

Genetic dissection of fruit weight and size in an F₂ peach (*Prunus persica* (L.) Batsch) progeny

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Abstract Fruit weight is a quantitative trait influenced by the combined action of several genes and environmental factors. Knowledge of the quantitative trait loci (QTLs) associated with fruit weight and size is a priority to support breeding programmes in peach (*Prunus persica* (L.) Batsch) because of commercial interest in larger fruits. To this end, we built a genetic map of an F₂ progeny of 117 individuals from the cross PI91459 ('NJ Weeping') × 'Bounty' using a single nucleotide polymorphism (SNP) genotyping array for peach (9K SNP array v1). Data for fruit weight, height,

width, and depth were recorded for the progeny and both parents over 2 years (2011, 2012). Correlations between the traits fruit weight and size were positive and significant for both years. A SNP map was constructed comprising 1,148 markers distributed over eight linkage groups. The map spans 536.6 cM with an average distance between markers of 0.52 cM, covering 93.6 % of the physical length of the peach genome, thus representing an ideal basis for QTL mapping. QTL analysis led to the identification of a total of 28 QTLs for the considered traits, eleven of which remained stable in both years. We also observed clusters of QTLs, some of which were mapped for the first time, while others correspond to loci previously identified in different progenies and following different approaches.

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Introduction

Fruit weight and size are quantitative traits resulting from the combined action of several genes and environmental factors such as plant health, weather conditions (e.g. temperature fluctuations) and cultural practices (fruit load per tree), soil moisture, and plant nutrition. From a commercial point of view, these

traits are important for market success, since consumers are naturally driven to aesthetically homogeneous fruits of large size without physiological and pathological alterations (Infante et al. 2008).

In peach, fruit growth is represented by a double sigmoid curve with distinct stages: stage I, characterised by cell division; stage II, with limited growth and lignification of the pit (endocarp); stage III, with cellular expansion; and stage IV, full ripening (Chalmers and van den Ende 1975; El-Sharkawy et al. 2007; Lombardo et al. 2011). In stage I, fruit growth occurs rapidly due to cell division (Marini 2002; Liu et al. 2006). This stage is very sensitive to water deficit, and towards the end of this phase, the fruit has reached about 20–25 % of its final size (Girona et al. 2012). The duration of stage II depends on the cultivar, ranging from a few days for early cultivars to weeks for late cultivars (Marini 2002; El-Sharkawy et al. 2007; Lombardo et al. 2011; Dardick et al. 2010). In stage III, the fruit grows due to cell expansion in the last weeks (or months) before harvest, accumulating water, sugars, organic acids, and mineral elements in the cell vacuole (Marini 2002, Coombe 1976). In stage IV, the fruit reaches the final full size (Lombardo et al. 2011).

Studies on peach, apple, and sweet cherry to evaluate fruit growth in the different stages showed that the final weight and size of the ripe fruit depends on the number and size of mesocarp cells and intercellular spaces (Yamaguchi et al. 2002; Harada et al. 2005; Olmstead et al. 2007).

Fruit weight and size are highly correlated and inherited as complex traits (Zhang et al. 2010; Pereira Silva et al. 2013). Studies to identify quantitative trait loci (QTLs) associated with these traits have been carried out in peach and several other species. In peach, QTLs associated with fruit weight were mapped by Dirlewanger et al. (1999) who identified a QTL in linkage group (LG) 6, linked to the fruit flat shape trait (*S*), by assessing an F₂ cross from the commercial cultivars ‘Ferjalou Jalousia’ × ‘Fantasia’. Using an F₁ progeny derived from the commercial cultivars ‘Bolero’ × ‘OroA’, fruit weight was shown to be highly correlated with maturity date and other traits such as juice soluble solids and acidity (Eduardo et al. 2011). Analysing an F₂ progeny from the cross between the commercial cultivars ‘Contender’ × ‘Ambra’, a stable QTL, was mapped on LG4, which explained approximately 22.5 and 31.5 %

of the observed phenotypic variation in the first and second year of the analysis, respectively, another stable QTL mapped on LG6 explained 11.8 and 17.6 % for the 2 years of analysis. A significant correlation between FW and maturity date was also observed in this progeny (Eduardo et al. 2011). Yamamoto et al. (2001) detected three QTLs on LG3 and one QTL on LG6 using an F₂ population from an intraspecific cross between rootstock peach ‘Akame’ and flower ornamental cultivar ‘Juseiton’.

In sweet cherry, Zhang et al. (2010) detected three QTLs associated with fruit size and one QTL for the number of mesocarp cells. In apple, Devoghlaere et al. (2012) found six QTLs for fruit weight using the ‘Royal Gala’ × ‘Braeburn’ and ‘Starkrimson’ × ‘Granny Smith’ genetic maps. In other species, similar studies were carried out, e.g. 14 QTLs associated with fruit weight and shape were identified in papaya, with phenotypic effects ranging from 5 to 23 % (Blas et al. 2012). Four QTLs were associated with fruit weight in cucumber (Yuan et al. 2008). In tomato, a total of 28 loci have been associated with variation in fruit weight and size (Grandillo et al. 1999; Paran and van der Knaap 2007; Zhang et al. 2012).

In peach, an important limitation of these previous studies was that the number and the locations of the genes or QTLs which controlled the traits were imprecise and showed low resolution. The low density of linkage maps and the use of diverse markers, such as RFLPs and AFLPs, made it difficult to integrate and accurately compare data from these studies (Martínez-García et al. 2012). However, the *Prunus* reference map (Dirlewanger et al. 2004) and the recent publication of the peach genome sequence (International Peach Genome Initiative 2013) provide a new basis for comparative approaches allowing alignment of different genetic maps to the reference sequence. In addition, a new SNP genotyping platform, i.e. 9K SNP array v1, has been developed for peach (<http://www.rosaceae.org/peach/genome>; Verde et al. 2012) resulting in dense linkage maps and greater precision in QTL mapping while also enabling high-resolution mapping and candidate gene studies (Yang et al. 2012; Eduardo et al. 2013; Pirona et al. 2013; Frett et al. 2014; Romeu et al. 2014).

The aims of the present study were (i) to construct a linkage map using the SNP genotyping array for peach (9K SNP array v1) and (ii) to identify QTLs associated

with fruit weight and size, using an F₂ progeny from a cross of two highly contrasting accessions for these traits.

Materials and methods

Plant Material and DNA extraction

An F₂ progeny of 117 individuals (WB) was obtained from self-pollination of an F₁ seedling from the cross ‘NJ Weeping (PI91459)’ × ‘Bounty’. ‘NJ Weeping’ is a late ripening ornamental peach with small fruits of white flesh colour, probably of Japanese origin, introduced from the USA. ‘Bounty’ is a medium season peach with large yellow flesh fruits bred in the USA.

Individuals of the F₂ progeny were located in the experimental field in Faenza (RA) in Emilia Romagna region (lower Po valley, northern Italy). The trees were planted with spacing of 1 m within and 4 m between rows. The progeny segregates for maturity date (Pirona et al. 2013), fruit weight and size, flesh texture and colour, peduncle length, flower type and colour, and tree habit. In the present study, only the data concerning fruit weight and size were evaluated.

DNA was extracted from young leaves, using the DNeasy Plant Mini Kit (Qiagen) according to the manufacturer’s instructions. For DNA quantification, Quant-iT Picogreen (Invitrogen) was used, and the DNA concentration of each sample was estimated based on a standard concentration curve. The final concentrations of all DNA samples were adjusted to 50 ng/μl.

Analysis of phenotypic characteristics

Pruning was performed yearly, and standard cultural practices were applied. In order to obtain fruits showing maximum phenotypic expression, in spring 2011 and 2012, the trees were heavily thinned before pit hardening to no more than one to three fruits for each fruiting branch, according to their vigour. At the beginning of physiological ripening (analysed in the field based on visual background skin colour and manual evaluation of firmness), the 10 largest fruits from each tree were harvested, and the three heaviest were chosen. The scope of the heavy thinning and the choice of the three heaviest fruits only for data

collection favoured the expression of the effective phenotypic potential of FW. Fruit height, width, and depth were measured with a calliper. FH is the distance between the tip and the stem base of the fruit, FWD is the distance between the two sides (cheeks) of the fruit, and FD is the distance between the suture and the opposite side at the equatorial zone (Supplementary Figure 1).

In order to estimate the broad-sense heritability (h^2), we collected a total of 30 fruits from three trees (10 fruits/tree) in the parents NJ Weeping and Bounty. In the F₁ hybrid that originated the WB F₂ progeny, we also collected 30 fruits from two trees (15 fruits/tree), and FW and size were measured. The variance of the parents and F₁ hybrid was calculated considering all 30 fruits collected. The variance of the 117 WB F₂ progeny was calculated considering the means of the three largest fruits collected for each plant.

