL&SafeCityName=College_Station&StateCode=TX&Units=none&IATA=CLL. Accessed 8 July 2018.

44. Koebner RMD: Marker Assisted Selection in the Cereals: The Dream and the Reality. In: Cereal Genomics. Edited by Gupta PK, Varshney RK. Dordrecht: Springer Netherlands; 2005: 317-329. https://doi.org/10.1007/1-4020-2359-6_10.

45. Daines RH, Hough LF. Artificial inoculation of peach seedlings with *Xanthomonas pruni*. Phytopathol. 1951;41(1):8-9.

46. Bailey CH, Hough LF. A hypothesis for the inheritance of season of ripening in progenies from certain early ripening peach varieties and selections. Proc Amer Soc Hort Sci. 1959;73:125-133.

47. Yu M, Ma R, Tang X. Inheritance of ripening season in F_1 hybrids of peach. Jiangsu J Agr Sci. 1997;13:176-181.

48. Dirlewanger E, Moing A, Rothan C, Svanella L, Pronier V, Guye A *et al*. Mapping QTLs controlling fruit quality in peach (*Prunus persica* (L.) Batsch). Theor Appl Genet. 1999;98(1):18-31. https://doi.org/10.1007/s001220051035.

49. Joobeur T, Viruel MA, de Vicente MC, Jáuregui B, Ballester J, Dettori MT *et al*. Construction of a saturated linkage map for *Prunus* using an almond×peach F_2 progeny. Theor Appl Genet. 1998;97(7):1034-1041. https://doi.org/10.1007/s001220050988.

50. Weather History for Fresno, CA http://www.wunderground.com/history/airport/KFAT/2012/8/10/MonthlyHistory.html. Accessed 10 Sep 2018

51. Verma S, Evans K, Guan Y, Luby JJ, Rosyara UR, Howard NP *et al*. Two large-effect QTLs, Ma and Ma3, determine genetic potential for acidity in apple fruit: breeding insights from a multi-family study. Tree Genet & Genomes. 2019;15:18. https://doi.org/10.1007/s11295-019-1324-y.

52. Hernández Mora JR, Micheletti D, Bink MCAM, Van de Weg WE, Bassi D, Nazzicari N *et al*. Discovering peach QTLs with multiple progeny analysis. Acta Hort. 2017(1172):405-410. https://doi.org/10.17660/ActaHortic.2017.1172.77.

53. Olukolu BA, Trainin T, Fan S, Kole C, Bielenberg DG, Reighard GL *et al*. Genetic linkage mapping for molecular dissection of chilling requirement and budbreak in apricot (*Prunus armeniaca* L.). Genome. 2009;52(10):819-828. https://doi.org/10.1139/g09-050.

54. Sánchez-Pérez R, Dicenta F, Martínez-Gómez P. Inheritance of chilling and heat requirements for flowering in almond and QTL analysis. Tree Genet & Genomes. 2012;8(2):379-389. https://doi.org/10.1007/s11295-011-0448-5.

55. Yamane H, Ooka T, Jotatsu H, Hosaka Y, Sasaki R, Tao R. Expressional regulation of PpDAM5 and PpDAM6, peach (*Prunus persica*) dormancy-associated MADS-box genes, by low temperature and dormancy-breaking reagent treatment. J Expt Bot. 2011;62(10):3481-3488. https://doi.org/10.1093/jxb/err028.

56. Jiménez S, Reighard GL, Bielenberg DG. Gene expression of DAM5 and DAM6 is suppressed by chilling temperatures and inversely correlated with bud break rate. Plant Mol Biol. 2010;73(1):157-167. https://doi.org/10.1007/s11103-010-9608-5.

57. Gregis V, Andrés F, Sessa A, Guerra RF, Simonini S, Mateos JL *et al*. Identification of pathways directly regulated by SHORT VEGETATIVE PHASE during vegetative and reproductive development in *Arabidopsis*. Genome Biology. 2013;14(6):R56. https://doi.org/10.1186/gb-2013-14-6-r56.

58. Rounsley SD, Ditta GS, Yanofsky MF. Diverse roles for MADS box genes in *Arabidopsis* development. The Plant Cell. 1995;7(8):1259-1269. https://doi.org/10.1105/tpc.7.8.1259.

59. Tapia-López R, García-Ponce B, Dubrovsky JG, Garay-Arroyo A, Pérez-Ruíz RV, Kim S-H *et al*. An AGAMOUS-related MADS-box gene, XAL1 (AGL12), regulates root meristem cell proliferation and flowering transition in *Arabidopsis*. Plant Physiol. 2008;146(3):1182-1192. https://doi.org/10.1104/pp.107.108647.

60. Smaczniak C, Immink RGH, Angenent GC, Kaufmann K. Developmental and evolutionary diversity of plant MADS-domain factors: insights from recent studies. Dev. 2012;139(17):3081-3098. https://doi.org/10.1242/dev.074674.

61. Morris ER, Chevalier D, Walker JC. DAWDLE, a forkhead-associated domain gene, regulates multiple aspects of plant development. Plant Physiol. 2006;141(3):932-941. https://doi.org/10.1104/pp.106.076893.

62. Seymour G, Poole M, Manning K, King GJ. Genetics and epigenetics of fruit development and ripening. Current Opinion Plant Biol. 2008;11(1):58-63. https://doi.org/10.1016/j.pbi.2007.09.003.

63. Katz A, Oliva M, Mosquna A, Hakim O, Ohad N. FIE and CURLY LEAF polycomb proteins interact in the regulation of homeobox gene expression during sporophyte development. Plant J. 2004;37(5):707-719. https://doi.org/10.1111/j.1365-313X.2003.01996.x.

64. Zhang Y-C, He R-R, Lian J-P, Zhou Y-F, Zhang F, Li Q-F *et al*. OsmiR528 regulates rice-pollen intine formation by targeting an uclacyanin to influence flavonoid metabolism. Proc Natl Acad Sci. 2020;117(1):727-732. https://doi.org/10.1073/pnas.1810968117.

65. Khan JA, Wang Q, Sjölund RD, Schulz A, Thompson GA. An early nodulin-like protein accumulates in the sieve element plasma membrane of *Arabidopsis*. Plant Physiol. 2007;143(4):1576-1589. https://doi.org/10.1104/pp.106.092296.

66. Kim G-T, Tsukaya H, Uchimiya H. The CURLY LEAF gene controls both division and elongation of cells during the expansion of the leaf blade in *Arabidopsis thaliana*. Planta. 1998;206(2):175-183. https://doi.org/10.1007/s004250050389.

67. Zemach A, Kim MY, Hsieh P-H, Coleman-Derr D, Eshed-Williams L, Thao K *et al*. The *Arabidopsis* nucleosome remodeler DDM1 allows DNA methyltransferases to access H1-containing heterochromatin. Cell. 2013;153:193-205. https://doi.org/10.1016/j.cell.2013.02.033.

68. Park H-Y, Seok H-Y, Park B-K, Kim S-H, Goh C-H, Lee B-h *et al*. Overexpression of *Arabidopsis* ZEP enhances tolerance to osmotic stress. BioChem Biophysical Res Communications. 2008;375(1):80-85. https://doi.org/10.1016/j.bbrc.2008.07.128.

