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Identification and characterization of QTLs for blush, soluble solids concentration (SSC), and titratable acidity (TA) in peach through a multi-family approach

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Abstract

Background: Fruit quality traits have a significant effect on consumer acceptance and subsequently on peach (*Prunus persica* (L.) Batsch) consumption. Determining the genetic bases of key fruit quality traits is essential for industry to improve fruit quality and increase consumption. A Bayesian approach embedded in the FlexQTL software increases the accuracy of QTL mapping and the probability of identifying new and validating known QTLs across a wide range of genetic backgrounds.

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. One major QTL for fruit blush was found on linkage group 4 (LG4) at 40–46 cM that explained from 20 to 32% of the total phenotypic variance and showed three QTL alleles of different effects. For SSC, one QTL was mapped on LG5 at 60-72cM and explained from 17 to 39% of the phenotypic variance. A major QTL for TA that co-localized with the major locus for low-acid fruit (*D*-locus) was mapped at the proximal end of LG5 and explained 35 to 80% of the phenotypic variance. The new QTL for TA on the distal end of LG5 explained 14 to 22% of the phenotypic variance. This QTL co-localized with the QTL for SSC and affected TA only when the first QTL is homozygous for high acidity (epistasis). Haplotype analyses revealed SNP haplotypes and predictive SNP marker(s) associated with desired QTL alleles.

Conclusions: A multi-family-based QTL discovery approach enhanced the ability to discover a new TA QTL and validated other QTLs which were reported in previous studies. Identified predictive SNPs and their original sources will facilitate the selection of parents and/or seedlings that have desired haplotype alleles. Our findings will help peach breeders develop new predictive, DNA-based molecular marker tests for routine use in marker-assisted breeding (MAB).

Keywords: FlexQTL, Peach QTL, haplotype, Pedigree-based Analysis, Titratable acidity, Soluble solids concentration, Blush.

Background

Peach [*Prunus persica* (L.) Batsch] is the third most important temperate fruit crop globally in terms of production [1]. Peach fruit quality traits such as flesh texture, color, sweetness, acidity, and other organoleptic attributes affect consumer preference and consumption [2]. Most of these traits are quantitatively inherited and their genetic control is still unclear [3].

In the last decade, the rate of fresh consumption has decreased from 2.3 to 1.3 kg per capita per year in the U.S. [4]. The lack of consistent quality (poor firmness, lack of flavor, low level of sweetness, and non-ripening fruit) is a main reason consumers do not purchase peaches [5]. The primary reason for poor quality is harvesting at immature stages, a lack of good postharvest handling practices, the need for high yields but not necessarily high quality to make production profitable and the relative ease for selecting for external versus internal fruit traits. Consumers are willing to pay more for fruits of better quality [6] which is the reason for developing branded fruits that consistently provide high quality fruit [7]. Although much progress was made over the last century in the improvement of fruit size, appearance, and firmness, the improvement of internal quality traits such as sugar content, antioxidant content, and tolerance to internal breakdown (IB) has lagged behind [8]. A better understanding of the inheritance of these quality traits will improve breeding efficiency and thereby accelerate the development of new cultivars with improved fruit quality [9].

The genetic map construction with Quantitative Trait Loci (QTL) analysis is vital for detecting candidate genes and predictive molecular markers associated with quality traits. In peach this work has been facilitated by its short juvenile period [10], a simple genome in terms of ploidy level (2x) and size (265 Mb), and the availability of a high-quality reference genome sequence [11]. In the last two decades, abundant genetic maps of important crops have been established including peach [10, 12, 13].

QTLs of SSC have been mapped to linkage groups (LGs) 2 – 6 [3, 14] and QTLs for organic acids have been mapped to LGs 1, 2, and 4 – 6 [14, 15]. QTLs associated with chilling injury and maturity date have been reported on multiple LGs with diverse levels of reliability [14]. For some of these QTLs, predictive molecular markers are available and used in breeding [16, 17].

Blush on the skin surface is an important trait that enhances the aesthetic appeal to consumers. In addition, the anthocyanin compounds that create the skin color may have health benefits as a source of antioxidants [18] which may in turn be an element for promoting the peach commercially [2]. Several studies have reported QTLs associated with blush on peach fruits [3, 13, 16] on LG3, LG4, LG5, and LG6. The interval on LG3 where the major QTL for blush (*Blush.Pp.ZC-3.1*) is located contains the candidate genes for skin and flesh coloration of peach (*PprMYB10*), apple (*MdMYB1/MdMYBA/ MdMYB10*), and cherry (*PavMYB10*) [19].

Peach fruits are expected to have a sweet taste, and consumer acceptance is associated with ripe soluble solids concentration (RSSC) reaching 10-12% for high acid and 15-16% for low acid cultivars [20]. Soluble solids concentration (SSC) has low to moderate heritability, which allows for enhancing sugar content even with the environmental, maturity, and production variations [12]. A major SSC QTL was consistently detected in the middle region of LG4 close to the maturity date (MD) locus in intraspecific full-sib families [3]. Minor QTLs have also been reported on LG1, 2, 3, 5, 6, 7, and 8 [14, 21].

Fruit acidity, like SSC, impacts consumer acceptance and is considered a major selection criterion in breeding [2, 22]. Low fruit acidity is associated with the major locus *D*-locus located on the proximal end of LG5 [15, 21, 22]. Several additional QTLs with minor effects have been mapped on five other LGs: LG1 and LG6 [15] and LG2, 3, and 7 [21].

Although various QTLs have been identified, only a few have been translated into diagnostic DNA tests. For example, eight DNA tests have been made available and used for several peach traits [23]. Fruit blush DNA test (Ppe-R_f-SSR) which predicts skin color accumulation is available and used for

targeting a major R_f locus on LG3 which explained up to 70% of blush [17]. A DNA test for acidity in peach (CPPCT040) is also available to target the *D* locus at LG5 [22]. Regarding SSC, no DNA test has been developed yet for this trait, even though several QTLs have been mapped [3, 14, 15, 24].

The main goals of this study are to identify new and validate previously reported QTL(s), to estimate QTL genotypes for important breeding parents, and to identify predictive single SNP or haplotype alleles for desired QTL alleles for three important fruit quality traits: SSC, titratable acidity (TA), and blush (BL) through a multi-family approach (Pedigree-Based Analysis, i.e. PBA) on Texas low to medium chill peach/nectarine germplasm. Results from this work will facilitate the design of DNA tests linked to these QTL(s) or genes to be used for MAB.

Results

Genome-wide QTL analysis

Blush

Narrow sense heritability (h^2) of blush ranged between low (0.31) for BL-CA11 to moderate (0.55) for BL-mean. Although candidate QTLs were identified on four linkage groups (LG1, 4-6) across the four environments (site × year combinations) and their overall mean, only the QTL located on LG4 passed our pre-defined inclusion threshold, showing strong to decisive evidence in each environment, except for CA11 when it did not give any signal (Table 1 and Additional file 1: Fig. S1). The proportion of phenotypic variation explained (PVE) by this QTL ranged between 20% and 32%, while the posterior QTL intensity was between 0.24–0.92, and the additive effect ranged between 0.53 and 0.63. (Table 2). Peaks for this QTL co-localized across locations and years, having their mode at 42 and 44 cM, and their interval between 40 to 46 cM corresponding with the coordinates 10,194,038 to 11,208,347 bp on the peach genome v2.0 [11] (Table 2, Additional file 1: Fig. S2, and Additional file 2: Table S1).