The Genes software (Cruz 2013) was used for estimating the broad-sense heritability and variance components: phenotypic (σ_p^2), environmental (σ_E^2), and genotypic variance (σ_G^2), where phenotypic variance $\sigma_p^2 = \sigma_{F_2}^2$; environment variance $\sigma_E^2 = 1/4 [\sigma_{P_1}^2 + 2\sigma_{F_1}^2 + \sigma_{P_2}^2]$; genotypic variance $\sigma_G^2 = \sigma_p^2 - \sigma_E^2$; with $\sigma_{P_1}^2$ being the variance of the first parent, $\sigma_{P_2}^2$ the variance of the second parent, $\sigma_{F_1}^2$ the variance of F₁, and $\sigma_{F_2}^2$ the variance of F₂. Broad-sense heritability (h^2) for the traits FW, FH, FWD, and FD was estimated by the model proposed by Allard (1960): $h^2 = \sigma_G^2 / \sigma_p^2$.

A variance analysis (ANOVA) was performed in order to detect significant differences between the parents. A total of 30 fruits from three tree (10/tree) were collected in the parents NJ Weeping and Bounty. We measured the traits FW, FH, FWD, and FD, and the means of the three tree in each parent were used in the analysis.

To evaluate whether the data followed a normal distribution, a normality analysis by Shapiro–Wilk test was performed with the PAST statistic package (Hammer et al. 2001), which was also used to construct histograms of frequency distribution and to perform correlation analyses.

Genotyping

The 117 F₂ plants were genotyped by means of the 9K SNP array v1 (Illumina Peach Infinium Chip),

developed by the International Peach SNP Consortium (IPSC), and including a total of 8,144 SNPs distributed over the eight peach chromosomes (Verde et al. 2012). Genotyping was performed at IASMA Research and Innovation Centre (San Michele all'Adige, Italy) following the manufacturer's recommendations as described by Verde et al. (2012).

SNP data were scored using GenomeStudio Data Analysis software (Illumina Inc.) with a GenCall threshold of 0.15 and SNPs showing severe segregation distortion (χ^2 test, $p < 10^{-6}$), and >1 % missing data were excluded. Similarly to the study by Eduardo et al. (2013), SNPs were excluded when genTrain scores were below 0.6.

Construction of the genetic map

The WB genetic map was built using JoinMap v.4.0 (Van Ooijen 2006), considering the markers segregating in the F_2 progeny. The recombination threshold value was set to 0.40. The Kosambi mapping function was used to convert recombination frequencies into map distances. Linkage groups were defined using a minimum logarithm of odds (LOD) value of 10.0. SNP markers that showed segregation distortion were not removed from the analysis because the method used for the construction of linkage groups adopted by the JoinMap software is based on the independence of the LOD score which is not affected by segregation distortion (Van Ooijen 2006; Alheit et al. 2011). The WB map was checked for double recombination events using the function "genotype probabilities" in JoinMap v.4.0 (Van Ooijen 2006).

Comparison of the WB linkage map with the peach genome sequence v1.0

Similarly to Yang et al. (2012) and Frett et al. (2014), positions of markers in the WB linkage map were aligned with their position on the peach genome sequence v1 (International Peach Genome Initiative 2013) using MapChart 2.2 (Voorrips 2002).

Comparison of the WB map with the ZC^2 linkage map

Positions of markers in the WB linkage map were aligned with their position on the ZC^2 linkage map by Frett et al. (2014), using MapChart 2.2 (Voorrips 2002).

Identification of QTLs

The MapQTL v 6.0 software (Van Ooijen 2009) was used for detecting QTLs based on the WB linkage map. Significance thresholds were calculated by random permutation test (PT) with 10,000 replicates considering the genome-wide LOD scores corresponding to $p = 0.05$. Then, interval mapping (IM) and multiple QTL mapping (MQM) were employed. The detection of QTLs was performed via IM with 95 % significance ($p < 0.05$) to identify QTLs with significant main effects. Subsequently, the module MQM was used applying the option "automatic cofactor selection" (ACS) in order to test cofactor markers around the putative QTL positions. The percentage of variation explained by each QTL and the total variance explained by all QTLs affecting a trait were obtained in the MQM model. Similarly to Mahbubul Alam et al. (2014) and Pacheco et al. (2014), we consider a QTL as "stable" for a specific trait, if it exhibited a significant effect and is located in overlapping positions between the 2 years of analysis (2011 and 2012). The MapChart 2.1 software (Voorrips 2002) was used to draw the mapped QTLs and the LOD plots on LGs.

Following the recommendations for Standard QTL Nomenclature and Reporting in the Rosaceae (Recommendations for Standard QTL Nomenclature and Reporting in the Rosaceae 2014), the QTL nomenclature reflects:

$$qP - TcL.1^m$$

where q = quantitative trait; P = Prunoideae, followed by a dash; Tc = trait code (Fw, Fh, Fwd, and Fd); L = *Prunus persica* linkage group; 1 = first chronological QTL for this trait reported on this chromosome; and m = number of years that a QTL was mapped.

Results

Phenotypic traits

In order to genetically dissect FW and size, we developed an F_2 progeny of 117 seedlings from a cross between PI91459 (NJ Weeping), an ornamental white peach introduced from Rutgers University (New Jersey, USA) with small fruits, and 'Bounty', a yellow

peach characterised by large fruits (Bassi and Rizzo 2002).

The results of the phenotypic evaluations in 2011 and 2012 are summarised in Supplementary Table 1. The parents exhibit significantly different values for fruit weight (FW), height (FH), width (FWD), and depth (FD). NJ Weeping is characterised by low values of fruit weight and dimensions, while Bounty fruits, in 2011, were approximately 3.8× heavier and 1.5× larger. In 2012, Bounty fruits were approximately 5.5× heavier and 2× larger than fruits of the NJ Weeping.

In the WB F₂ progeny, all traits follow a normal distribution according to Shapiro–Wilk test (Fig. 1; Supplementary Table 1). Transgressive segregation was observed in 2011 for FH, FWD, and FD with values higher than those observed in Bounty (Fig. 1). Transgressive segregation was also observed in 2012 for some F₂ individuals showing FW, FWD, and FD values lower than NJ Weeping (Fig. 1).

In the WB F₂ progeny, average fruit size values of the F₂ progeny were significantly lower in 2012 compared to 2011 (Supplementary Table 1). All correlations between traits were positive and significant for the 2 years (Supplementary Table 2), where a very high correlation was found between FWD and FD. The traits related to fruit size were correlated with FW in both years, demonstrating that heavier fruits have higher values of FH, FWD, and FD, as expected. Concerning the correlation between years, FH showed the highest correlation coefficient (0.737) and FWD the lowest (0.634). FW showed a correlation coefficient of 0.673.

The evaluated traits showed heritability above 80 %, indicating a high contribution of genetic variance to phenotypic variation (data not shown). The highest heritability was found for FD (93.3 %) and FH (90.7 %) in 2011 and 2012, respectively.

Construction of a highly saturated genetic map for the WB F₂ progeny

The parents and 117 individuals of the WB F₂ progeny were genotyped with the 9K SNP peach array v1, with a total of 8,144 SNPs distributed over the eight peach chromosomes (Verde et al. 2012). After SNP filtering and eliminating missing data (876 markers), markers with GenTrain score lower than 0.6 (415 markers), monomorphic markers (4,363 markers), elimination of

SNPs that showed high recombination frequencies (larger than 0.6) classified as “suspect linkages” by the JoinMap software (Van Ooijen 2006) and checked for double recombination patterns, a total of 1,148 SNPs were retained (Supplementary Figure 2). The final 1,148 SNPs were distributed in eight linkage groups (LGs 1–8), which correspond to the eight chromosomes of the peach genome v1.0 (Verde et al. 2012), with a total map distance of 536.6 cM and an average distance between markers of 0.52 cM (Supplementary Figure 2).

In almost all LGs, the total number of markers was above 100, with the exception of LG2, which showed a total number of 51. The highest numbers of markers were found on LG1 and LG4, with 246 and 170 markers, respectively (Table 1). The size of LGs ranged from 57.9 cM of LG2 to 107.3 cM of LG1 (Table 1). The maximum genetic distance between pairs of adjacent markers was 6.879 cM on LG2 (between SNP_2_22274363 and SNP_IGA_286418 located at 22,274,363 and 24,588,955 on scaffold 2). Large gaps were not observed, indicating that the map is highly saturated, thus providing an ideal basis for QTL detection.

Comparison of the WB map with the peach genome sequence v1.0

Although overall marker order was in agreement with the peach genome reference (International Peach Genome Initiative 2013), inversions in the marker order were observed in LGs 1, 4, 7, and 8, when compared to the peach genome sequence v1.0 (Fig. 2; Table 1). In addition, SNP markers Pp20Cl, SNP_IGA_435668, SNP_IGA_435720 putatively located on scaffold 4 and SNP_IGA_919869 located on scaffold 15 were mapped on LG1. Other SNPs putatively located on scaffold 10 (SNP_IGA_897342), scaffold 4 (SNP_IGA_435008, SNP_IGA_431437, Pp12Cl, SNP_IGA_428047, SNP_IGA_429127), and scaffold 9 (SNP_IGA_894039) of the peach genome were mapped on LG3. Finally, the SNPs SNP_IGA_335205 and SNP_IGA_335123 putatively located on scaffold 3 were mapped on LG7. Positions of markers which showed inverse order in the WB map compared to the peach genome sequence v1.0 and markers that were putatively located in one scaffold, but mapped in other LGs, are in agreement with the proposed reassembly of the peach genome (International Peach Genome Initiative 2013).