69. Tzafrir I, Pena-Muralla R, Dickerman A, Berg M, Rogers R, Hutchens S *et al*. Identification of genes required for embryo development in *Arabidopsis*. Plant Physiol. 2004;135(3):1206-1220. https://doi.org/10.1104/pp.104.045179.

70. Shen Y, Li C, McCarty DR, Meeley R, Tan B-C. Embryo defective12 encodes the plastid initiation factor 3 and is essential for embryogenesis in maize. Plant J. 2013;74(5):792-804. https://doi.org/10.1111/tpj.12161.

71. Ciavatta VT, Morillon R, Pullman GS, Chrispeels MJ, Cairney J. An aquaglyceroporin is abundantly expressed early in the development of the suspensor and the embryo proper of loblolly pine. Plant Physiol. 2001;127(4):1556-1567. https://doi.org/10.1104/pp.010793.

72. Qi Z, Xiong L. Characterization of a purine permease family gene OsPUP7 involved in growth and development control in rice. J integrative plant Biol 2013;55(11):1119-1135. https://doi.org/10.1111/jipb.12101.

73. Jung S, Ficklin SP, Lee T, Cheng CH, Blenda A, Zheng P *et al*. The genome database for Rosaceae (GDR): year 10 update. Nucleic Acids Res. 2014;42(Database issue):D1237-1244. https://doi.org/10.1093/nar/gkt1012.

74. Zhou H, Lin-Wang K, Wang H, Gu C, Dare AP, Espley RV *et al*. Molecular genetics of blood-fleshed peach reveals activation of anthocyanin biosynthesis by NAC transcription factors. Plant J. 2015;82(1):105-121. https://doi.org/10.1111/tpj.12792.

75. Connor A, Stephens M, Hall H, Alspach P. Variation and heritabilities of antioxidant activity and total phenolic content estimated from a red raspberry factorial experiment. J Amer Soc Hort Sci. 2005;130:403-411. https://doi.org/10.21273/JASHS.130.3.403.

76. Hallauer AR, Carena MJ, Filho JBM: Means and Variances. In: Quantitative Genetics in Maize Breeding. Edited by Hallauer AR, Carena MJ, Filho JBM. New York, NY: Springer; 2010: 33-67. https://doi.org/10.1007/978-1-4419-0766-0_2.

77. Holland JB, Nyquist WE, Cervantes-Martínez CT: Estimating and Interpreting Heritability for Plant Breeding: An Update. In: Plant Breeding Rev. Edited by Janick J, vol. 22: Wiley; 2003: 9-112. https://doi.org/10.1002/9780470650202.ch2.

78. Illumina Inc.: GenomeStudio Genotyping module v1.0, User Guide. In. San Diego, CA, USA.: Illumina Inc.; 2010.

79. Vanderzande S, Howard NP, Cai L, Da Silva Linge C, Antanaviciute L, Bink MCAM *et al*. High-quality, genome-wide SNP genotypic data for pedigreed germplasm of the diploid outbreeding species apple, peach, and sweet cherry through a common workflow. PLOS ONE. 2019;14(6):e0210928. https://doi.org/10.1371/journal.pone.0210928.

80. Verde I, Jenkins J, Dondini L, Micali S, Pagliarani G, Vendramin E *et al*. The Peach v2.0 release: high-resolution linkage mapping and deep resequencing improve chromosome-

scale assembly and contiguity. BMC Genomics. 2017;18:225. https://doi.org/10.1186/s12864-017-3606-9.

81. Bink MCAM, Boer MP, ter Braak CJF, Jansen J, Voorrips RE, van de Weg WE. Bayesian analysis of complex traits in pedigreed plant populations. Euphytica. 2008;161(1-2):85-96. https://doi.org/10.1007/s10681-007-9516-1.

82. Sorensen D, Gianola D: Likelihood, Bayesian, and MCMC methods in quantitative genetics. New York, NY: Springer, New York, NY; 2002. https://doi.org/10.1007/b98952.

83. Kass RE, Raftery AE. Bayes Factors. J Amer Stat Assn. 1995;90(430):773-795. https://doi.org/10.2307/2291091.

84. Voorrips RE. MapChart: Software for the graphical presentation of linkage maps and QTLs. J Hered. 2002;93(1):77-78. https://doi.org/10.1093/jhered/93.1.77.

838 **Tables and Figures**

Table 1. QTLs mapped for the bloom date (BD), ripening date (RD), and fruit development period (FDP) traits evaluated in four environments (CA11, CA12, TX12, and TX13), and the overall mean for 143 peach seedlings.

									2ln(BF)		
Trait	***MCMC***	***Records***	μ	σ^2_p	σ^2_e	σ^2_A	h^2	***LG***	***1/0***	***2/1***	***3/2***
BD-CA11	150,000	82	42.3	15.2	8.5	6.7	0.44	1	6.6	0.1	0.0
BD-CA12	250,000	138	43.8	10.5	2.2	8.3	0.79	1	11.4	2.7	0.2
								4	10.4	0.3	-0.5
								7	29.5	1.0	-0.1
BD-TX12	150,000	114	49.3	76.3	23.5	52.9	0.69	1	5.1	1.3	0.7
								4	3.9	1.0	0.4
								7	15.6	1.3	0.6
BD-TX13	150,000	124	50.2	89.3	23.5	65.7	0.74	1	14.1	-0.4	-0.3
								4	29.6	-1.3	na
BD-mean	3,600,000	143	47.0	42.6	7.6	35.1	0.82	1	13.9	5.5	-1.2
								4	4.6	-2.0	na
								7	14.6	-0.9	na
RD-CA11	100,000	104	157.4	313.9	97.6	216.3	0.69	4	28.0	3.9	0.6
RD-CA12	200,000	138	147.3	239.0	41.5	197.5	0.83	4	na	18.6	0.2
RD-TX12	100,000	94	129.2	278.8	112.6	166.1	0.60	4	29.3	0.6	-0.4
								7	2.3	0.2	na
RD-TX13	500,000	114	141.8	293.7	119.8	173.8	0.59	4	27.6	4.5	0.7
RD-mean	100,000	135	142.9	187.9	67.4	120.5	0.64	4	na	10.0	1.0
FDP-CA11	100,000	59	115.3	285.2	97.7	185.7	0.65	4	27.0	4.4	1.1
FDP-CA12	100,000	138	103.5	249.9	46.2	203.1	0.82	4	na	30.9	0.3
FDP-TX12	250,000	94	81.2	286.5	91.6	194.8	0.68	4	29.0	1.8	1.0
								6	4.5	1.3	0.0
FDP-TX13	150,000	114	91.3	321.0	105.5	215.4	0.67	4	28.2	3.6	1.0
FDP-mean	100,000	138	95.5	246.4	71.7	174.7	0.71	4	na	11.7	1.8

Bloom date, ripening date, and fruit development period in Julian days.

CA11 = Fowler, California 2011, CA12 = Fowler, California 2012, TX12 = College Station, Texas 2012, TX13 = College Station, Texas 2013.

Markov chain Monte Carlo (MCMC) run length, phenotypic mean (μ), phenotypic variance (σ^2_P), residual variance(σ^2_e), additive variance(σ^2_A), narrow-sense heritability (h^2), the linkage groups (LG) that QTLs were mapped on.