Soluble Solids Concentration

The narrow sense heritability of SSC ranged between low (0.29) for CA11 to moderate (0.47) for CA12 (Table 1). QTLs were identified on three LGs across three environments (except TX12) and their overall mean (Additional file 1: Fig. S3). Only the QTL located at the distal end of LG5 passed our inclusion threshold. It showed consistency across environments and in the overall mean analysis with its reliability supported by trace plot patterns. Its PVE ranged from 17 to 39% (Table 2). The highest posterior QTL intensity (0.90) was for CA12 and the lowest (0.27) was for CA11. The highest additive effect (2.32 °Brix) was associated with TX13. The peaks of the SSC QTL co-localized across locations and years, having their mode at 60 and 66 cM at the distal end of LG5, and having their interval within the 58 to 72 cM or 14,538,721 to 18,236,497 bp region (Table 2, Fig. 1, and Additional file 2: Table S1). Also, a minor QTL was mapped on LG4 in TX13 and the overall mean with positive and strong evidence, respectively.

Titratable Acidity

The narrow sense heritability of TA ranged between low (0.33) in TA-TX12 to high (0.86) in TA-CA12 (Table 1). Three candidate QTLs were detected on LG5: one to three QTLs per environment of which two passed our inclusion criteria. A QTL on the proximal end (*qTA5a*) was common to all three environments examined (TA data was not taken for the 4th environment TX13) and their overall mean (Table 2; Additional file 1: Fig. S4). A second QTL on the distal end (*qTA5b*) was environment specific detected only in CA and not in TX. Their PVE ranged from 35 to 80% (*qTAG5a*), and 14-22% (*qTAG5b;* Table 2). *qTA5a* interval ranged from 2–8 cM (557,504 – 2,028,804 bp) with a peak at 4 or 6 cM (Fig. 1 and Additional file 2: Table S1). *qTAG5b* had its interval from 58-72 cM (14,538,721 - 18,236,497 bp) and its peak at either 60 or 66 cM (Table 2 and Additional file 2: Table S1). The highest posterior QTL intensity (1.59) was associated with *qTA5a*-CA12, and the lowest intensity (0.64) with

qTA5-TX11, while the highest value of additive (0.49) and dominant effects (-0.13) were recorded for *qTA5a*-mean and *qTA5b*-mean, respectively (Table 2).

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

Blush

A total of 14 SNPs in the predicted *qBLG4* region (42.33 - 44.83 cM) (Additional file 2: Table S2) chosen for haplotyping revealed four SNP haplotypes across the seven parents in which H1 and H3 were the most prevalent and H2 was the only haplotype associated with high blush (Table 3).

The analyses on estimated diplotype effects revealed the presence of three statistically distinct phenotype classes (Fig. 2a). H1 had a greater effect on blush than H4 in the comparisons H1**H1**<>H1**H4** and **H1**H3 <> **H4**H3. Likewise, H2 had a larger effect than H1, H3 and H4 in the comparisons H4**H2**<>H4**H1,** H1**H2** <> H1**H1,** H4**H2**<>H4**H3,** H1**H2** <> H1**H3,** and H1**H2**<>**H4**H1; H3 had a larger effect than H4 (H1**H3** <> **H4**H1). Also, the effects of H1 and H3 could not be differentiated when comparing H1**H1** to H1**H3** and H4**H3** to H4**H1**. The haplotype effects can thus be ordered as H2 > H1 & H3 > H4, thus indicating the presence of three functional QTL alleles with different effects that were coined as Q_1, Q_2 and *q*, respectively.

The four haplotypes could be differentiated from each other by various pairs of SNP markers, like the two adjacent SNP markers [ss_409901 (42.33 cM, 10.5 Mb) and ss_410134 (42.51 cM, 10.6 Mb)] (Additional file 2: Table S2), where H2 has the SNP genotype *BB,* H1 *BA,* H3 *AB* and H4 *AA*.

Q_1 (H2) was found only in parent Y426-371 and some of its descendants, Q_2*'s* H1 is from F_Goldprince, F_TXW1490_1, 'Galaxy', TX2B136 and Y435-246, and Q_2*'s* H3 is from 'Galaxy', Y426-371, and Y434-40, and *q* (H4) is from the selection Fla3-2 through 'Tropic Beauty' (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 to us for genotyping.

Soluble Solids Concentration

Eight SNPs in the predictive QTL region (58.15– 72.95 cM) were chosen for haplotyping (Additional file 2: Table S2). There were six SNP haplotypes across the seven parents, of which H1 and H3 were the most prevalent (Table 3). The analyses on estimated diplotype effects identified two parents as segregating (heterozygous) for the QTL (TX2B136 and 'Galaxy') and associated three haplotypes to the *Q*-allele (H1, H2, and H6) and three to the *q*-allele (H3-H5) (Table 3). Diplotype effects analyses was consistent with a bi-allelic QTL (Fig. 2b). The *Q*-allele was associated with an increase of ~1.7 °Brix (Fig. 2) and was associated with the *AB* haplotype of the pair of adjacent SNP markers ss_600256 (14.6 Mb, 58.48cM) and ss_600509 (14.9 Mb and 59.55cM) (Additional file 2: Table S2).

Titratable Acidity

There were 12 SNP markers in the QTL region (2.23-8.12 cM) (Additional file 2: Table S2) for *qTA5a*. Five SNP haplotypes were discovered among the seven parents, in which H2 and H4 were the most prevalent. FlexQTL indicated that H2, H4, and H5 were associated with high TA, and H1 and H3 with low TA (Table 3). The observed high intensity for *qTA5a* (1.59) implies that FlexQTL actually assigned two QTLs to the *qTA5a* QTL interval. The distance between them averaged just 2.7 cM across all sampled models that included both. This distance is too short to be genetically meaningful with our current population size and might have affected FlexQTL's QTL genotype assignments. Moreover, to distinguish the individual effects of *qTA5a* and *qTA5b*, both QTLs have to be considered simultaneously, e.g. through phenotypic means of their compound genotypes. Therefore, we deviated from our previous analysis workflow by examining QTL-allele – SNP haplotype associations and haplotype effects through a compound diplotype analysis for each family separately (Table 4). The analyses were hampered by the small family sizes, and hence a very low representation of various compound diplotypes. Nevertheless *qTA5a*-H2 is clearly associated with high TA, and H1 and H3 with low TA. While less information was available for H4 and H5, their effect seemed to be similar to that of