Fig. 1 Frequency distribution of FW, FH, FWD, and FD in the F₂ progeny (WB)

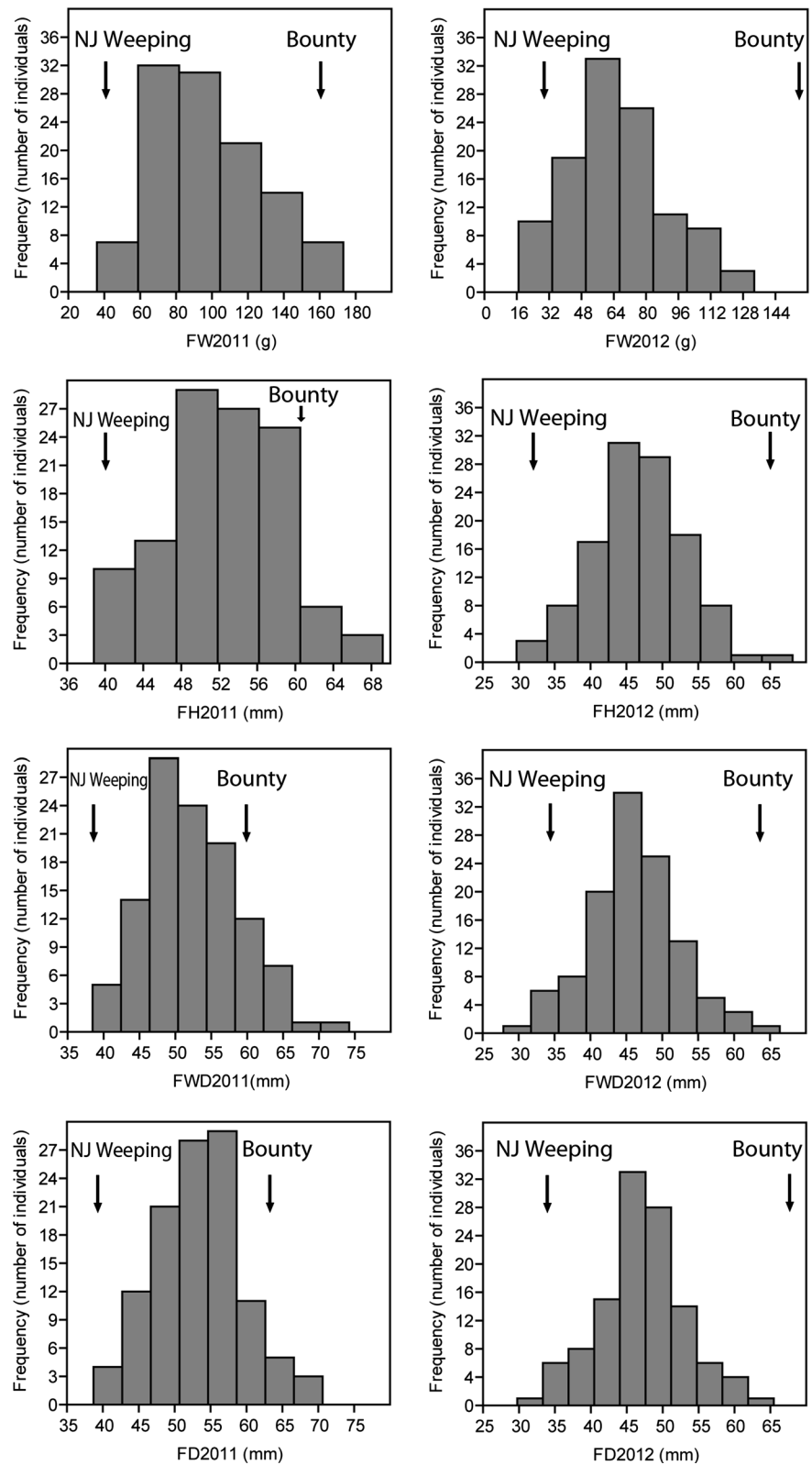


Table 1 Comparison of the WB map with the peach genome sequence v1.0

Group	WB map					Marker density		Average coverage kb/cM
	SNPs markers	Markers inverted	Genetic distance	Physical length	Physical coverage	cM	Kb	
LG1	246	24	107.3	46.75	0.99	0.436	190.04	435.69
LG2	51	–	57.9	25.19	0.94	1.135	493.92	435.06
LG3	156	–	62.8	21.1	0.96	0.403	135.26	335.99
LG4	170	7	61.9	27.75	0.92	0.364	163.24	448.30
LG5	112	–	62.4	16.41	0.90	0.557	146.52	262.98
LG6	139	–	58.6	26.39	0.92	0.422	189.86	450.34
LG7	143	18	65.3	20.55	0.90	0.457	143.71	314.70
LG8	131	5	60.4	19.91	0.96	0.461	151.98	329.64

The map covers approximately 93.6 % of the peach genome v 1.0 physical length. Scaffold 1 showed the largest coverage (99.0 %), while the scaffold 5 and 7 demonstrated the lowest coverage (90.0 %). The values estimated of the average coverage per marker on the scaffolds ranged from approximately 1/262 kb on LG1 to 1/450 kb on LG6.

Comparison of the WB map with ZC² map

The WB map was also compared to the ZC² map (Frett et al. 2014) by common markers and by order of markers between the maps (Supplementary Figure 3). The ZC² map has a total of 190 markers and covers a genetic distance of approximately 452.51 cM with an average marker spacing of 2.38 cM/marker, while the WB map has a genetic distance of 536.6 cM and an average distance between markers of 0.52 cM. Comparing the two maps, we observed 17 common markers in LG1, 9 common markers in LG3, and 6, 2, 3, 4, and 13 common markers in LGs 4, 5, 6, 7, and 8, respectively, which directly allowed the alignment with the ZC² map. In LG2, no common markers were observed between the two maps.

QTL analyses for fruit weight and dimensions

A total of 1,148 SNPs were used for constructing a WB map. For QTL analysis, we used a total of 877 SNPs markers, omitting 271 markers that co-mapped in the same loci and would increase the computation time substantially.

The critical LOD values (thresholds) obtained by permutation test varied from 3.5 to 3.6 (Table 2) for all

traits in both years (2011 and 2012). Similar to Eduardo et al. (2013) and Pacheco et al. (2014), we also considered as stable a FW QTL with a LOD score close to the threshold 3.6. In our study, one QTL (qP-Fw1.2² on LG1) was significant in 2011, with a LOD score of 7.46, and showed a LOD score of 3.26 close to the threshold 3.6 in 2012.

In total, 65 significant associations were mapped for FW and size (Table 2; Supplementary figure 4) in the 2 years analysed. Some of these associations had overlapping confidence intervals in the 2 years of the analysis or were mapped for more than one trait. These associations were counted only once, reducing the number of 65 significant associations to 28 (Supplementary Table 3). Eleven QTLs were stable (Fig. 3) across the 2 years (QTLs that showed overlapping confidence intervals over the 2 years were considered as stable), and 17 QTLs were mapped only in 1 year. Out of the eleven stable QTLs, one main QTL associated with the marker SNP_IGA_776826 explained the largest fraction of phenotypic variance for FW, FH, FWD, and FD.

QTLs for individual traits

Analysing individually the QTLs mapped for each trait, eight QTLs were mapped for FW, accounting for 62.7 % of the phenotypic variation in 2011.

In 2012, nine QTLs for FW were mapped, explaining 57.1 % of the observed phenotypic variation.

The QTLs qP-Fw1.1², qP-Fw1.2², qP-Fw2.1², qP-Fw3.1², qP-Fw5.1², qP-Fw6.1², and qP-Fw7.1² were stable in both years. The main QTL qP-Fw7.1² (LG7)

Fig. 2 Alignment of the WB linkage map and the peach genome sequence v1.0. Peach genome scaffolds and WB linkage groups are shown on the *left* and *right* of each pair, respectively