2ln(BF). Bayes Factor, a measure quantifies the support from the data for the number of QTLs in the model (QTL evidence), after pair-wise model comparison (1/0, 2/1, and 3/2) such as 'one-QTL model' vs. 'zero-QTL
839

Table 2. QTL name, linkage group, interval, mode peak, intensity, additive effect, and phenotypic variance explained (PVE) for the bloom date (BD), ripening date (RD), and fruit development period (FDP) traits evaluated in four environments (CA11, CA12, TX12, and TX13), and the overall mean for 143 peach seedlings.

QTL name	***Linkage Group***	***Interval (cM)***	***Mode peak (cM)***	***Intensity***	***Additive Effect (d)***	***PVE***
qBD1-CA11	1	[174, 182]	178	0.94	5	54
qBD1-CA12	1	[172, 180]	176	0.43	2	19
qBD1-TX12	1	[172, 182]	178	0.72	5	17
qBD1-TX13	1	[172, 182]	174	0.86	6	20
qBD1-mean	1	[172, 182]	178	0.96	5	35
qBD4-CA12	4	[70, 78]	76	0.60	2	18
qBD4-TX12	4	[48, 52]	50	0.21	4	11
qBD4-TX13	4	[48, 52]	50	0.85	10	55
qBD4-mean	4	[48, 54]	50	0.42	4	14
qBD7-CA12	7	[62, 70]	66	0.87	2	17
qBD7-TX12	7	[62, 70]	64	0.89	5	18
qBD7-mean	7	[62, 68]	66	0.91	3	11
qRD4-CA11	4	[42, 46]	44	1.40	17	46
qRD4-CA12	4	[42, 46]	45	1.80	19	75
qRD4-TX12	4	[42, 46]	44	0.85	18	54
qRD4-TX13	4	[40, 46]	44	1.21	17	52
qRD4-mean	4	[42, 46]	44	1.50	17	57
qFDP4-CA11	4	[42, 46]	45	1.10	16	42
qFDP4-CA12	4	[42, 46]	45	1.60	19	71
qFDP4-TX12	4	[46, 52]	50	0.79	18	56
qFDP4-TX13	4	[42, 46]	44	1.10	20	62
qFDP4-mean	4	[40, 46]	44	1.04	14	40

Bloom date, ripening date, and fruit development period in Julian days

CA11 = Fowler, California 2011, CA12 = Fowler, California 2012, TX12 = College Station, Texas 2012, TX13 = College Station, Texas 2013.

Posterior intensity is the accumulated probability of QTL presence in a successive series of 2 cM bins (chromosome segments) based on Bayesian analysis.

For each QTL reported, the evidence [*2ln(BF)*] is either positive (2-5), strong (5-10), or decisive (>10).

840

Table 3. QTL genotypes for bloom date (BD), ripening date (RD), and fruit development period (FDP) traits for seven breeding parents, with associated linkage groups, haplotype names, the haplotype's SNP sequences, and original sources. QTL alleles for each parent cultivar are presented with ♀ and ♂ for maternal and paternal parent sources, respectively. Parents that are heterozygous for the QTL are in bold. Allele(s) for predictive SNP marker(s) associated with *Q* or *q*-alleles for increasing or decreasing a given trait, respectively, are shown in **underscored bold**. *Q*/*q* of different effect magnitude are indicated by subscript numbers. The identity of the SNP markers and their physical and genetic location is given in Additional file 1: Table S7.

Trait/LG/Pos	Parents	QTL allele	Hap.	SNP haplotype Allele sequence	Successive ancestors (founders in bold)
BD LG1 [172.23-182.34]	Galaxy	*Q* ♀	H4	**AB**ABBBBBAAB	**Galaxy**
	Galaxy	*Q* ♂	H4	**AB**ABBBBBAAB	**Galaxy**
	Y426-371	Q_1 ♀	H1	**AB**ABBBAAAAB	**Y426-371**
	Y426-371	Q_1 ♂	H7	**BA**BBBBAAABB	**Y426-371**
	Y434-40	Q_4 ♂	H2	**AB**ABBBAAABB	**Y434-40**
	Victor	*Q* ♂	H5	**AB**BBBBABBBA	Goldprince > **F_Goldprince**
	Y435-246	Q_3 ♀	H6	**BA**ABBBBBAAB	**Y435-246**
	Y435-246	Q_2 ♂	H3	**AB**ABBBABBBA	**Y435-246**
	Y434-40	*q* ♀	H8	**BB**ABBBBB**AAA**	**Y434-40**
	Victor	*q* ♀	H8	**BB**ABBBBB**AAA**	TropicBeauty > **Fla3-2**
	TX2B136	*q* ♀	H8	**BB**ABBBBB**AAA**	**TX2B136**
	TX2B136	*q* ♂	H8	**BB**ABBBBB**AAA**	**TX2B136**
	TXW1490_1	*q* ♀	H8	**BB**ABBBBB**AAA**	TropicBeauty > **Fla3-2**
	TXW1490_1	*q* ♂	H8	**BB**ABBBBB**AAA**	**F_TXW1490_1**
BD LG4 [47.83-54.54]	TX2B136	*Q* ♀	H3	AB**A**A**A**AB**BA**ABAB	**TX2B136**
	TX2B136	*Q* ♂	H3	AB**A**A**A**AB**BA**ABAB	**TX2B136**
	TXW1490_1	*Q* ♀	H3	AB**A**A**A**AB**BA**ABAB	TropicBeauty > **Flordaprince**
	TXW1490_1	*Q* ♂	H3	AB**A**A**A**AB**BA**ABAB	**F_TXW1490_1**
	Y426-371	*Q* ♀	H3	AB**A**A**A**AB**BA**ABAB	**Y426-371**
	Victor	*Q* ♂	H3	AB**A**A**A**AB**BA**ABAB	Goldprince > **F_Goldprince**
	Y435-246	*Q* ♂	H2	B**A**BBBB**A**ABBA**B**A	**Y435-246**
	Galaxy	*Q* ♂	H2	B**A**BBBB**A**ABBA**B**A	**Galaxy**
	Y435-246	*q* ♀	H1	B**BB**BBBBABAAAA	**Y435-246**
	Y426-371	*q* ♂	H1	B**BB**BBBBABAAAA	**Y426-371**
	Galaxy	*q* ♀	H1	B**BB**BBBBABAAAA	**Galaxy**
	Y434-40	*q* ♂	H1	B**BB**BBBBABAAAA	**Y434-40**
	Y434-40	*q* ♀	H4	A**BB**BBBBABAAAA	**Y434-40**
	Victor	*q* ♀	H5	A**BB**ABABABBBAB	TropicBeauty > **Fla3-2**