193 H2. Two families (4 and 5) indicated that the effect of H2 was larger at double than at single dose.
194 Compound diplotypes where H2 occurred together with H4 or H5 showed higher TA values than
195 compound genotypes in which one of these three haplotypes were combined with H1 or H3. All
196 together this indicates that *qTA5a*'s H2, H4 and H5 are associated with a *Q*-allele for high acidity, and
197 that H1 and H3 are associated with a *q*-allele for low acidity. This outcome of the diplotype analyses
198 was consistent with the *Q/q* allele assignments by FlexQTL. For *qTA5b*, H6 was associated with
199 increased TA values in the presence of a double *Q*-dose at *qTA5a*. The overview on compound *qTA5a-*
200 *qTA5b* diplotypes (Table 4) could be simplified by converting qTA5s diplotypes to QTL genotypes
201 (Additional file 2: Table S3). Our results indicating an epistatic effect of *qTA5a* over *qTA5b*, however, a
202 few compound genotypes carrying both *qTA5b*-Q (H6) and *qTA5a-QQ* (H2H2, H2H4, or H2H5) did
203 not show increased TA levels (TA>1.0). This might be due to experimental variation, as the between
204 years variation of a progeny increased with increasing TA levels (Additional file 1: Fig. S5), while a
205 genetic contribution cannot be excluded. With regard to recombination, few events confined on *qTA5a*
206 whereas many recombination events occurred on *qTA5b* but with wide recombination intervals
207 resulting in many recombinant haplotypes for the later one with a frequency of 1.
208 Concerning predictive markers for *qTA5a,* each of two SNP markers can distinguish the *Q* and *q* alleles
209 (ss_544428 at 557,504 bp and ss_544495 at (610,569 bp) (Table 3, Additional file 2: Table S2). Three
210 breeding parents ('Victor', TX2B136 and TXW1490-1) were homozygous for the *Q*-allele, while the
211 remaining four parents were heterozygous. The lower TA values were in individuals with diplotypes
212 containing H1 and H3 and were present in Y435-246, Y426-371, Y434-40, and 'Galaxy'. For *qTA5b*'s,
213 QTL genotypes could be predicted by various pairs of SNP markers that includes ss_600509 combined
214 with one of the six markers ss_600072, ss_600169, ss_600230, ss_600256, ss_603047, or ss_604283).
215
216

Discussion

Blush

A high percentage of red blush on the fruit surface is desirable for the fresh market peaches and nectarines in the U.S [25]. Blush, a quantitative trait, is expressed during the final stage of fruit development and when the fruit is directly exposed to sunlight [16]. QTLs for blush have been reported on the linkage groups 2-7 [3, 13, 14, 16], indicating the polygenic nature of inheritance.

In this study, the narrow sense heritability of blush was between 0.31 to 0.52 (Table 1), thus falling between previously reported values of 0.19 [26], 0.70 [27], and 0.71 [14]. Heritability is germplasm and environment specific thus different h^2 values may be expected among studies [28].

In this study, one QTL for blush was found on LG4 between 10.2-11.2 Mb which explained 20–32% of the phenotypic variation (Table 2 and Additional file 2: Table S1). This genomic region is close to positions for major blush QTL previously reported on different peach germplasm, like the region around 11.8 Mb for a QTL with a PVE of ~69% in the family 'Venus' × 'BigTop' [13], or the 11.2-14.1 Mb region in a multi-parent population [18]. Also two minor QTLs for blush on LG4 have been reported for the 3.5-4.4 Mb and 7.5-8.8 Mb region in an F_2 family from a 'Zin Dai' × 'Crimson Lady' progeny [29] that had a major QTL on LG3 with an PVE of up to 84%. The QTL resulted from an interval mapping approach, where the minor ones were not validated through a co-factor analysis. The mapped QTL in our study could be the same as these previously reported major QTLs, whereby the variation in QTL positions could be due to the differences in genetic background, differences in mapping methods and coincidental variation in phenotypic distributions.

Examination of the relative effects of haplotypes and estimated QTL genotypes revealed for the first time a series of QTL alleles of different effect that we coined Q_1, Q_2, and q. Q_1 had the largest effect, and was present in just one parent (Y426-371), and the q allele for low blush was present in two parents and inherited in both cases from a single source **Fla3-2.** These findings underline the narrow genetic base of our germplasm for high and low blush. Q_2 had a less strong effect and was present in

each of our parents, underlining its general occurrence in our breeding program. The use of multi-parent populations for finding multiple functional alleles of different effect was also reported for two acidity QTLs/genes in apple by [30].

Our interval for *qBL4* co-localizes with major QTL for ripening date around the markers ss_410398 (10.7 Mb) [31] and ss_411147 (10.9 Mb) [32]. Also, moderate correlation between ripening date and blush have been found in this study (r=-0.42, Additional file 1: Fig. S6) as in other studies with r = -0.57 [27] and -0.24 and -0.56 [3] that may be explained by either the presence of a single QTL with pleiotropic effects or by the linkage between separate QTLs for these traits [15].

More insight in the inheritance may be gained in future through a multi-parent study in which the known major QTLs are segregating and which is of sufficient size to allow good representation of the various compound QTL genotypes.

A deviation in QTL detection on the environment level was noticed in this study, as no QTL was found in one of the three environments (CA11). We were unable to determine the reason for this behavior; however, it could be due to that data CA11was taken from 2nd leaf trees which were very vigorous and might increase shading and thereby decrease blush development [33] as less sunlight exposure of the fruit would depress the activity of the light-inducible MYB gene regulating anthocyanin biosynthesis pathway [34].

Soluble solids concentration

The narrow sense heritability (h^2) of SSC ranged from low (0.29) to moderate (0.47) which agrees with previous reports [12, 27]and with SSC being strongly influenced by multiple environmental factors including temperature, canopy position, water availability, crop load, and agricultural practices during fruit development period [33].

In this study, we mapped a QTL associated with SSC at the distal end of LG5 between ss_600072 and ss_604283 corresponding to the 14.5–18.2 Mb or 58-72cM interval, and which exhibited a PVE from

17 to 39% (Table 2 and Additional file 2: Table S1). The interval overlapped with the QTL reported [14], and might be different from a QTL reported by [24] that had its peak around the SNP markers ss_572589 and ss_585182 located at 5.8 and 9.2 Mb, respectively, with a PVE between 13 to 17%. The mapped QTL of this study also overlapped with *G*-locus for controlling fruit type (pubescence vs. glabrous) at the distal end of LG5, spanned from 15.1 to 16.3Mb on the peach genome [35]. Haplotype analysis revealed that the H6 had a greater effect than other haplotypes on increasing SSC in peaches and was inherited from TX2B136. Furthermore, two minor QTLs (SSC-TX13 and SSC-overall) were mapped on LG4 with positive and strong evidence, were located between ss_410794 and ss_414387 (43.56 – 48.43 cM, 10.8 – 12.1 Mb). Our results are in correspondence with previously reported findings [3, 14]. No QTL was detected for TX12, probably because of a low number of records in this environment and year (n=53).

Titratable Acidity

The narrow sense heritability (h^2) of TA was moderate (0.33) to high (0.86) (Table 1) which was similar to that previously reported [36]. This suggests the proportion of variation in this trait within our population is more attributed to the genetic component than the environment effects.

