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Identification of *loci* controlling phenology, fruit quality and post-harvest quantitative parameters in Japanese plum (*Prunus salicina* Lindl.)

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ABSTRACT

Japanese plums are popular fruits since they are exceptionally nutritious with high fiber and antioxidant content. This work has aimed to analyze the most critical phenology, fruit quality and postharvest parameters from a genomic point of view to identify molecular markers closely linked to the most significant Quantitative trait loci (QTLs). A genetic linkage map of an F1 population of 151 individuals from the cross '98–99' × 'Angeleno' was constructed using previously reported Single Nucleotide polymorphism (SNP) data and 25 additional Simple Sequence Repeat (SSR) markers. Twenty-three phenotypic traits evaluated during three harvest seasons were assayed to estimate best linear unbiased predictors by using two genomic association QTL analysis approaches: General Linear Model-based single marker-trait associations (GLM) and Multiple QTL Model analyses (MQM). In addition, loss of weight and chlorophyll degradation between days 1 and 7 as well as fruit softening for days 1, 4, and 7 were monitored during two consecutive seasons. The most significant identified QTLs were linked to fruit development period and fruit weight in Linkage Groups (LG) 4 and 2, respectively. Regarding postharvest parameters, the identified QTLs related to chlorophyll degradation and loss of weight showed lower significance than phenology or fruit quality traits. In contrast, minor QTLs for fruit firmness evolution using destructive and non-destructive methods were confirmed in LG 4 and 5.

1. Introduction

Japanese plum (*Prunus salicina* Lindl.) is the second more cultivated stone fruit species in the world after peach, reaching a global production of 12,608,678 tons per year (FAOSTAT, 2018). Throughout the last decades, many breeding programs have been focused on releasing new early ripening varieties with high fruit quality and proper response to postharvest regimes (Ruiz et al., 2016; Minas et al., 2015). It is well known that high fruit quality is related to a balance between soluble solids content and acidity, as well as a low softening rate linked to a longer potential market life (Crisosto et al., 2004). The global market demands that growers and retailers should focus on fruit postharvest behavior, allowing sending the product to distant markets while keeping the highest organoleptic condition. For this reason, it is necessary to use efficiently cold storage facilities and ethylene inhibitor treatments during postharvest and transport (Pan et al., 2016; Velardo-Micharet et al., 2017; Singh and Singh, 2017; Shi et al., 2013; Verde et al., 2012). However, although cold storage combined with 1-MCP applications favor a more extended shelf-life period, the response to ethylene inhibitor treatments is quite genotype-dependent (Candan et al., 2008). In this context, genomic studies suppose an interesting approach to reveal the physiological machinery involved in the manifestation of postharvest parameters.

There is a high interest to associate important and heritable agronomic traits with genomic regions to develop molecular markers suitable for Marker Assisted Selection (MAS). To date, there is a high amount of information about quantitative trait loci (QTLs) for different traits related to different agronomic traits in *Prunus* species (Salazar et al., 2014; Fresnedo-Ramírez et al., 2015; Bielenberg et al., 2015; Castède et al., 2014; Desnoues et al., 2016; Zeballos et al., 2016; Salazar et al., 2016, 2017; Cai et al., 2017), but not so much in terms of postharvest and fruit quality, especially in Japanese plum.

In the case of the QTL identification linked to postharvest parameters in Japanese plum, the most significant challenges include: i) the

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difficulty to monitor and establish an adequate phenotyping methodology in a progeny, and ii) the polygenic/quantitative nature of these traits.

On the other hand, the most relevant postharvest parameter in the fruit's shelf-life period is by far the fruit softening. Some difference in flesh firmness is explained by the different ages of the individual fruit (i.e., the time from flowering to harvest), as young fruit reach the rapid softening phase later than those formed earlier. These observations explain why shaded fruit may be delayed by seven days in reaching commercial maturity compared to more light exposed fruit (Bonora, 2013), as well as why no direct relationship is found regarding light conditions (Lewallen and Marini, 2003). In addition, other studies on peaches have confirmed that softening rates follow the same pattern in all fruit and that variations are determined by the degree of maturity of each fruit. These findings also indicated that the softening rate has a strong genetic component compared to the other maturity parameters. Consequently, when ripeness monitoring starts at the time of the "color break," flesh firmness is a reliable indicator for predicting the onset of the harvest time in advance (Pinto et al., 2016).

At the genomic level, the high synteny between *Prunus* species (Shi et al., 2013) allows the use of the peach genome (Verde et al., 2013) as a positional and functional reference in genomic or transcriptomic sequencing applications, i.e., in re-sequencing projects aimed to find new genomic variants segregating in progenies or populations of interest, which allows the generation of markers suitable for marker-trait association studies. At this moment, there are more advanced and new molecular tools adequate for trait associations at the genomic level through QTL mapping. For example, there are useful genotyping platforms as 9 K IPSC array (Verde et al., 2012), SNPlex (De la Vega et al., 2005; Chen et al., 2012) or Genotype by Sequencing (Elshire et al., 2011; Salazar et al., 2017) allowing us to compare and associate thousands of Single Nucleotide polymorphisms (SNPs) with phenotypical traits. These genotyping technologies allow the use of Genome-Wide Association Studies and Genomic Selection for different phenotyping studies (Cao et al., 2016; Schulz et al., 2016; Biscarini et al., 2016).