Table 3. (*Cont.)*

Trait/LG	Parents	QTL allele	Hap.	SNP haplotype Allele sequence	Successive ancestors (founders in bold)
BD LG7 [62.05-68.91]	Y435-246	*Q* ♂	H3	AB**A**AABAABBABB	**Y435-246**
	Galaxy	*Q* ♀	H6	BB**A**BABBABABBA	**Galaxy**
	Victor	*Q* ♂	H6	BB**A**BABBABABBA	Goldprince > **F_Goldprince**
	TX2B136	*Q* ♀	H1	BB**A**BBAAAABAAB	**TX2B136**
	Y426-371	*Q* ♂	H2	BB**A**BBAAAABABA	**Y426-371**
	Y435-246	*Q* ♀	H2	BB**A**BBAAAABABA	**Y435-246**
	Y434-40	*Q* ♀	H2	BB**A**BBAAAABABA	**Y434-40**
	Galaxy	*q* ♂	H4	AA**B**ABBBBBABAB	**Galaxy**
	Y426-371	*q* ♀	H4	AA**B**ABBBBBABAB	**Y426-371**
	Y434-40	*q* ♂	H5	AA**B**AAAAAABABA	**Y434-40**
	Victor	*q* ♀	H7	AA**B**BBAAAABABA	TropicBeauty > **Flordaprince**
	TX2B136	*q* ♂	H7	AA**B**BBAAAABABA	**TX2B136**
	TXW1490_1	*q* ♀	H7	AA**B**BBAAAABABA	TropicBeauty > **Flordaprince**
	TXW1490_1	*q* ♂	H7	AA**B**BBAAAABABA	**F_TXW1490_1**
RD and FDP LG4 [42.33-45.19]	**Y426-371**	Q_1 ♂	H3	B**AA**A**A**A**AA**AB**A**AAA**B**	**Y426-371**
	Y434-40	Q_1 ♂	H3	B**AA**A**A**A**AA**AB**A**AAA**B**	**Y434-40**
	Galaxy	Q_1 ♀	H3	B**AA**A**A**A**AA**AB**A**AAA**B**	**Galaxy**
	Victor	Q_2 ♀	H4	A**AA**B**A**B**AA**BA**A**BBB**B**	TropicBeauty > **Fla3-2**
	TXW1490_1	Q_2 ♀	H4	A**AA**B**A**B**AA**BA**A**BBB**B**	TropicBeauty > **Fla3-2**
	Y435-246	q_1 ♀	H1	A**BB**B**B**B**BB**BA**B**BBB**A**	**Y435-246**
	Y435-246	q_1 ♂	H1	A**BB**B**B**B**BB**BA**B**BBB**A**	**Y435-246**
	Y434-40	q_1 ♀	H1	A**BB**B**B**B**BB**BA**B**BBB**A**	**Y434-40**
	Galaxy	q_1 ♂	H1	A**BB**B**B**B**BB**BA**B**BBB**A**	**Galaxy**
	Victor	q_1 ♂	H1	A**BB**B**B**B**BB**BA**B**BBB**A**	Goldprince > **F_Goldprince**
	TX2B136	q_1 ♀	H1	A**BB**B**B**B**BB**BA**B**BBB**A**	**TX2B136**
	TX2B136	q_1 ♂	H1	A**BB**B**B**B**BB**BA**B**BBB**A**	**TX2B136**
	TXW1490_1	q_1 ♂	H1	A**BB**B**B**B**BB**BA**B**BBB**A**	**F_TXW1490_1**
	Y426-371	q_2 ♀	H2	B**BB**B**B**B**BB**BA**B**BBB**A**	**Y426-371**

843

844

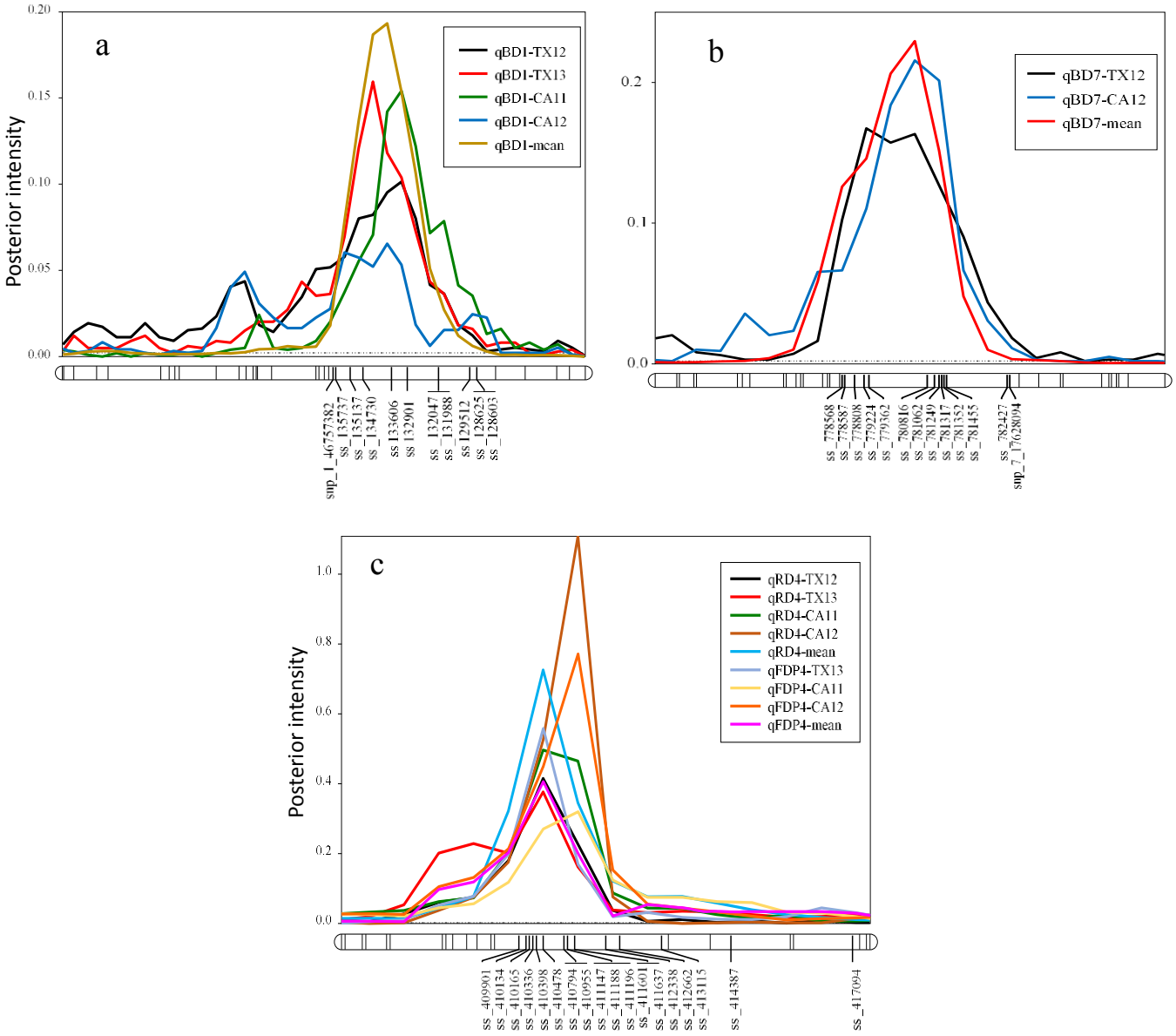

Fig. 1. Position of putative QTLs and peaks controlling the bloom date (BD) in peach at linkage group 1 (LG1) (a) and LG7 (b) and the ripening date (RD) and fruit development period (FDP) at LG4 (c) from four environments (CA11, CA12, TX12, TX13), and the overall combined mean generated using MapChart software [84].