FlexQTL detected two QTLs associated with TA, *qTA5a* and *qTA5b.* The first QTL was at the upper part of LG5, showed recessive inheritance for high acidity and had PVEs between 35–80%, indicating this locus had a high contribution to the observed trait variation (Table 2, Fig. 1, and Additional file 2: Table S1). Our findings are consistent with literature, as *qTA5a* co-localizes with the *D*-locus for fruit acidity in peaches [22], explained 60-87 % of the phenotypic variance [15, 24, 36], and was generally considered to be dominant for low acidity [22].

Our data did not allow adequate estimation of dominance levels for the two TA QTLs as one of the three QTL genotypes was lacking in our study population (*qTA5a-qq*, and *qTA5b-QQ*). In the absence of *qTA5a-qq,* FlexQTL's dominance estimates (Table 2) are calculated under the assumption that *qq*

progenies would not have any TA. The true level of negative dominance is likely to be higher as individuals probably have some base level of TA > 0. The *qTA5a* region has been frequently associated with TA with high PVEs indicating that the *D*-locus has a major effect across a wide range of environments. From a breeding viewpoint, dominance is useful when the dominant allele is directed towards the desired trait level. A single *Q*-dose is sufficient for a relatively large effect which means less need for homozygosity, making breeding goals easier to achieve while at the same time giving flexibility to bring in other traits through the 2nd homolog. However, dominance complicates breeding when it is directed to the less desired trait level. Our breeding program aims at a range of acidity levels, which is reflected by the *qTA5a* genotypes of the seven parents: some were *QQ* (*dd*) for high acidity, others were *Qq* (*dD*) for low acidity but with the potential to raise acidic progenies, and none were *qq* (*DD*) for low acidity

The new, second QTL for TA, *qTA5b* mapped at the lower part of LG5 between ss_600072 and ss_604283 within the chromosomal positions between 14.5–18.2 Mb (Additional file 2: Table S1). It explained 14-22% of the phenotypic, was only detected in CA data sets and segregating in 'TX2B136' families (Tables 2 and 3) . CA11 had lower statistical power for the presence of the second QTL compared to CA12 which may be attributed to the low number of phenotypic data (95 vs.131 records) especially for those progenies that had H*6* (*Q*-allele) (8 vs. 14 progenies) of increasing TA. Hence, averages over years were used to reduce the experimental error and obtain more progenies with phenotypic data.

Also, the fact that this QTL was only mapped in CA could suggest that fruits were picked at a less mature stage (firmer state) which contain higher levels of TA compared to TX. The temperature could also be another factor as CA had cooler temperatures (15 °C) during fruit development compared to TX (20 °C).This QTL has not been previously reported. Further research on larger families is needed to confirm its presence and mode of action.

Parent TX2B136 as a source for SSC and TA

The only QTL for SSC discovered in this study co-localized with the *qTA5b* QTL. Both QTLs had the parent TX2B136 as the source for their *Q*-allele, and both were in coupling phase with each other. The co-localization between *qSSC5* and *qTA5b* may indicate that there is a single QTL with pleiotropic effects rather than two functionally independent but genetically linked QTLs.

Limitations of this study

The low number of FS families combined with the small family sizes resulted in the lacking/under-representation of compound QTL genotypes, hampered final conclusions on the haplotype effects of the interplay between the two TA QTLs: *qTA5a* and *qTA5b*. The other limitation lays in the lack of genotyped pedigrees for most of our parents, making progenies from different families difficult to link genetically through the identity by descent concept. This reduces the power of QTL discovery and consistent assignment of *Q/q*-alleles.

To overcome limitations of this research, a larger total population size is needed to allow larger representation of QTL genotype classes for estimating QTL effects in case of the presence of G×G interaction and/or multiple QTL alleles at a locus. Additional QTL mapping (PBA) across a wider range of breeding germplasm is also crucial to validate the QTLs of this study and those reported in literature in numerous genetic backgrounds. Such research would enhance the estimation of haplotype effects and assigned QTL genotypes along with the original sources of the desired *Q*-alleles of the traits of interest. Fine-mapping and/or the candidate gene (CG) approach should be used in future studies to develop markers useful for MAS.

Conclusions

Pedigree-Based Analysis successfully detected the location of QTLs associated with BL, SSC, and TA among low-medium chill peach/nectarine germplasm. This technique allows the use of multiple segregating full-sib families with diverse genetic background to enhance the ability to identify both

major and minor QTLs that are associated with quality traits. Our analysis detected a BL associated QTL at the central part of LG4 which agreed with previous studies [14, 16]. This genomic region was associated with ripening date in this study and supported by other research [29, 31, 32]. Also, multiple functional alleles of different effects were present in our germplasm for BL. The proximal end of LG5 was related to TA and co-localized with the major locus for low-acid fruit (*D*-locus), while the distal end of LG5 was associated with both TA and SSC. Moreover, the results from haplotype analysis revealed predominant SNP haplotypes associated with increasing or decreasing levels of each trait. We were able to identify predictive SNPs and haplotype alleles for desired QTL alleles and their original sources. The employment of these predictive SNPs to develop DNA tests will facilitate the selection of parents that have desired haplotype alleles for population development and in seedling selection to discard undesired seedlings as small plants before planting into the field.

Future studies

Our findings will help peach breeders develop new predictive DNA-based molecular marker tests by converting the trait linked SNP haplotypes to easy-to-use, (semi) high throughput markers such as simple sequence repeat (SSR), Kompetitive Allele Specific PCR (KASP), or Sequence Characterized Amplified Region (SCAR) markers for routine use in MAB for enhancing peach quality traits. Also, conducting additional pedigree-based analysis (PBA) to discover molecular markers for other fruit quality traits of interest will be useful.

Methods

Plant materials

This study included 162 seedlings from seven related F_1 families derived from seven parents descending from 12 founders (Additional file 1: Fig. S7). Parents were medium to low chill selections from the Texas A&M University breeding program, and medium- to high-chill selections from the USDA Stone Fruit Breeding Program in Parlier, CA. The number of seedlings in each family ranged

362 from 8 to 36 with an average of 20. These seedlings, along with parental genotypes, were budded onto
363 'Nemaguard' peach rootstocks and planted in College Station, TX, and Fowler, CA. Each site included
364 one replicate of each seedling and three (Fowler) to four (College Station) replicates of each parent.

365 **Plot establishment and design**

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

372 College Station is located in east central Texas with a sub-humid and warm temperate climate with
373 mild winters and warm to hot, humid summers. Fowler is located in the San Joaquin Valley in central
374 California and is ideal for peach production with a semi-arid Mediterranean climate. The minimum
375 average January temperature and the maximum average July temperature was 4.0 °C and 36.5 °C for
376 Fowler and 7.0 °C to 35.0 °C for College Station, respectively. College Station has greater rainfall than
377 Fowler (1022 versus 248 mm), higher humidity (67.5% versus 55.1%), warmer night temperatures
378 during fruit development (15.8 °C versus 12.4 °C), and more cloudy days (College Station receives
379 27% less sunlight per year) [37]. In addition, College Station is more subject to late spring freezes, low
380 chill accumulation and has a heavy textured soil. These environmental factors make College Station
381 much less suitable for stone fruit production as compared to Fowler.