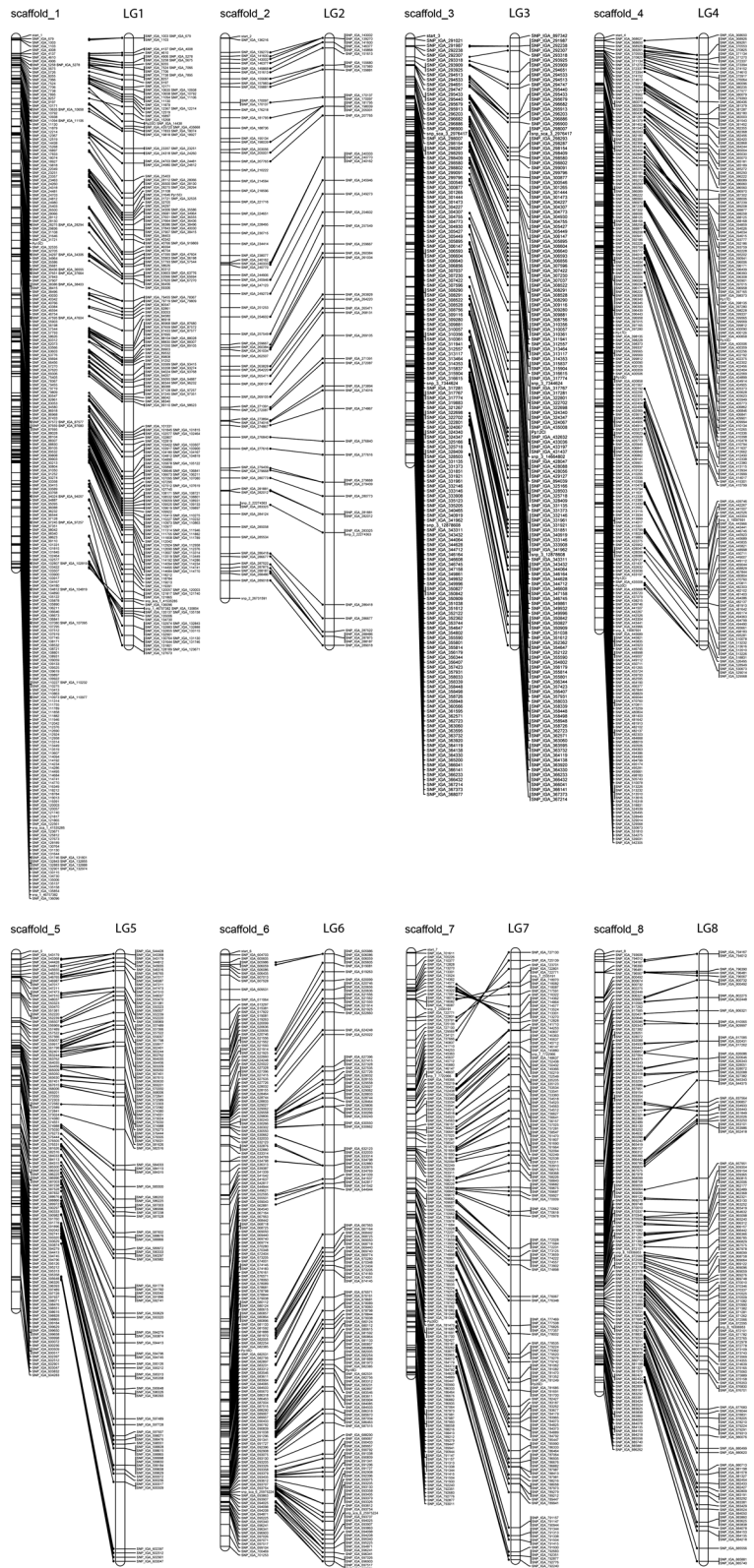


Table 2 Mapped QTLs in WB F₂ progeny by multiple QTL mapping; trait, permutation test, QTL name, year analysed, linkage group (LG), start and final position of the QTL in WB map (cM), cofactor marker, genomic position of cofactor marker, LOD and variations explained (R²) by the QTLs which control fruit weight and size in the WB F₂ progeny in 2011 and 2012

Trait	Permutation test		Multiple QTL mapping							LOD	R ²
	Genome-wide threshold for $p > 0.05$	2011 = 3.6 2012 = 3.6	QTL ^a	Year	LG	Start (cM)	Final (cM)	Cofactor marker	Genomic position ^b		
Fruit weight (FW)			qP-Fw1.1 ²	2011	1	30.86	30.86	SNP_IGA_32535	11,810,777	7.18	7.6
			qP-Fw1.1 ²	2012	1	30.86	30.86	SNP_IGA_32535	11,810,777	8.32	7.6
			qP-Fw1.2 ²	2011	1	100.783	100.783	SNP_IGA_135854	46,726,253	7.46	7.9
			qP-Fw1.2 ²	2012	1	100.783	100.783	SNP_IGA_135854 ^c	46,726,253	3.26	2.7
			qP-Fw2.1 ²	2011	2	5.024	6.518	SNP_IGA_205001	5,472,142	4.29	4.3
			qP-Fw2.1 ²	2012	2	6.518	6.518	SNP_IGA_205001	5,472,142	4.46	3.8
			qP-Fw2.1 ²	2012	2	5.024	5.656	SNP_IGA_170137	3,084,510	5.53	4.8
			qP-Fw3.1 ²	2011	3	50.223	50.653	SNP_IGA_356179	16,952,454	3.8	3.3
			qP-Fw3.1 ²	2012	3	50.223	50.223	SNP_IGA_355590	16,843,422	9.55	9
			qP-Fw4.1 ¹	2011	4	34.771	36.2	SNP_IGA_410336	10,665,019	4.81	4.8
			qP-Fw4.2 ¹	2012	4	11.235	11.664	SNP_IGA_385272	3,790,399	4.37	3.7
			qP-Fw4.3 ¹	2012	4	30.34	30.34	SNP_IGA_406846	9,889,664	3.85	3.2
			qP-Fw5.1 ²	2011	5	11.675	12.973	SNP_IGA_574551	6,287,541	4.82	4.8
			qP-Fw5.1 ²	2012	5	9.491	11.675	SNP_IGA_572303	5,696,058	4.5	3.7
Fruit height (FH)			qP-Fw6.1 ²	2011	6	28.76	29.189	SNP_IGA_669440	18,315,130	9.29	10.3
			qP-Fw6.1 ²	2012	6	28.76	28.76	SNP_IGA_669050	18,228,936	6.8	6
			qP-Fw7.1 ²	2011	7	40.147	40.147	SNP_IGA_776826	15,293,347	15.5	19.7
			qP-Fw7.1 ²	2012	7	40.147	40.147	SNP_IGA_776826	15,293,347	12.52	12.6
			qP-Fh1.1 ¹	2011	1	99.055	100.484	SNP_IGA_136096	46,846,766	10.58	10.8
			qP-Fh1.3 ¹	2012	1	97.756	98.756	SNP_IGA_121865	40,845,002	7.91	8.9
			qP-Fh1.2 ²	2011	1	28.254	30.561	SNP_IGA_31108	11,662,916	5.1	4.6
			qP-Fh1.2 ²	2012	1	29.561	30.561	SNP_IGA_31108	11,662,916	3.91	4
			qP-Fh2.1 ²	2011	2	11.39	11.39	SNP_IGA_240162	10,707,212	12.65	13.5
			qP-Fh2.1 ²	2012	2	11.39	11.39	SNP_IGA_240162	10,707,212	7.07	7.8
			qP-Fh3.1 ²	2011	3	37.821	38.561	SNP_IGA_341962	12,836,182	3.71	3.3
			qP-Fh3.1 ²	2012	3	36.821	38.561	SNP_IGA_341962	12,836,182	7.16	7.9
			qP-Fh4.1 ¹	2011	4	58.04	59.339	SNP_IGA_513616	26,221,760	4.03	3.6
			qP-Fh7.1 ²	2011	7	40.147	40.147	SNP_IGA_776826	15,293,347	19.48	24.3
		qP-Fh7.1 ²	2012	7	40.147	40.147	SNP_IGA_776826	15,293,347	16.09	21.6	

Table 2 continued

Trait	Permutation test		Multiple QTL mapping				Final (cM)	Cofactor marker	Genomic position ^b	LOD	R ²
	Genome-wide threshold for $p > 0.05$	2011 = 3.6 2012 = 3.5	QTL ^a	Year	LG	Start (cM)					
Fruit width (FWD)	2011 = 3.6 2012 = 3.5	qp-Fwd1.1 ²	2011	1	30.86	30.86	SNP_IGA_32535	11,810,777	6.64	5.4	
		qp-Fwd1.1 ²	2012	1	30.86	30.86	SNP_IGA_32535	11,810,777	7.05	5.1	
		qp-Fwd1.2 ¹	2011	1	101.212	101.212	SNP_IGA_135137	46,478,138	7.23	6	
		qp-Fwd2.1 ¹	2011	2	20.941	24.483	SNP_IGA_263828	15,656,993	10.21	9	
		qp-Fwd2.2 ¹	2011	2	38.455	41.753	SNP_IGA_277616	19,589,778	4.7	3.2	
		qp-Fwd2.3 ¹	2012	2	17.911	19.65	SNP_IGA_259667	14,631,909	13.08	10.8	
		qp-Fwd3.1 ²	2011	3	41.587	41.587	SNP_IGA_344628	13,758,717	3.82	2.9	
		qp-Fwd3.1 ²	2012	3	41.158	41.587	SNP_IGA_344628	13,758,717	5.61	4	
		qp-Fwd4.1 ¹	2011	4	55.894	56.323	SNP_IGA_448998	19,896,554	5.01	3.9	
		qp-Fwd4.2 ¹	2012	4	14.973	15.47	SNP_IGA_386970	4,410,111	4.05	2.9	
		qp-Fwd5.1 ¹	2011	5	12.675	12.973	SNP_IGA_574551 ^D	6,287,541	5.33	4.2	
		qp-Fwd5.2 ¹	2012	5	3.885	4.314	SNP_IGA_556969	3,427,197	5.06	3.2	
		qp-Fwd6.1 ¹	2011	6	30.477	33.109	SNP_IGA_676161	20,546,646	12.8	11.9	
		qp-Fwd6.2 ¹	2012	6	1.724	2.586	SNP_IGA_620099	4,809,346	12.51	10.2	
		qp-Fwd7.1 ²	2011	7	40.147	40.147	SNP_IGA_776826	15,293,347	18.93	20.3	
		qp-Fwd7.1 ²	2012	7	40.147	40.147	SNP_IGA_776826	15,293,347	10.69	8.4	
qp-Fwd7.2 ¹	2012	7	0	0.871	SNP_IGA_725139	4,203,103	4.31	3			
qp-Fwd8.1 ¹	2012	8	32.443	33.549	SNP_IGA_870010	15,538,262	9.96	7.7			
Fruit depth (FD)	2011 = 3.6 2012 = 3.5	qp-Fd1.1 ²	2011	1	30.86	30.86	SNP_IGA_32535	11,810,777	8.03	6.7	
		qp-Fd1.1 ²	2012	1	30.86	30.86	SNP_IGA_32535	11,810,777	7.41	5.3	
		qp-Fd1.2 ¹	2011	1	101.212	101.212	SNP_IGA_135137	46,478,138	7.89	6.5	
		qp-Fd2.1 ¹	2011	2	20.941	24.483	SNP_IGA_263828	15,656,993	10.77	9.5	
		qp-Fd2.2 ¹	2011	2	38.455	41.753	SNP_IGA_277616	19,589,778	4.87	3.8	
		qp-Fd2.3 ¹	2012	2	17.911	19.65	SNP_IGA_259667	14,631,909	11.85	9.3	
		qp-Fd3.1 ²	2011	3	41.587	41.753	SNP_IGA_344628	13,758,717	4.61	3.5	
		qp-Fd3.1 ²	2012	3	41.158	41.587	SNP_IGA_344628	13,758,717	5.22	3.6	
		qp-Fd4.1 ¹	2011	4	55.894	56.323	SNP_IGA_448998	19,896,554	3.89	2.9	
		qp-Fd4.2 ¹	2012	4	14.973	15.47	SNP_IGA_386970	4,410,111	3.69	2.6	
qp-Fd5.1 ¹	2011	5	12.675	12.973	SNP_IGA_574551	6,287,541	5.47	4.3			
qp-Fd5.2 ¹	2012	5	3.885	4.314	SNP_IGA_556969	3,427,197	4.8	3.2			
qp-Fd6.1 ¹	2011	6	30.477	33.109	SNP_IGA_676161	20,546,646	12.12	11			