CA11, CA12 = Fowler, California 2011 and 2012; TX12, TX13 = College Station, Texas 2012 and 2013.

845

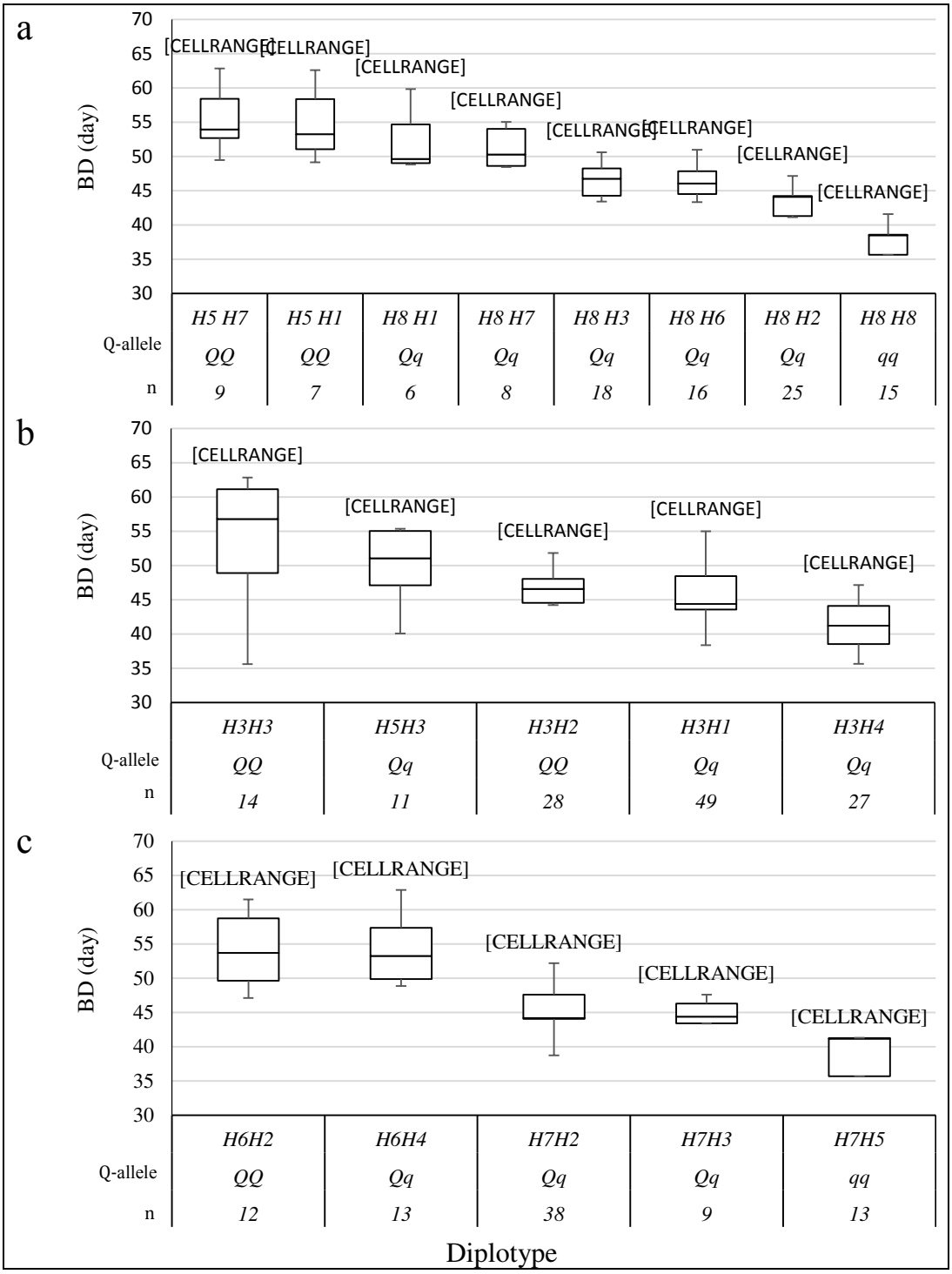

Fig. 2. Diplotype effect of the most common haplotypes associated with bloom date (BD) for the three QTLs mapped on LG1 (a), LG4 (b), and LG7 (c).
Means not connected by the same letter are significantly different ($P<0.05$) within each linkage group.
n = Diplotype sample size