382 **Phenotypic Evaluations**

383 All seedlings and parents were evaluated over two years (Fowler CA for 2011 and 2012, and College
384 Station, TX for 2012 and 2013) for blush, SSC, and TA. TA was not taken in Texas for 2013. When
385 fruits reached the physiological maturity (manually and visually assessment of firmness and

386 background skin color), samples of five fruits were placed in either paper or plastic bags and stored at
387 1-4 °C for later evaluation.
388 Subjective scales were used to evaluate fruit blush (0 - 5 scale, 0 = none, 3 = 40-60%, 5 > 90% red
389 blush on fruit surface) as described by TJ Frett [38]. For biochemical traits, a longitudinal slice of the
390 fruit, approximately 2 cm wide, was taken to extract juice with a juicer for the measurement of SSC
391 using a digital refractometer, and to measure TA using an automatic titrator (DL 22 Food and Beverage
392 analyzer, Mettler Toledo, Columbus, OH, USA). TA was obtained by the titration of 2 mL peach juice
393 to pH 8.2 with 0.1N NaOH, expressed as milliequivalents of malic acid, and calculated as:

394 $$Titrateable\ acidity\ (\%) = \frac{[NaOH\ titrated\ (ml) \times 0.1\ N\ (NaOH) \times milliequivalent\ factor \times 100}{6\ g\ of\ juice}$$

395 with 0.067 as the milliequivalent factor for malic acid [39].

396 **SNP genotyping and genetic linkage map**

397 Individuals were previously genotyped as part of the US Peach Crop Reference Set and Breeding
398 Pedigree Set [40] using the IPSC 9K SNP Array for Peach [41]. The raw iScan data was initially
399 processed into the GenomeStudio software v2010.3 [42] using the Genotyping Module with a Gen Call
400 threshold of 0.15. Parentage records and SNP data curation was performed as described before [43].
401 After filtering null alleles and non-Mendelian error conflicts across our germplasm 1,487
402 informative SNPs were retained. Their physical position on the peach genome v2.0 [11] was assessed
403 and scaled to an approximate genetic map by using a conversion factor where every 1 Mb corresponded
404 to 4 cM [43]. The markers were evenly distributed over the eight chromosomes.

405 **QTL detection**

406 Genotypic and phenotypic data for the seedlings were combined and analyzed following a Bayesian
407 approach as embedded in the FlexQTL software [44]. FlexQTL analyses were conducted on data from
408 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) [45] 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 2,500,000 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 [44]. Traits were first tested with a mixed model (allowing QTLs with additive and dominant effects).

As BL and SSC showed no dominance, they were reanalyzed with an additive model. The statistical evidence for QTLs was evaluated by twice the natural logarithm of the obtained of Bayes Factors (BF) [*2ln(BF)*] [46]; 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 greater than 5 for at least one data set, or that had a 2lnBF greater than 2 for at least two data sets with overlapping intervals of at least 2 cM and explained at least 15% of the phenotypic variation. QTL intervals were defined as a series of successive 2-cM bins with intensities corresponding to 2lnBF > 2.

Additive variance (σ_A^2) for each trait was calculated by subtracting the residual variance (σ_e^2) from the phenotypic variance (σ_P^2) and the narrow sense heritability (h^2) was calculated as follows:

$$h^2 = \frac{\sigma_A^2}{\sigma_P^2}$$

$PVE = \frac{\sigma_A^2}{\sigma_P^2} \times 100$ where:

$\sigma_A^2 = additive\ variance\ of\ QTL$ additive model

$PVE = \frac{\sigma_A^2 + \sigma_D^2}{\sigma_P^2} \times 100$ where:

$\sigma_A^2 = additive\ variance\ of\ QTL\ for\ mixed\ model$

$\sigma_D^2 = dominant\ variance\ of\ QTL\ for\ mixed\ model$

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

Our QTL nomenclature is a modification of that of Fan et al. [47]. 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 [48].

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.

Additional files

Additional file 1: Supplemental figures S1 – S7

Additional file 2: Supplemental Tables S1 – S3

Abbreviations

BF: Bayes factor; BL: Blush; 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; IB: Internal breakdown; LG: Linkage group; MAB: Marker-assisted breeding; Mb: Megabase pair; MCMC: Markov Chain Monte Carlo; MD: Maturity date; PBA: Pedigree-based analysis; PVE: Phenotypic variance explained; QTL: Quantitative trait loci; RSSC: Ripe soluble solids concentration; SNP: Single nucleotide polymorphism; SSC: Soluble solids concentration; TA: Titratable acidity.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

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

Competing interests

The authors declare that they have no competing interest.

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Authors' contributions

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

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Table 1. QTL mapped for the blush (BL), soluble solids concentration (SSC), and titratable acidity (TA) traits evaluated in different environments (CA11, CA12, TX12, TX13), and the overall combined 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***
BL-CA11	150,000	103	3.08	0.56	0.38	0.18	0.32	1	2.6	0.6	0.3
BL-CA12	150,000	138	2.79	0.60	0.29	0.31	0.52	4	13.2	1.1	0.8
								5	2.4	1.8	0.0
								6	3.9	1.0	-0.2
BL-TX12	150,000	62	3.18	0.62	0.41	0.20	0.33	4	5.7	0.9	0.8
BL-TX13	150,000	110	3.48	0.83	0.49	0.33	0.40	4	5.1	1.7	1.6
BL-mean	100,000	143	3.06	0.47	0.21	0.26	0.55	4	16.1	1.6	-0.5
								6	2.0	1.1	-0.9
SSC-CA11	100,000	105	11.87	4.94	3.52	1.42	0.29	5	2.6	0.9	na
SSC-CA12	100,000	137	11.61	3.35	1.79	1.56	0.47	5	13.8	4.0	1.3
SSC-TX13	100,000	111	12.84	6.63	4.59	2.04	0.31	4	2.3	0.4	0.8
								5	9.6	1.0	0.1
SSC-mean	100,000	137	11.90	2.46	1.43	1.03	0.42	4	6.1	0.3	-2.0
								5	11.8	0.9	-0.5
TA-CA11	100,000	95	0.78	0.14	0.03	0.11	0.79	5	7.6	4.2	2.1
TA-CA12	2,500,000	131	0.71	0.14	0.02	0.12	0.86	5	11.8	6.0	5.4
TA-TX12	150,000	43	0.55	0.06	0.04	0.02	0.33	5	5.9	0.1	-0.6
TA-mean	500,000	137	0.72	0.13	0.03	0.10	0.77	5	na	6.8	5.6

Blush = blush visually based on % coverage of red blush on skin using 0-5 scale (0 = 0% red coverage, 1 = 1%-20%, 2 = 21%-50%, 3 = 51%-80%, 4 = 81%-99%, 5 = 100%); SSC = soluble solids concentration in ºBrix; TA = titratable acidity %.

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, and the QTL evidence [*2ln(BF)*] which is hardly any (0-2); positive (2-5); strong (5-10); and decisive (>10).