Table 2 continued

Trait	Permutation test	Multiple QTL mapping						LOD	R ²	
		QTL ^a	Year	LG	Start (cM)	Final (cM)	Cofactor marker			Genomic position ^b
Genome-wide threshold for $p > 0.05$		qP-Fd6.2¹	2012	6	1.724	2.586	SNP_IGA_620099	4,809,346	11.87	9.3
		qP-Fd7.1²	2011	7	40.147	40.147	SNP_IGA_776826	15,293,347	19.61	21.1
		qP-Fd7.1²	2012	7	40.147	40.147	SNP_IGA_776826	15,293,347	12.39	9.8
		qP-Fd7.2¹	2012	7	0.871	0.871	SNP_IGA_725139	4,203,103	3.55	2.3
		qP-Fd8.1¹	2012	8	32.443	33.549	SNP_IGA_870010	15,538,262	9.99	7.5

Stable QTLs in both years are in bold

q quantitative trait, P Prunoideae, followed by a dash, Tc = trait code (Fw, Fh, Fwd, Fd), L *Prunus persica* linkage group, l 1th chronological QTL for this trait reported on this chromosome, m Number of years which the QTL was mapped

^a The QTL nomenclature according to the recommendations for Standard QTL Nomenclature and Reporting in the Rosaceae where: qP-TcL.1^m

^b Genomic position of cofactor marker was reported in bp

^c QTL peak with a LOD value lower than genome-wide threshold, considered as a significant QTL because of localising in the same position in respect to a significant QTL (with LOD higher than threshold) from the other season

showed the highest LOD score and highest percentage of explained phenotypic variation in both years. The seven stable QTLs explained 57.9 and 50.2 % of the total FW phenotypic variation in 2011 and 2012, respectively.

For FH, the main QTL qP-Fh7.1² (LG7) explained the highest percentage of the phenotypic variation in 2011 (24.3 %) and 2012 (21.6 %). All QTLs together explained 60.1 and 50.2 % of the phenotypic variation in 2011 and 2012, respectively. In the 2 years, four QTLs qP-Fh7.1² (LG7), qP-Fh2.1² (LG2), qP-Fh1.2² (LG1), and qP-Fh3.1² (LG3) remained stable, explaining 45.7 and 41.3 % of the phenotypic variation in 2011 and 2012, respectively.

In 2011, nine QTLs were mapped for FWD, and the phenotypic variation explained was 66.8 % overall. The main QTL qP-Fwd7.1² (LG7) showed the largest effect and explained 20.3 % of the FWD observed variation. In the second year, nine QTLs were mapped for this trait which explained approximately 55.3 % of the phenotypic variation; the QTL qP-Fwd2.3¹ was associated with the largest effect (10.8 %). Three QTLs (qP-Fwd7.1², qP-Fwd1.1², and qP-Fwd3.1²) remained stable and accounted for 28.6 and 17.5 % of the variation in 2011 and 2012, respectively.

The significant associations mapped for FD were located in overlapping confidence intervals as observed for FWD, differing in LOD scores and percentage of explained phenotypic variation. Nine QTLs were detected for this trait in 2011 and explained 69.3 % of the FD variation, the latter being attributed to the QTL qP-Fd7.1², which explained 21.1 % of the FD variation. In 2012, nine QTLs were mapped for FD and explained 52.9 % of the observed variation. Within the 2 years, three stable QTLs (qP-Fd7.1², qP-Fd1.2², and qP-Fd3.1²) explained 31.3 and 18.7 % of the observed variation in the first and second year, respectively.

QTL clusters

Considering all traits in the 2 years analysed, we observed that some QTLs were mapped in the same region forming clusters of QTLs. Clusters of stable QTLs were mapped on LGs 1, 3, 5, and 7 (Fig. 3). The QTLs in the cluster on LG7 (associated with the marker SNP_IGA_776826) were mapped in both years and explain the majority of phenotypic variation for FW, FH, FWD, and FD. In the cluster located on

◀ **Fig. 3** Stables QTLs controlling FW, FH, FWD, and FD. Marker names are listed at the right side of each LG, and the genetic distances (in centimorgans) are listed at the left of each one. QTLs are drawn at the right of each LG and are represented as central bar representing the “LOD minus two” interval (maximum LOD score, minus two), and the external *thin line* represents the whole significance interval of the QTL. QTLs for FW, FH, FWD, and FD are represented with different *bar fills*: red for FW, green for FH, blue for FWD, pink for FD. (Color figure online)

LG1, QTLs for FW, FWD, and FD were associated with the marker SNP_IGA_32535.

QTLs for FW, FWD, and FD were also mapped in the cluster associated with the marker SNP_IGA_574551, located on LG5. However, for FWD and FD, QTLs in this position were mapped only in 2011. Cluster of QTLs for FWD and FD was mapped on LG3 (SNP_IGA_344628).

Together, the sum of the R^2 in the QTL in the clusters on LG7, LG1, and LG5 (associated with the markers SNP_IGA_776826, SNP_IGA_32535 and SNP_IGA_574551, respectively) explained 32.1 and 23.9 % of the observed phenotypic variation for FW in 2011 and 2012, respectively.

For FH, QTLs in the cluster on LG7 (associated with the markers SNP_IGA_776826) explained 24.1 and 21.6 % of the observed phenotypic variation for FH in the 2 years analysed.

For FWD and FD, the values explained by the QTLs in the clusters on LG7, LG1, and LG3 (associated with the markers SNP_IGA_776826, SNP_IGA_32535 and SNP_IGA_344628, respectively) were 28.6 and 31.3 % in 2011 and 17.5 and 18.7 % in 2012.

Discussion

Linkage mapping and comparison of WB linkage map and peach genome sequence v1

In the present work, the WB linkage map was used to detect QTLs associated with fruit weight and size in peach. This map provides a valuable tool for genetic analysis of other traits segregating in the WB F_2 progeny, such as maturity date (Pirone et al. 2013), flesh texture and colour, peduncle length, flower type and colour, and tree habit. In line with previous work (Eduardo et al. 2013; Romeu et al. 2014), the use of the 9 K peach SNP array v1 (Verde et al. 2012) developed

by the International Peach SNP Consortium (IPSC) provided a large number of high quality SNPs for map construction, resulting in a total map distance of 536.6 cM and an average distance between markers of 0.52 cM (Table 1; Supplementary Figure 2). The WB map has higher map coverage and lower average distance between markers in comparison with other SNP maps (Yang et al. 2012; Frett et al. 2014) produced with the 9K peach SNP array v1 (Verde et al. 2012). Yang et al. (2012) using a cross between ‘O’Henry’ and ‘Clayton’ obtained a total map length of 421.4 cM (covering 63 % of the pseudomolecules/scaffolds of peach genome v1.0) with an average density of 1.6 cM/marker. The map by Frett et al. (2014) spanned 452.51 cM (covering 61.6 % of the pseudomolecules/scaffolds of peach genome v1) with an average marker spacing of 2.38 cM/marker.

In the WB map, large gaps were not observed; the largest gap was 6.879 cM between adjacent markers SNP_2_22274363 and SNP_IGA_286418 in LG2. This value is lower than the one obtained by Yang et al. (2012), who observed two gaps larger than 15 cM in LG3 and LG5, and the one by Frett et al. (2014), who observed the largest gaps in LG3 and LG4 (16.5 and 13.7 cM, respectively).