849

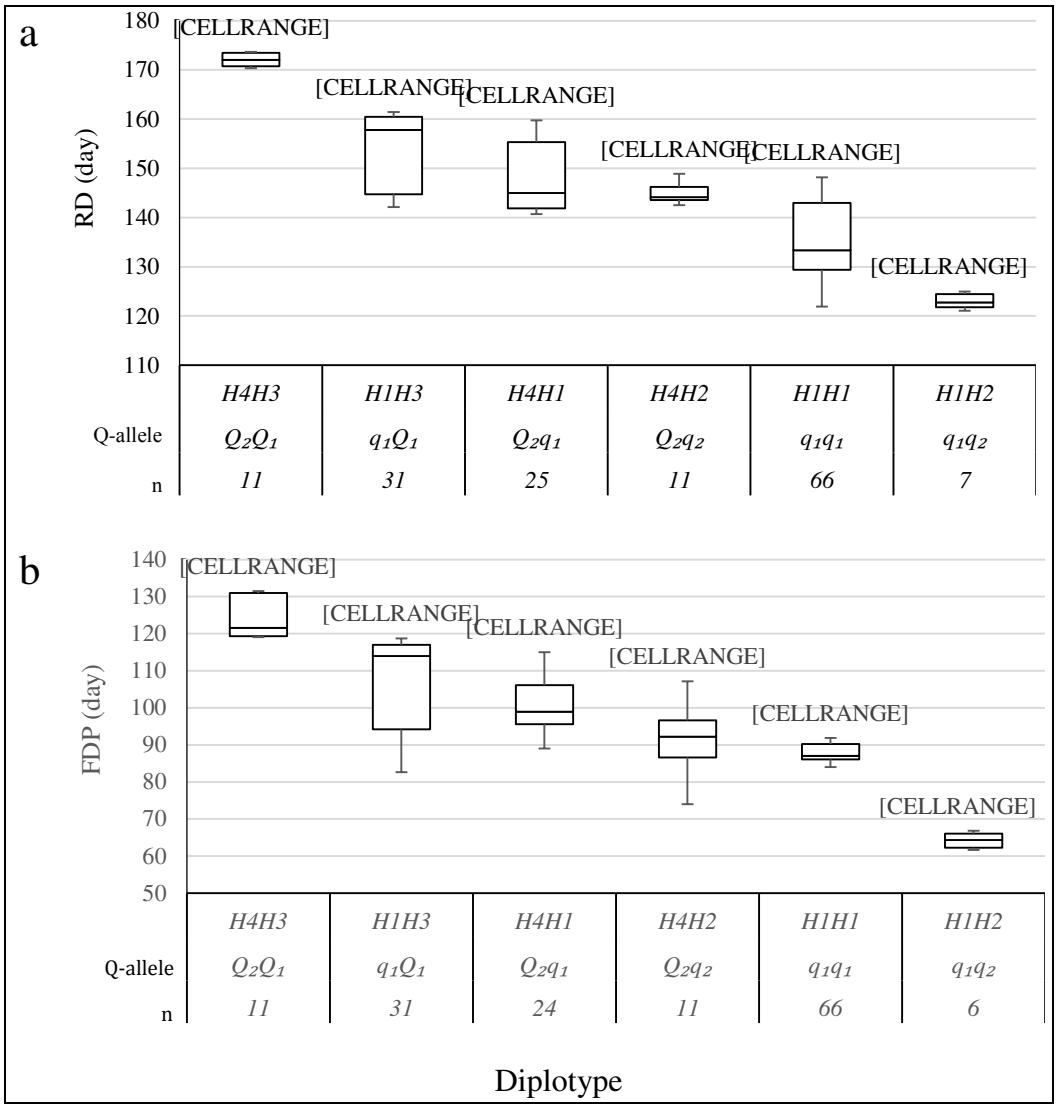

Fig. 3. Diplotype effect of the most common haplotypes associated with ripening date (RD) (a) and fruit development period (FDP) (b) for the QTLs mapped on LG4.

Means not connected by the same letter are significantly different ($P<0.05$) within each linkage group.

n = Diplotype sample size

850

851

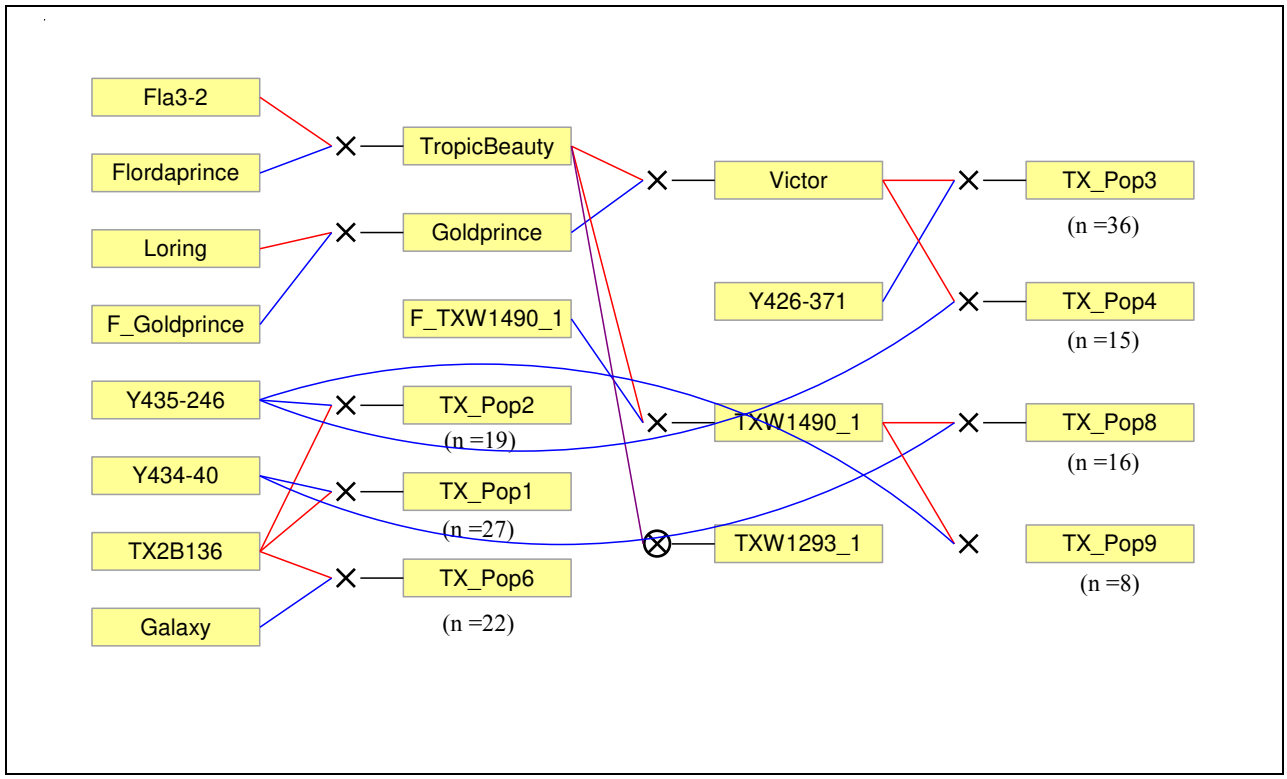

Fig. 4. Pedigree of the seven peach families and their progeny number. Red and blue lines link progeny to female and male parents, respectively.