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Table 2. QTL name, linkage group, interval, mode peak, intensity, additive effect, dominant effect, and phenotypic variance explained (PVE) for the blush (BL), soluble solids concentration (SSC), and titratable acidity (TA) traits evaluated in four environments (CA11, CA12, TX12, TX13), and the overall combined mean for 143 peach seedlings.

QTL name	***Linkage Group***	***Interval (cM)***	***Mode peak (cM)***	***Intensity***	***Additive Effect***	***Dominant Effect***	***PVE (%)***
qBL4-CA12	4	[42, 46]	44	0.92	0.63	-	32
qBL4-TX12	4	[42, 46]	44	0.24	0.62	-	31
qBL4-TX13	4	[40, 46]	42	0.43	0.57	-	20
qBL4-mean	4	[42, 46]	44	0.85	0.53	-	30
qSSC5-CA11	5	[58, 72]	66	0.27	1.31	-	17
qSSC5-CA12	5	[60, 72]	66	0.90	1.27	-	22
qSSC5-TX13	5	[58, 72]	60	0.91	2.32	-	38
qSSC5-mean	5	[58, 72]	66	0.91	1.42	-	39
qTA5a-CA11	5	[2, 8]	6	0.64	0.33	-0.10	35
qTA5a-CA12	5	[2, 8]	6	1.16	0.47	-0.02	74
qTA5b-CA12	5	[58, 72]	66	0.68	0.26	-0.10	22
qTA5-TX12	5	[2, 8]	4	0.66	0.32	-	72
qTA5a-mean	5	[4, 8]	6	0.90	0.49	-0.04	80
qTA5b-mean	5	[58, 72]	60	0.70	0.25	-0.13	14

Blush = blush visually based on % coverage of red blush on skin using 0-5 scale (0 = 0% red coverage, 1 = 1%-20%, 2 = 21%-50%, 3 = 51%-80%, 4 = 81%-99%, 5 = 100%); SSC = soluble solids concentration in ºBrix; TA = titratable acidity %.

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

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

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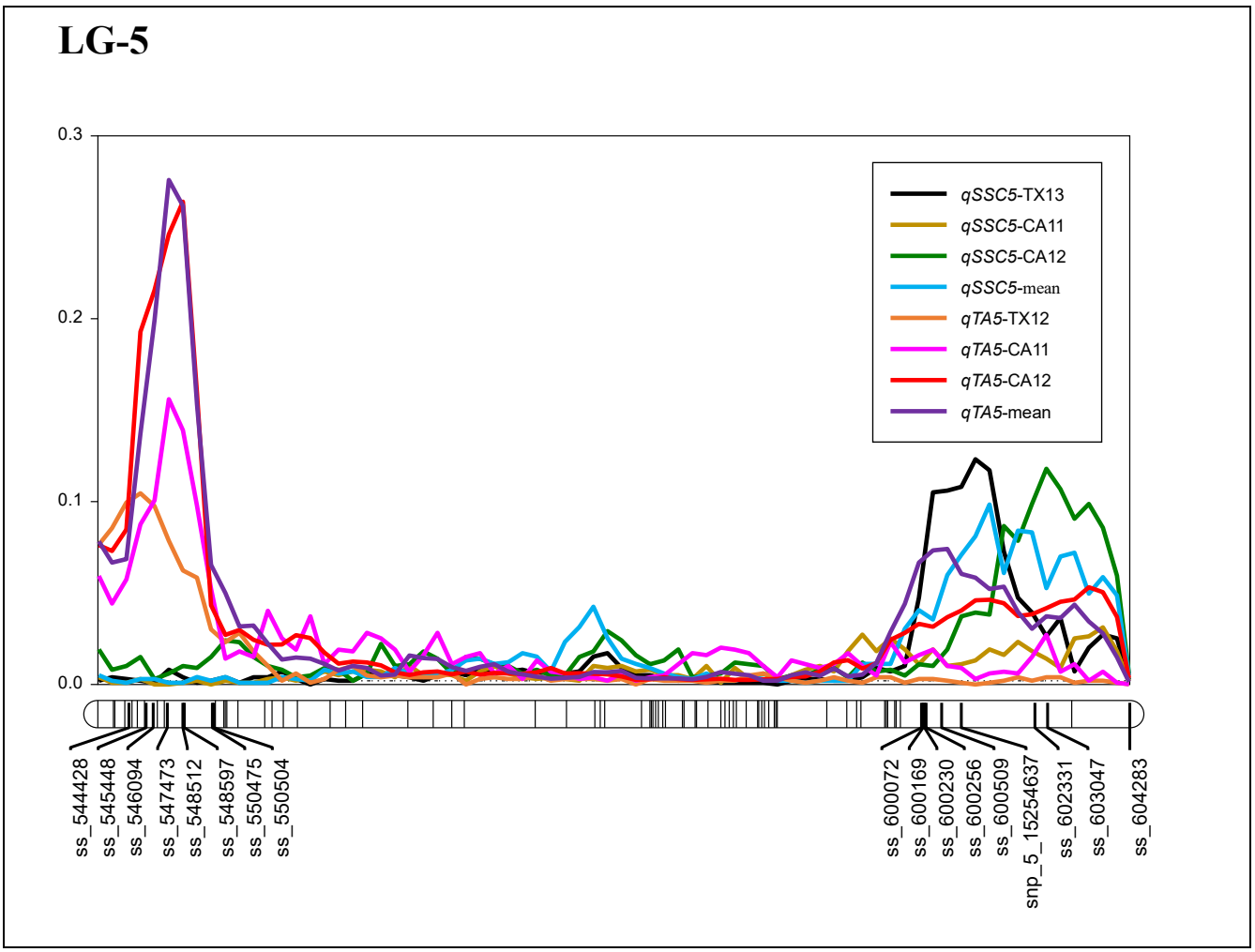

Fig. 1. The position of putative QTLs and peaks (large bold font) controlling the soluble solids concentration (SSC), and titratable acidity (TA) for LG5 in peach from four environments (CA11, CA12, TX12, TX13), and the overall combined mean generated using MapChart software [49].

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

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Table 3. QTL genotypes for blush (BL), soluble solids concentration (SSC), and titratable acidity (TA) for seven important peach breeding parents, with associated linkage groups, haplotype names, the haplotype's SNP sequences, and origin 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**. The identity of the SNP markers and their physical and genetic location is given in Additional file 2: Table S2.