SNP maps with high coverage and marker density such as WB map are important because they allow a higher precision and accuracy for mapping QTLs (Van Ooijen 2006; Martínez-García et al. 2012; Eduardo et al. 2013).

Anchoring to the reference genome sequence (International Peach Genome Initiative 2013) shows that the WB map covers 93.6 % of the peach genome, facilitating integration and comparisons with other maps and QTL studies. While approximately 95 % of the SNP markers in WB were in accordance with their position when compared to the peach genome sequence v1.0, discrepancies in some marker positions were observed. Inversions in the marker order were observed in LG1, LG4, LG7, and LG8 (Fig. 2; Table 1). In addition, some markers that are putatively located on scaffold 4 and scaffold 15 were mapped on LG1. Other SNPs putatively located on scaffold 3 were mapped in LG7, and finally, some SNPs putatively located on scaffold 10, scaffold 4, and scaffold 9 of the peach genome were mapped on LG3. The positions of the majority of markers which showed inverse order in the WB map compared to the peach genome sequence v1 and the positions of

markers which were putatively located in one scaffold, but mapped in other LGs, are in agreement with the proposed reassembly of the peach genome (International Peach Genome Initiative 2013).

Phenotypic data, correlation, and heritability of the traits

All the studied traits showed a normal distribution. Transgressive segregation in FW and size was observed in the F₂ progeny compared to NJ Weeping and Bounty parents. This may be due to a contribution of alleles of the two parents or as a result of the different types of interaction between them (Swamy et al. 2012). Analysing the same traits and using an advanced backcross from an interspecific cross (*Prunus persica* × *P. davidiana*), Quilot et al. (2004) also observed the presence of a normal distribution, but did not detect the presence of transgressive segregation for FW and size.

Significantly lower values were recorded for fruit weight and size in 2012 compared to 2011 (Supplementary Table 1). Since these traits are strongly affected by environmental factors (e.g. mineral nutrients and water availability, air temperature, soil management), the weight and the final size of the fruit can be extremely affected in situations where one of these factors is limiting. Thus, a possible explanation for the observed values in 2012 would be the high temperatures and low rainfall reported from June to August. June 2012 was above the average maximum temperature with up to 39 °C, and rainfall was significantly lower than normal for this period. Similar situations were also observed in July and August 2012, when low rainfall, high average and maximum temperatures, exceptionally low soil water content, and higher evaporation/transpiration values than normal were reported (ARPA 2013). However, despite the reduction in fruit weight and size observed in 2012, high correlation between years was detected for all traits (Supplementary Table 2), and stable QTLs were identified for all traits across the 2 years (Table 2). This suggests that these QTLs mapped for FW and size have a strong effect on the trait independently of environmental conditions.

The broad-sense heritability coefficients were above 80 % for all traits in both years. Considering the 2 years, heritability coefficients reported in the

present study are markedly higher than those obtained by Hansche et al. (1972), whose estimates were 31, 26, and 29 % for fruit length, cheek, and suture (corresponding to FH, FWD, and FD in the present study), respectively. The value for FW, despite the lowest heritability (86.78 %) in 2012, is higher than the 50 % reported by Hansche (1986). Moreover, the broad-sense heritability results reported in the present study show a high contribution of the genotypic to the phenotypic variance. Most importantly, high heritability coefficients increase the detection power of QTLs, as well as the reliability of QTL position and effect estimation, allowing for efficient dissection of complex traits (Zhao et al. 2007).

All traits presented high, positive, and significant correlation coefficients. This is an expected result, since fruits with higher weight have a larger size as well. For example, high correlation between fruit weight and size has been observed in sweet cherry (Zhang et al. 2010) and between FH and FWD in peaches grown in subtropical regions (Pereira Silva et al. 2013). In the present study, consistent with high correlation coefficients, some QTLs coincided among correlated traits (clusters of QTLs). Concerning the correlation between years, FW showed a coefficient of 0.673, similar to the value of 0.62 observed by Eduardo et al. (2011) in a different F₂ progeny for two consecutive years.

Maturity date (MD), which segregates in the WB F₂ progeny, could also affect FW. Eduardo et al. 2011 showed that FW is highly correlated with MD. Clusters of QTLs associated with these traits were mapped in peach, and due to a major pleiotropic effect of MD, the identification of other QTLs for traits such as FW could be masked. In the WB F₂ progeny, the major MD locus was previously identified on LG4 by Pirona et al. (2013) (therein, the WB F₂ progeny was referred to as WxB_y). In our work, the results obtained by QTL mapping suggest that the MD locus did not exert a strong influence in the genetic dissection of FW in the WB F₂ progeny. Only one QTL qP-Fw4.1¹ (associated with the marker SNP_IGA_410336) was mapped in the region reported by Pirona et al. (2013), which explained approximately 92 % of the phenotypic changes observed in the MD of the WB F₂ progeny. The qP-Fw4.1¹ explained approximately 4.8 % of the observed FW variation and was mapped only in 2011.

QTL analyses for fruit weight and dimensions

Previous QTL analyses of fruit weight and size in tomato and sweet cherry have identified several loci involved in genetic control of these traits with some major QTL accounting for 25–30 % of observed phenotypic variation (Frery et al. 2000; Zhang et al. 2010). In the present work, considering the 2 years analysed, 10 FW QTLs were identified with effects ranging from 2.7 to 19.7 % of phenotypic variation. While none of our QTLs accounted for over 25 % of phenotypic variance, one main QTL (qP-Fw7.1²) was identified which accounted for the largest fraction of phenotypic variance in both years, explaining 19.7 % in 2011 and 12.6 % in 2012.

We observed specific QTLs for FW, indicating that the genetic basis of this trait is partially distinct from fruit size. On LG1, the QTL qP-Fw1.2² explained about 7 % of the phenotypic variation observed in 2011 and 2.7 % in 2012. In contrast to previous studies (Dirlewanger et al. 1999; Eduardo et al. 2011; Quilot et al. 2004), QTLs associated with FW were not mapped in this region.

The stable QTL qP-Fw2.1² (mapped between 3,812,067 and 5,472,142 on scaffold 2) explained approximately 4 % of the observed phenotypic variation of FW in both years. QTLs mapped on LG2 in previous studies do not correspond to the same region reported in the present work. In a ‘Contender’ × ‘Ambra’ F₂ progeny, a QTL for FW was previously mapped in LG2 by Eduardo et al. (2011) near marker UDP98-025 (position 10,872,102 in scaffold 2). Analysing an F₁ progeny derived from ‘Bolero’ × ‘OroA’, Eduardo et al. (2011) mapped another QTL near marker UDP98-406 in the position 24,408,936 of scaffold 2. In the study by Quilot et al. (2004) on an advanced backcross population from an interspecific cross (*Prunus persica* × *P. davidiana*), a QTL for fruit weight, fruit cheek diameter, and fruit suture diameter was mapped at 13 cM below the marker pchgms1 (position 21,255,417 on scaffold 2), associated to the marker CC115.

Several authors mapped QTLs for FW on LG6. Using the ‘Ferjalou Jalousia’ × ‘Fantasy’ F₂ progeny, Dirlewanger et al. (1999) mapped a QTL associated with marker PGL1 (position 25,400,375 on peach genome v1.0). In the ‘Contender’ × ‘Ambra’ F₂ progeny, one stable QTL was mapped next to marker UDP98-412 (position 24,753,353 in scaffold 6)

(Eduardo et al. 2011). In the association mapping study conducted by Cao et al. (2012), a QTL associated with marker CPPCT008 (position 4,898,50 on peach genome v1.0) was mapped. In our study, a stable QTL (qP-Fw6.1²) was found in the interval 18,064,690–18,315,130 on scaffold 6 (markers SNP_IGA_669440 in 2011 and SNP_IGA_669050 in 2012).

Finally, a FW QTL (qP-Fw3.1²) was mapped on LG3. Based on comparative mapping with the *Prunus* reference map (Illa et al. 2010), candidate genes such as 1-aminocyclopropane-1- carboxylate oxidase and glutathione S-transferase involved in fruit growth and maturity exist in this region in peach.

Considering only the trait FH, the stable QTL qP-Fh2.1² (LG2) matches a previously described QTL for FW in an F₂ progeny from ‘Contender’ × ‘Ambra’ (Eduardo et al. 2011). The stable QTLs qP-Fh1.2² and qP-Fh3².1 were mapped specifically for the trait FH, suggesting that these loci are uniquely associated with this trait.

For the highly correlated FW and size (FH, FWD, and FD) traits reported in the present study, clusters of QTLs may reflect the presence of one or several linked genes or a common genetic mechanism which results in an increase in both fruit mass and dimensions. Clusters of QTLs were also reported for FW and size in sweet cherry (Zhang et al. 2010), cashew apple (Santos et al. 2010), and pear (Zhang et al. 2013). In peach, overlapping QTLs associated with FWD and FD were reported by Cantín et al. (2010) on LG4.