852
853

854 **Additional files**

855 Additional file 1: Supplemental Tables S1 – S8

856 Additional file 2: Supplemental Fig. S1 – S5

Figures

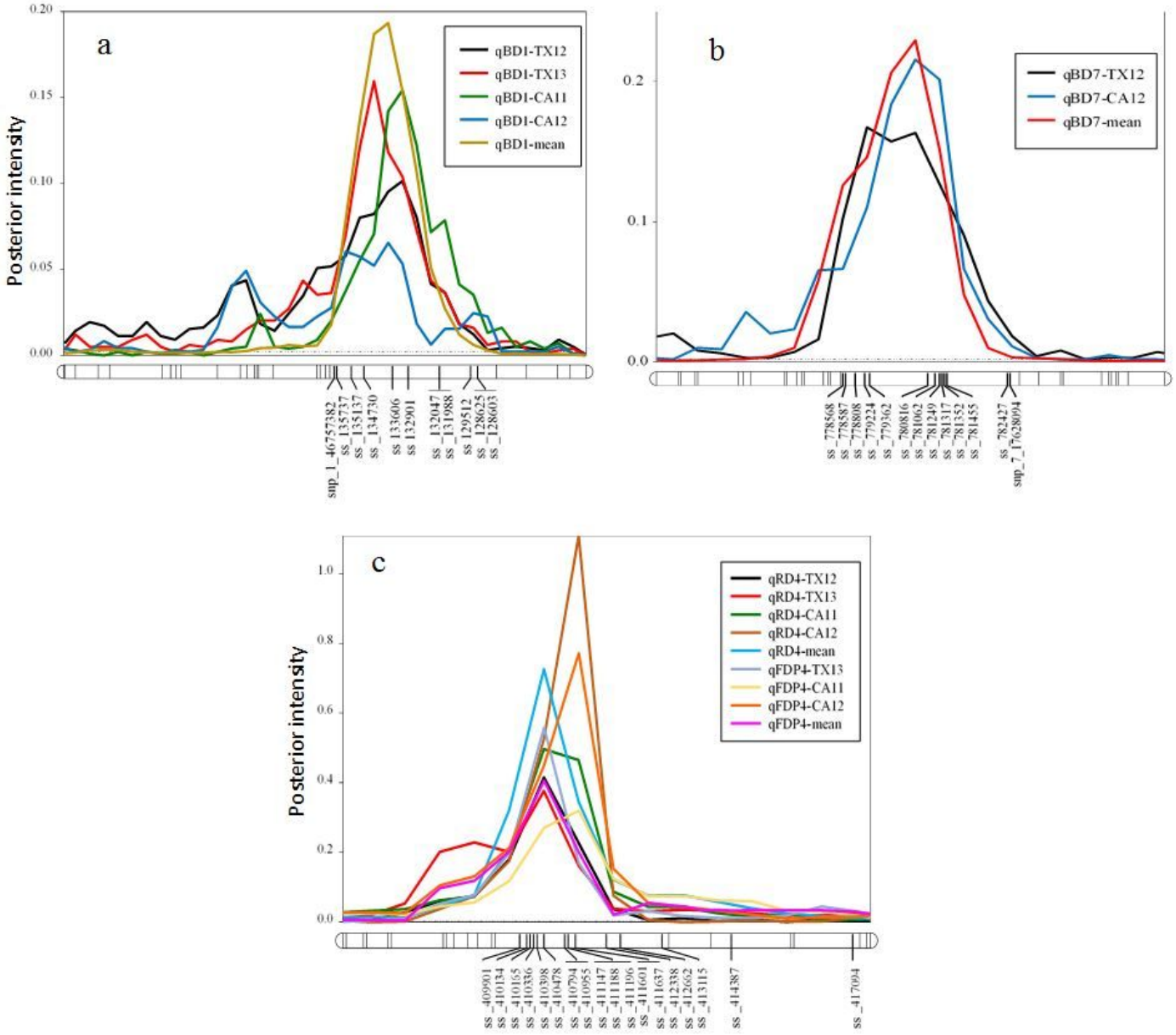

Figure 1

Position of putative QTLs and peaks controlling the bloom date (BD) in peach at linkage group 1 (LG1) (a) and LG7 (b) and the ripening date (RD) and fruit development period (FDP) at LG4 (c) from four environments (CA11, CA12, TX12, TX13), and the overall combined mean generated using MapChart software [84]. CA11, CA12 = Fowler, California 2011 and 2012; TX12, TX13 = College Station, Texas 2012 and 2013.

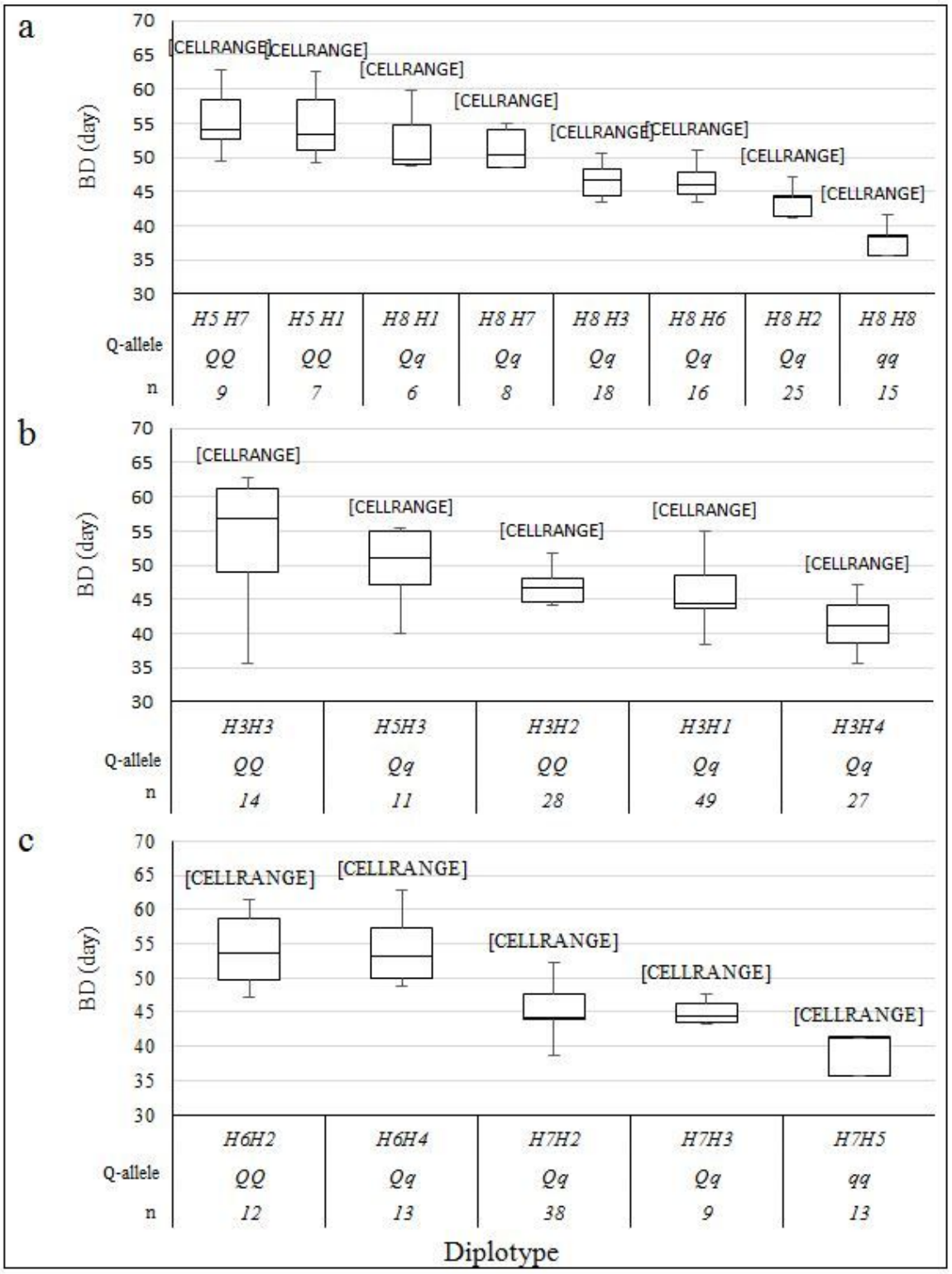

Figure 2

Diplotype effect of the most common haplotypes associated with bloom date (BD) for the three QTLs mapped on LG1 (a), LG4 (b), and LG7 (c). Means not connected by the same letter are significantly different ($P<0.05$) within each linkage group. n = Diplotype sample size