Trait	Parents	QTL allele	Hap.	SNP haplotype	Successive ancestors
				Allele sequence	(founders in bold)
BL4	**Y426-371**	Q_1 ♀	H2	**BB**BBBBBBBABBBB	**Y426-371**
	Y426-371	Q_2 ♂	H3	BAAAAAAAA**B**AAAA	**Y426-371**
	Y434-40	Q_2 ♂	H3	BAAAAAAAA**B**AAAA	**Y434-40**
	Galaxy	Q_2 ♀	H3	BAAAAAAAA**B**AAAA	**Galaxy**
	Y435-246	Q_2 ♀	H1	**A**BBBBBBBBABBBB	**Y435-246**
	Y435-246	Q_2 ♂	H1	**A**BBBBBBBBABBBB	**Y435-246**
	Y434-40	Q_2 ♀	H1	**A**BBBBBBBBABBBB	**Y434-40**
	Galaxy	Q_2 ♂	H1	**A**BBBBBBBBABBBB	**Galaxy**
	Victor	q ♀	H4	**A**AABABAABAABBB	TropicBeauty > **Fla3-2**
	Victor	Q_2 ♂	H1	**A**BBBBBBBBABBBB	Goldprince > **F_Goldprince**
	TX2B136	Q_2 ♀	H1	**A**BBBBBBBBABBBB	**TX2B136**
	TX2B136	Q_2 ♂	H1	**A**BBBBBBBBABBBB	**TX2B136**
	TXW1490_1	q ♀	H4	**A**AABABAABAABBB	TropicBeauty > **Fla3-2**
	TXW1490_1	Q_2 ♂	H1	**A**BBBBBBBBABBBB	**F_TXW1490_1**
SSC5	**TX2B136**	Q ♂	H6	AAAB**AB**BB	**TX2B136**
	Y435-246	Q ♀	H1	BBBA**AB**BB	**Y435-246**
	Y426-371	Q ♀	H1	BBBA**AB**BB	**Y426-371**
	Y426-371	Q ♂	H1	BBBA**AB**BB	**Y426-371**
	Y434-40	Q ♀	H1	BBBA**AB**BB	**Y434-40**
	Y434-40	Q ♂	H1	BBBA**AB**BB	**Y434-40**
	Galaxy	Q ♂	H1	BBBA**AB**BB	**Galaxy**
	Y435-246	Q ♂	H2	BBBA**AB**BA	**Y435-246**
	Victor	q ♀	H3	AAABBBAB	TropicBeauty > **Fla3-2**
	TX2B136	q ♀	H3	AAABBBAB	**TX2B136**
	TXW1490_1	q ♀	H3	AAABBBAB	TropicBeauty > **Fla3-2**
	TXW1490_1	q ♂	H3	AAABBBAB	**F_TXW1490_1**
	Galaxy	q ♀	H4	AAABBBBA	**Galaxy**
	Victor	q ♂	H5	BBBAAABA	Goldprince > **F_Goldprince**

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Table 3. (*Cont.)*

Trait	Parents	QTL allele	Hap.	SNP haplotype Allele sequence	Successive ancestors (founders in bold)
TA5a	**Y435-246**	*Q* ♂	H2	**AB**BBBAABBBBB	**Y435-246**
	Y426-371	*Q* ♂	H2	**AB**BBBAABBBBB	**Y426-371**
	Y434-40	*Q* ♂	H2	**AB**BBBAABBBBB	**Y434-40**
	Galaxy	*Q* ♂	H2	**AB**BBBAABBBBB	**Galaxy**
	Victor	*Q* ♂	H2	**AB**BBBAABBBBB	Goldprince > **F_Goldprince**
	Victor	*Q* ♀	H4	**AB**BBBAABBBBA	TropicBeauty > **Flordaprince**
	TX2B136	*Q* ♂	H5	**AB**ABBABBABAB	**TX2B136**
	TX2B136	*Q* ♀	H4	**AB**BBBAABBBBA	**TX2B136**
	TXW1490_1	*Q* ♀	H4	**AB**BBBAABBBBA	TropicBeauty > **Flordaprince**
	TXW1490_1	*Q* ♂	H4	**AB**BBBAABBBBA	**F_TXW1490_1**
	Y435-246	*q* ♀	H1	**BA**AAABBAAAAB	**Y435-246**
	Y426-371	*q* ♀	H1	**BA**AAABBAAAAB	**Y426-371**
	Y434-40	*q* ♀	H3	**BA**ABBAABBBBB	**Y434-40**
	Galaxy	*q* ♀	H1	**BA**AAABBAAAAB	**Galaxy**
TA5b	**TX2B136**	*Q* ♂	H6	**AAABA**B**BB**	**TX2B136**
	TX2B136	*q* ♀	H3	AAABBBAB	**TX2B136**
	Victor	*q* ♀	H3	AAABBBAB	TropicBeauty > **Fla3-2**
	TXW1490_1	*q* ♀	H3	AAABBBAB	TropicBeauty > **Fla3-2**
	TXW1490_1	*q* ♂	H3	AAABBBAB	**F_TXW1490_1**
	Galaxy	*q* ♀	H4	AAABBBBA	**Galaxy**
	Victor	*q* ♂	H5	BBBAAABA	Goldprince > **F_Goldprince**
	Galaxy	*q* ♂	H1	BBBAABBB	**Galaxy**
	Y435-246	*q* ♀	H1	BBBAABBB	**Y435-246**
	Y435-246	*q* ♂	H2	BBBAABBA	**Y435-246**
	Y426-371	*q* ♀	H1	BBBAABBB	**Y426-371**
	Y426-371	*q* ♂	H1	BBBAABBB	**Y426-371**
	Y434-40	*q* ♀	H1	BBBAABBB	**Y434-40**
	Y434-40	*q* ♂	H1	BBBAABBB	**Y434-40**

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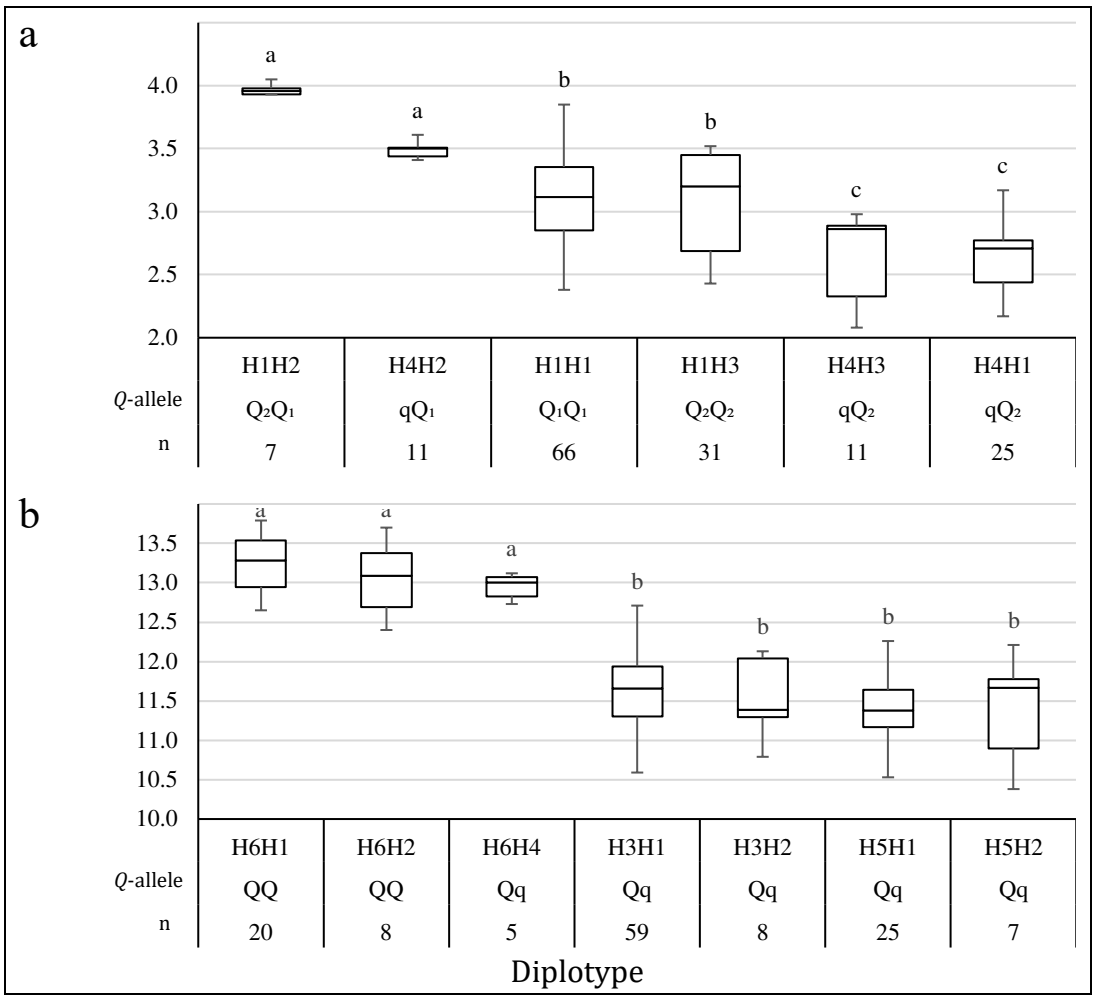