Noteworthy is the cluster of QTLs for FW, FH, FWD, and FD mapped on LG7 (SNP_IGA_776826, Table 2), which explained approximately 19 and 12 % of the phenotypic variation for FW in 2011 and 2012, respectively. This QTL also explains the majority of phenotypic variation for fruit size traits. Comparative mapping analyses suggest that the QTL cluster associated with SNP_IGA_776826 could be the FW QTL which Quilot et al. (2004) reported on the same LG in an advanced backcross from an interspecific cross (*Prunus persica* × *P. davidiana*). The QTL mapped by Quilot et al. (2004) was associated with CFF11 (whose physical position is not available in peach genome v1.0), located 3 cM above marker AG104A, at 15,131,908 in the peach genome (scaffold 7). The QTL cluster mapped in our study spans the region 15,293,347–15,492,141 on the same scaffold. Considering the stability of this cluster of QTLs and

the percentage of phenotypic variation explained, this genomic region is an interesting target for further dissection and candidate gene identification.

Another cluster of QTLs was associated with marker SNP_IGA_32535 on LG1 (11,739,016–11,810,777). QTLs for FW, FWD, and FD were mapped in this region in both years, explaining approximately 7.5 % of the phenotypic variation of FW. This position is distinct from the QTLs reported in LG1 for fruit weight and size identified in previous studies conducted by Quilot et al. (2004) and Eduardo et al. (2011). Quilot et al. (2004) mapped a QTL for fruit weight, cheek diameter, and suture diameter (corresponding to FWD and FD in the present study) associated to marker PC102, 12 cM below marker PC78 (position 9,166,621, scaffold 1), while Eduardo et al. (2011) mapped a QTL for FW near BPPCT016 (position 36,074,785, scaffold 1, in the peach genome v1) in a 'Contender' × 'Ambra' F₂ progeny.

On LG5, a FW QTL associated with markers SNP_IGA_574551 and SNP_IGA_572303 was mapped in both years, and overlapping QTLs for FWD and FD were obtained only in 2011. This cluster of QTLs may correspond to a major QTL for FW reported by Cao et al. (2012) who carried out an association mapping study with landraces from China, using 53 SSRs markers. Considering that this QTL was identified following two different approaches (linkage analysis and association mapping) in diverse genetic materials, this region represents an interesting source of allelic variation for fruit weight and size in peach.

Finally, a cluster of QTLs for FWD and FD was mapped in LG3 associated to marker SNP_IGA_344628. This QTL explained only 2.9 and 4 % of the observed phenotypic variation in the FWD values in 2011 and 2012, respectively. As for the values of FD, this QTL explained 3.5 and 3.6 % in the years 2011 and 2012. Cao et al. (2012) mapped a distinct QTL for FW on LG3, associated to marker BPPCT007 (position 27,411,939, scaffold 3). Considering the traits FWD and FD, QTLs were mapped in the same region and associated with the same markers. The existence of overlapping QTLs between these traits reflects the 98 % correlation detected between FWD and FD for both years.

The QTLs mapped in this study are an important source for further studies of FW. The choice of the study population had a strong influence: The parents of

the WB F₂ progeny are highly contrasting concerning FW, with one of the parents being an ornamental cultivar (NJ Weeping) which produces small fruits. This increased the segregation of FW and size in the population and the probability of mapping new QTLs associated with these traits in peach.

Due to the high segregation observed in FW and size in the WB F₂ progeny, we mapped stable QTLs which have not yet been reported in the literature, providing new relevant information for future breeding programmes. The clusters of QTLs for FW and size mapped on LG7 could correspond to the FW QTL reported by Quilot et al. (2004), who used an advanced backcross from an interspecific cross (*Prunus persica* × *Prunus davidiana*), and the knowledge of this genetic interval can facilitate manipulation of these traits. We also mapped QTLs that had been reported in other studies which did not use cultivars with small fruits as parents. One QTL previously mapped in an association mapping study was identified in the present work. Thus, our results show that at least some of the alleles segregating in the WB progeny also segregate in other crosses.

The information from the present study provides an excellent basis for future studies of candidate genes using the genetic intervals for stable QTLs mapped in the WB F₂ progeny.

Conclusions

The highly saturated SNP map for the WB F₂ progeny provides a powerful tool for QTL analysis. The results offer new information for the genetic dissection and manipulation of FW and size in peach. The traits FW, FH, FWD, and FD are highly correlated, and clusters of QTLs were mapped. Specific QTLs for each trait were also mapped. Eleven stable QTLs were obtained in the 2 years of evaluation. To our knowledge, seven out of the eleven stable QTLs mapped in this study have been mapped for the first time, while four had already been reported for different progenies and approaches. Considering the 2 years analysed, a cluster of QTL associated with marker SNP_IGA_776826 in LG7 explained the highest proportion of phenotypic variation for FW, FH, FWD, and FD (in average 16.2, 23.0, 14.4 and 15.5 %, respectively), highlighting its relevance in controlling these traits. It will be interesting to assess whether stable QTLs mapped in this study will be

validated in other progenies. The definition of restricted genetic intervals for these stable QTLs and the availability of the peach genome sequence will provide an ideal basis for the identification of candidate genes and, eventually, the analysis of the molecular basis of FW and size in peach. In parallel, an association mapping study is underway in order to confirm the QTLs mapped in the present work and to identify new QTLs associated with fruit weight and size in peach.

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References

- Alheit KV, Reif JC, Maurer HP, Hahn V, Weissmann EA, Miedaner T, Wurschum T (2011) Detection of segregation distortion loci in triticale (*x Triticosecale Wittmack*) based on a high-density DArT marker consensus genetic linkage map. *BMC Genom* 12:380
- Allard RW (1960) Principles of plant breeding. John Wiley, New York
- ARPA Agenzia regionale per la prevenzione e l’ambiente dell’Emilia Romagna (2013) Dati tempo reale. <http://www.arpa.emr.it/sim/>. Accessed 26 Feb 2014
- Bassi D, Rizzo M (2002) Fruit trait segregation in a peach cross between distant genotypes. *Acta Hort* 592:217–221
- Blas A, Yu Q, Veatch O, Paull R, Moore P, Ming R (2012) Genetic mapping of quantitative trait loci controlling fruit size and shape in papaya. *Mol Breed* 29:457–466
- Cantín CM, Crisostob CH, Ogundiwinb EA, Gradziel T, Torrentsc J, Morenoa MA, Gogorcena Y (2010) Chilling injury susceptibility in an intra-specific peach [*Prunus persica* (L.) Batsch] progeny. *Postharvest Biol Technol* 58:79–87
- Cao K, Wang L, Zhu G, Fang W, Chen C, Luo J (2012) Genetic diversity, linkage disequilibrium, and association mapping analyses of peach (*Prunus persica*) landraces in China. *Tree Genet Genomes* 8:1–16
- Chalmers DJ, van den Ende B (1975) Productivity of peach trees: factors affecting dry-weight distribution during tree growth. *Ann Bot* 39:423–432
- Coombe BG (1976) The development of fleshy fruits. *Ann Rev Plant Physiol* 27:507–528
- Cruz CD (2013) GENES - a software package for analysis in experimental statistics and quantitative genetics. *Acta Scientiarum* 35:271–276
- Dardick CD, Callahan AM, Chiozzotto R, Schaffer RJ, Piagnani MC, Scorza R (2010) Stone formation in peach fruit exhibits spatial coordination of the lignin and flavonoid pathways and similarity to Arabidopsis dehiscence. *BMC Biol* 8:13
- Devoghalaere F, Doucen T, Guitton B, Keeling J, Payne W, Ling TJ, Ross JJ, Hallett IC, Gunaseelan K, Dayatilake GA, Diak R, Breen KC, Tustin DS, Costes E, Chagne D, Schaffer RJ, David KM (2012) A genomics approach to understanding the role of auxin in apple (*Malus × domestica*) fruit size control. *BMC Plant Biol* 12:7
- Dirlewanger E, Moing A, Rothan C, Svanella L, Pronier V, Guye A, Plomion C, Monet R (1999) Mapping QTLs controlling fruit quality in peach (*Prunus persica* (L.) Batsch). *Theor Appl Genet* 98:18–31
- Dirlewanger E, Graziano E, Joobeur T, Garriga-Caldere F, Cosson P, Howad W, Arús P (2004) Comparative mapping and marker-assisted selection in Rosaceae fruit crops. *Proc Natl Acad Sci USA* 101:9891–9896
- Eduardo I, Pacheco I, Chietera G, Bassi D, Pozzi C, Vecchiatti A, Rossini L (2011) QTL analysis of fruit quality traits in two peach intraspecific populations and importance of maturity date pleiotropic effect. *Tree Genet Genomes* 7:323–335
- Eduardo I, Chietera G, Pirona R, Pacheco I, Troglio M, Banchi E, Bassi D, Rossini L, Vecchiatti A, Pozzi C (2013) Genetic dissection of aroma volatile compounds from the essential oil of peach fruit: QTL analysis and identification of candidate genes using dense SNP maps. *Tree Genet Genomes* 9:189–204
- El-Sharkawy I, Kim WS, El-Kereamy A, Jayasankar S, Svircev AM, Brown DC (2007) Isolation and characterization of four ethylene signal transduction elements in plums (*Prunus salicina* L.). *J Exp Bot* 58:3631–3643
- Frary A, Nesbitt TC, Frary A, Grandillo S, van der Knaap E, Cong B, Liu J, Meller J, Elber R, Alpert KB, Tanksley SD (2000) fw2.2: a quantitative trait locus key to the evolution of tomato fruit size. *Science* 289:85–88
- Frett TJ, Reighard GL, Okie WR, Gasic K (2014) Mapping quantitative trait loci associated with blush in peach [*Prunus persica* (L.) Batsch]. *Tree Genet Genomes* 10:367–381
- Girona J, Fereres E, Marsal J, Goldhamer DA, Naor A, Soriano MA (2012) Peach. In: Crop yield response to water FAO irrigation and drainage paper 66. Food and Agriculture Organization of the United Nations, Rome, pp 392–407
- Grandillo S, Ku HM, Tanksley SD (1999) Identifying the loci responsible for natural variation in fruit size and shape in tomato. *Theor Appl Genet* 99:978–987
- Hammer O, Harper DAT, Ryan RD (2001) PAST: paleontological statistics software package for education and data analysis. *Palaeontol Electron* 4:19
- Hansche PE (1986) Heritability of juvenility in peach. *HortScience* 21:1197–1198
- Hansche PE, Hasse CO, Beres V (1972) Estimates of genetic and environmental effects on several traits in peach. *J Am Soc Hort Sci* 97:76–79