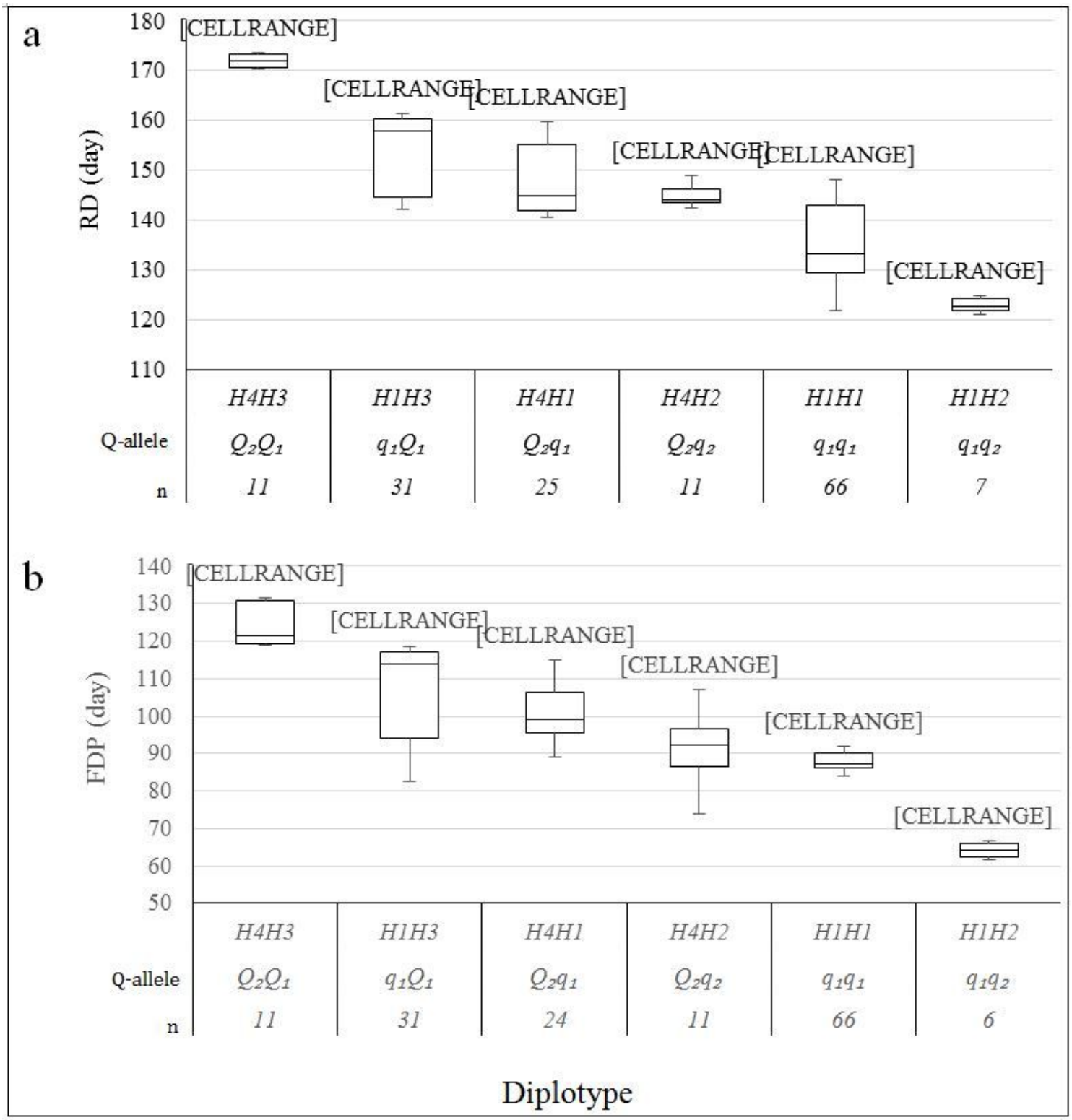

Figure 3

Diplotype effect of the most common haplotypes associated with ripening date (RD) (a) and fruit development period (FDP) (b) for the QTLs mapped on LG4. Means not connected by the same letter are significantly different ($P<0.05$) within each linkage group. n = Diplotype sample size

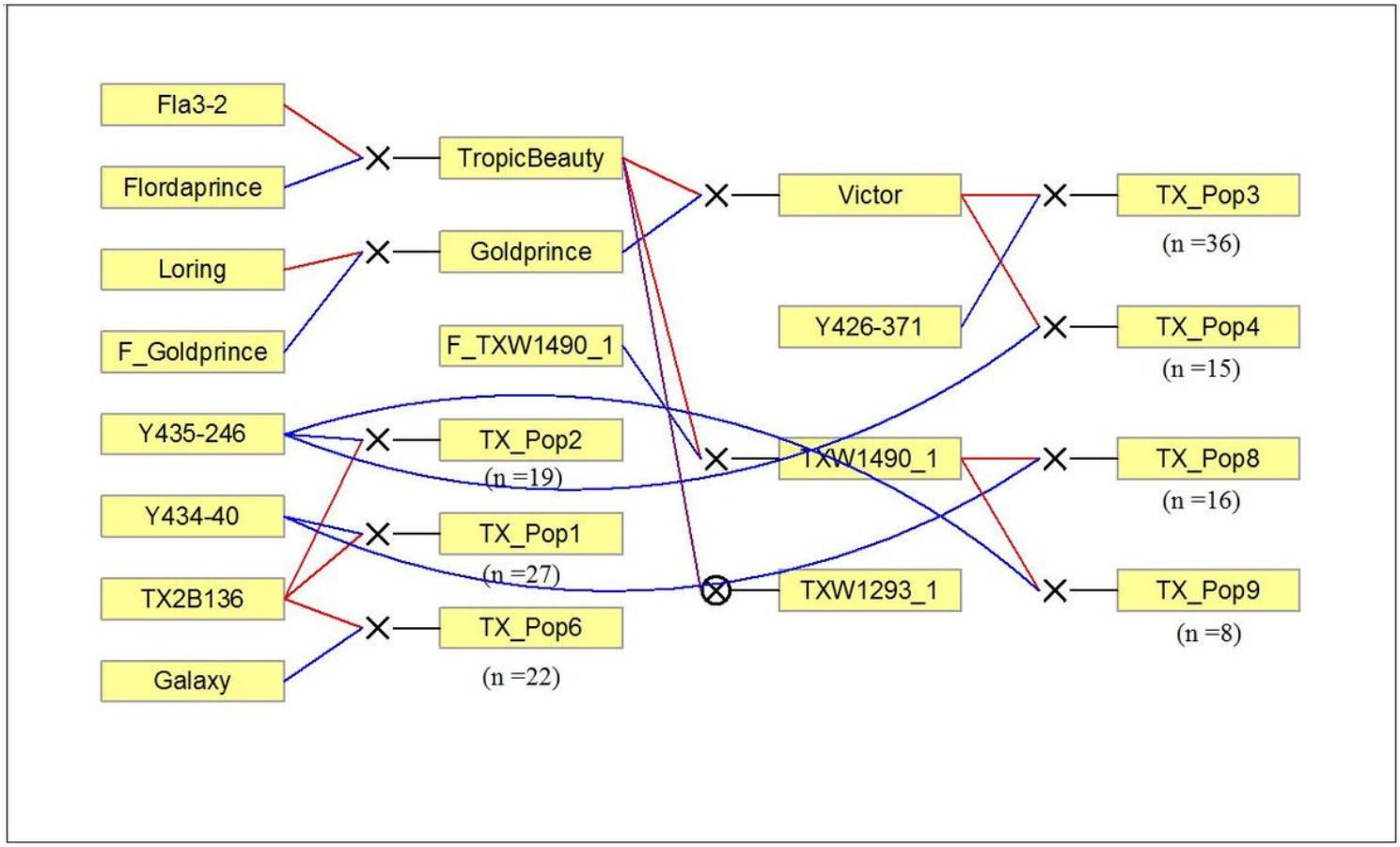

Figure 4

Pedigree of the seven peach families and their progeny number. Red and blue lines link progeny to female and male parents, respectively.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfile1.TablesS1S8.docx
- Additionalfile2.FiguresS1S5.docx