Fig. 2. Diplotype effect of the most common haplotypes associated with fruit blush (BL) (a) and soluble solids concentration (SSC) (b) QTLs mapped on peach LG4 and LG5, respectively.

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

n = Diplotype sample size

Table 4. Analysis of compound QTL (*qTA5a* and *qTA5b*) diplotypes in seven full-sib peach families for their average titratable acidity (TA) content from the two environments CA11 and CA12. Haplotypes that seemed to be associated with a *Q*-allele for increased TA are in bold. Underlined TA-values are deviating from the proposed genetic model in which *qTA5a* shows recessive inheritance and where expression of *qTA5b* requires *qTA5a* to be-*QQ*.

FS-Family			*qTA5a*									
			TA					***Individual count***				
		Diplotype	H3**H4**	H**3H5**	**H2H4**	**H2H5**	***Total***	H3**H4**	H3**H5**	**H2H4**	**H2H5**	***Total***
TX2B136 × Y434-40	*qTA5b*	H1H3	0.42	-	**0.75**	**0.98**	0.71	6	-	4	2	12
		H1H6	0.58	0.60	**1.35**	**1.45**	0.99	2	4	1	5	12
		Total	**0.50**	**0.60**	**1.05**	**1.21**	**0.85**	**8**	**4**	**5**	**7**	**24**
		Conclusions: 1) qTA5a: Effect H2 > H3, H4 ≡ H5										
		2) qTA5b: Effect H6 > H3*, Effective only in the presence of qTA5a*-H2										
			H1**H4**	H1**H5**	**H2H4**	**H2H5**	***Total***	H1**H4**	H1**H5**	**H2H4**	**H2H5**	***Total***
TX2B136 × Y435-246	*qTA5b*	H1H3	0.45	0.40	**0.70**	**-**	0.52	2	1	2	-	5
		H2H3	0.30	0.50	**-**	**1.05**	0.62	1	1	-	1	3
		H2**H6**	0.53	0.20	**0.95**	**-**	0.56	3	1	1	-	5
		Total	**0.43**	**0.37**	**0.83**	**1.05**	**0.56**	**6**	**3**	**3**	**1**	**13**
		Conclusions: 1) qTA5a: Effect H2 > H1, H4 ≡ H5										
		2) qTA5b: None, too few pairwise comparisons										
			H1**H2**	H1**H4**	**H2H2**	**H2H4**	***Total***	H1**H2**	H1**H4**	**H2H2**	**H2H4**	***Total***
Victor × Y426-371	*qTA5b*	H1H3	0.38	0.35	**0.90**	**0.77**	0.60	3	3	1	3	10
		H1H5	0.43	0.30	**1.08**	**0.97**	0.69	7	2	2	8	19
		Total	**0.41**	**0.33**	**0.99**	**0.87**	**0.65**	**10**	**5**	**3**	**11**	**29**
		Conclusions: 1) qTA5a: Effect H2 > H1, H2 ≡ H4, H2 at single dose has no effect										
		2) qTA5b: H5 possibly slightly > H3 in some genetic backgrounds										
			H1**H2**	H1**H4**	**H2H2**	**H2H4**	***Total***	H1**H2**	H1**H4**	**H2H2**	**H2H4**	***Total***
Victor × Y435-246	*qTA5b*	H1H3	-	0.35	**0.80**	**-**	0.58	-	1	1	-	2
		H1H5	0.35	0.55	**-**	**-**	0.45	1	1	-	-	2
		H2H3	-	-	**-**	**0.85**		-	-	-	2	2
		H2H5	0.40	0.30	**0.85**	**-**	0.52	1	1	4	-	6
		Total	**0.38**	**0.40**	**0.83**	**0.85**	**0.51**	**2**	**3**	**5**	**2**	**12**
		Conclusions: 1) qTA5a: Effect H2 > H1, H2 at single dose has no effect										
		2) qTA5b: None, too few pairwise comparisons										

Table 4. *Cont.*

FS-Family			*qTA5a*									
			TA					***Individual count***				
		Diplotype	H1**H4**	H1**H5**	**H2H4**	**H2H5**	***Total***	H1**H4**	H1**H5**	**H2H4**	**H2H5**	***Total***
TX2B136 × Galaxy	*qTA5b*	H1H3	0.30	0.35	**0.98**	**-**	0.54	1	1	2	-	4
		H1**H6**	0.50	**1.05**	**-**	**1.63**	1.06	1	1	-	3	5
		H4**H6**	0.33	-	**1.00**		0.67	3	-	2		5
		Total	**0.38**	**0.70**	**0.99**	**1.63**	**0.76**	**5**	**2**	**4**	**3**	**14**
		Conclusions: 1) qTA5a: Effect H2 > H1, *2) qTA5b:* None, too few pairwise comparisons H6 > H3										
			H3**H4**		**H2H4**		***Total***	H3**H4**		**H2H4**		***Total***
TXW1490_1 × Y434-40	*qTA5b*	H1H3	0.39		1.03		0.71	4		11		15
		Total	**0.39**		**1.03**		**0.71**	**4**		**11**		**15**
		Conclusion: qTA5a: Effect H2 > H3										
			H1**H4**		**H2H4**		***Total***	H1**H4**		**H2H4**		***Total***
TXW1490_1 × Y435-246	*qTA5b*	H1H3	0.33		0.75		0.54	2		3		5
		H1**H6**	0.70		0.88		0.79	1		2		3
		Total	**0.52**		**0.82**		**0.67**	**1**		**2**		**8**
		Conclusion: None, too few data										

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