- Harada T, Kurahashi W, Yanai M, Wakasa Y, Satoh T (2005) Involvement of cell proliferation and cell enlargement in increasing the fruit size of malus species. *Sci Hort (Amsterdam)* 105:447–456
- Illa E, Eduardo I, Audergon JM, Barale F, Dirlwanger E, Li XW, Moing A, Lambert P, Dantec LL, Gao ZS, Poëssel JL, Pozzi C, Rossini L, Vecchietti A, Arús P, Howad W (2010) Saturating the *Prunus* (stone fruits) genome with candidate genes for fruit quality. *Mol Breed* 28:667–682
- Infante R, Martínez-Gómez P, Predieri S (2008) Quality oriented fruit breeding: peach [*Prunus persica* (L.) Batsch]. *J Food Agric Environ* 6:342–356
- International Peach Genome Initiative (2013) The high-quality draft genome of peach (*Prunus persica*) identifies unique patterns of genetic diversity, domestication and genome evolution. *Nat Genet* 45:487–494
- Liu J, Nada K, Pang X, Honda C, Kitashiba H, Moriguchi T (2006) Role of polyamines in peach fruit development and storage. *Tree Physiol* 26:791–798
- Lombard VA, Osorio S, Borsani J, Lauxmann MA, Bustamante CA, Budde CO, Andreo CS, Lara MV, Fernie AR, Drincovich MF (2011) Metabolic profiling during peach fruit development and ripening reveals the metabolic networks that underpin each developmental stage. *Plant Physiol* 157:1696–1710
- Mahbubul Alam AKM, Somta P, Srinives P (2014) Identification and confirmation of quantitative trait loci controlling resistance to mungbean yellow mosaic disease in mungbean [*Vigna radiata* (L.) Wilczek]. *Mol Breed* 34:1497–1506
- Marini R (2002) How to grow big peaches, 2002. <http://njaes.rutgers.edu/peach/orchard/bigpeaches.pdf>. Accessed 26 Feb 2014
- Martínez-García P, Parfitt D, Ogundiwin E, Fass J, Chan H, Ahmad R, Lurie S, Dandekar A, Gradziel T, Crisosto C (2012) High density SNP mapping and QTL analysis for fruit quality characteristics in peach (*Prunus persica* L.). *Tree Genet Genomes* 9:19–36
- Olmstead JW, Iezzoni AF, Whiting MD (2007) Genotypic differences in sweet cherry fruit size are primarily a function of cell number. *J Am Soc Hort Sci* 132:697–703
- Pacheco I, Bassi D, Eduardo I, Ciaccioli A, Pirona R, Rossini L, Vecchietti A (2014) QTL mapping for brown rot (*Monilinia fructigena*) resistance in an intraspecific peach (*Prunus persica* L. Batsch) F₁ progeny. *Tree Genet Genomes*. doi:10.1007/s11295-014-0756-7
- Paran I, van der Knaap E (2007) Genetic and molecular regulation of fruit and plant domestication traits in tomato and pepper. *J Exp Bot* 58:3841–3852
- Pereira Silva DF, Pires Matias RG, Rodrigues Ribeiro M, Horst Bruckner C (2013) Correlação entre características quantitativas e qualitativas de frutos de pessegueiros na geração F₂ cultivados em região subtropical. *Revista Ceres* 60:53–58
- Pirona R, Eduardo I, Pacheco I, Da Silva Linge C, Miculan M, Verde I, Tartarini S, Dondini L, Pea G, Bassi D, Rossini L (2013) Fine mapping and identification of a candidate gene for a major locus controlling maturity date in peach. *BMC Plant Biol* 13:166
- Quilot B, Wu BH, Kervella J, Genard M, Foulongne M, Moreau K (2004) QTL analysis of quality traits in an advanced backcross between *Prunus persica* cultivars and the wild relative species *P. davidiana*. *Theor Appl Genet* 109:884–897
- Recommendations for Standard QTL Nomenclature and Reporting in the Rosaceae. http://www.rosaceae.org/system/files/QTL_nomenclature_recommendations.pdf. Accessed 05 Oct 2014
- Romeu JF, Monforte AJ, Sanchez G, Granell A, Garcia-Brunton J, Badenes ML, Rios G (2014) Quantitative trait loci affecting reproductive phenology in peach. *BMC Plant Biol* 14:52
- Santos FHC, Cavalcanti JJV, Silva FP (2010) Detection of quantitative trait loci for physical traits of cashew apple. *Crop Breed Appl Biotechnol* 10:101–109
- Swamy BPM, Kaladhar K, Rani NS, Prasad GSV, Viraktamath BC, Reddy GA, Sarla N (2012) QTL analysis for grain quality traits in 2 BC₂F₂ populations derived from crosses between *Oryza sativa* cv swarna and 2 accessions of *O. nivara*. *J Hered* 103:442–452
- Van Ooijen JW (2006) JoinMap 4 software for the calculation of genetic linkage maps in experimental populations. Kyazma BV, Wageningen, The Netherlands
- Van Ooijen JW (2009) MapQTL software for the mapping of quantitative trait loci in experimental populations of diploid species. Kyazma BV, Wageningen, The Netherlands
- Verde I, Bassil N, Scalabrin S, Gilmore B, Lawley CT, Gasic K, Micheletti D, Rosyara UR, Cattonaro F, Vendramin E, Main D, Aramini V, Blas AL, Mockler TC, Bryant DW, Wilhelm L, Troggo M, Sosinski B, Aranzana MJ, Arús P, Iezzoni A, Morgante M, Peace C (2012) Development and evaluation of a 9K SNP array for peach by internationally coordinated SNP detection and validation in breeding germplasm. *PLoS ONE* 7:e35668
- Voorrips RE (2002) MapChart Software for the graphical representations of linkage maps and QTLs. *J Hered* 93:77–78
- Yamaguchi M, Haji T, Miyake M, Yaegaki H (2002) Studies on the varietal differences and yearly deviation of mesocarp cell numbers and lengths and fruit weight among commercial peach [*Prunus persica* (L.) Batsch] cultivars and selections, wild types, and their hybrids. *J Jpn Soc Hortic Sci* 71:459–466
- Yamamoto T, Shimada T, Imai T, Yaegaki H, Haji T, Matsuta N, Yamaguchi M, Hayashi T (2001) Characterization of morphological traits based on a genetic linkage map in peach. *Breed Sci* 51:271–278
- Yang N, Reighard GL, Ritchie D, Okie WR, Gasic K (2012) Mapping quantitative trait loci associated with resistance to bacterial spot (*Xanthomonas arboricola* pv. *pruni*) in peach. *Tree Genet Genomes*. doi:10.1007/s11295-012-0580-x
- Yuan XJ, Li XZ, Pan JS, Wang G, Jiang S, Li XH, Deng SL, He HL, Si MX, Lai L, Wu AZ, Zhu LH, Cai R (2008) Genetic linkage map construction and location of QTLs for fruit-related traits in cucumber. *Plant Breed* 127:180–188
- Zhang G, Sebolt AM, Sooriyapathirana SS, Wang D, Bink MCAM, Olmstead JW, Iezzoni AF (2010) Fruit size QTL analysis of an F₁ population derived from a cross between a domesticated sweet cherry cultivar and a wild forest sweet cherry. *Tree Genet Genomes* 6:25–36

- Zhang N, Brewer MT, van der Knaap E (2012) Fine mapping of fw3.2 controlling fruit weight in tomato. *Theor Appl Genet* 125:273–284
- Zhang RP, Wu J, Li XG, Khan MA, Chen H, Korban SS, Zhang SL (2013) An AFLP, SRAP, and SSR genetic linkage map and identification of QTLs for fruit traits in pear (*Pyrus L.*). *Plant Mol Biol Report* 31:678–687
- Zhao K, Aranzana MJ, Kim S, Lister C, Shindo C, Tang C, Toomajian C, Zheng H, Dean C, Marjoram P, Nordborg M (2007) An Arabidopsis example of association mapping in structured samples. *PLoS Genet* 3:e4