The objective of this study is to build a saturated genetic map in the '98 − 99' × 'Angeleno' Japanese plum progeny using SNPs aligned to peach genome v2.1 and Simple Sequence Repeat (SSR) markers, to identify in a precise manner marker-trait associations and QTLs linked to the most important phenological, fruit quality and postharvest parameters.

2. Material and methods

2.1. Plant material

An F1 Japanese plum progeny was generated in Rinconada de Maipu Experimental Station (Santiago, Chile) in 2011. The selection '98 − 99' was the female parent and 'Angeleno' cultivar the male parent (Salazar et al., 2017). The selection '98 − 99' is a mid-early ripening plum with red skin and yellow flesh and with high fruit quality, while 'Angeleno' is a late-maturing cultivar with purple skin color and excellent postharvest performance due to ethylene suppression (Candan et al., 2008).

2.2. Phenology, fruit quality, and postharvest evaluation

Phenology and fruit quality traits were evaluated in the '98 − 99' × 'Angeleno' progeny during the years 2016, 2017, and 2018 (Table S1). Blooming date (BD) was considered as the date in which the plants reached a 50 % of anthesis while ripening time (RT) was determined when the skin chlorophyll absorption (I_{AD}) ranged from 1 to 1.4 units and firmness was close to 40 N (Contador et al., 2016) when the fruit had not yet reached its maturity of consumption. Both BD and RT were evaluated in Julian days (considering July 1 st as the first day) since the study was carried out in the southern hemisphere. Finally, the fruit developmental period (FDP) was considered as the difference in Julian days between BD and RT.

On the other hand, fruit quality traits were evaluated using ten homogeneous sample fruit per individual, including fruit weight (FW), fruit diameters, fruit shape (SHP), soluble solids content (SSC) and malic acid (MALIC). Fruit texture attributes were determined by a destructive method after harvest in two moments (1 and 7 days) to estimate the rate of fruit texture change. FW was determined with a precision digital balance in grams; fruit diameter was measured in the sutural (SUT), equatorial (EC) and polar (POL) fruit directions by a digital gauge in mm; SHP was visually determined as 1 (elongated), 2 (hearted), 3 (rounded) and 4 (flattened); SSC was measured on day 1 and 7 using ATAGO® manual refractometer calibrated as the percentage of sucrose at 20 °C and MALIC was determined as the percentage of malic acid by based-acid titration 0.1 N pH 8.1. Fruit texture attributes were evaluated by "TA.XT plus" texturometer (Texture Technologies Corp., USA) using a 7.9 mm plunger by penetration on one side of the fruit after removing the skin as the destructive method (stress area of 5 mm^2) at harvest (day 1) and seven days after harvest (day 7) including maximum stress force (FMAX in N; considered as the texture attribute that best defines fruit firmness), maximum force area (Amax) in N × mm, bioyield (BYD) as the first point on the force-deformation curve, elasticity (Young) as Young module in N/mm, peaks (PKS) as microcracks counting in the stress area, total force area (ATotal) in N × mm, final force in the stress area (FFinal) in N and linear distance (DLinear) in N × mm.

Finally, regarding postharvest parameters, a fruit sample was stored at 20 °C to monitor loss of weight (LW), skin chlorophyll degradation (I_{AD}_1 − 7), and fruit softening (Fmax_1-4-7) for the years 2017 and 2018 (Table S2), for all F1 individuals. As shown in Figs. 3 and 4, we noticed a high inter-season variability in the fruit softening parameters, therefore we decided to monitor fruit softening trait. LW was calculated as fruit weight loss percentage between day one and day seven, while I_{AD}_1-7 was determined as a chlorophyll index difference between days 1 and 7. According to fruit texture evolution, Fmax_1-4-7 was considered as a result of firmness by compression (N) as a non-destructive method for days 1, 4, and 7. I_{AD} was measured using DA-meter (Gottardi et al., 2009; Infante et al., 2011), and Fmax was quantified in Newton using a 20 mm diameter probe by compression (stress area of 3 mm^2) as a non-destructive method. Fruit softening was determined by several possible combinations, including softening by firmness difference between maturity states (Fmax_1-4, Fmax_4-7 and Fmax_1-7) and as a percentage (Fmax(%)_1-4, Fmax(%)_4-7 and Fmax(%)_1-7). The softening rate was calculated as the slope value (SLP_1-4, SLP_4-7, and SLP_1-7) or slope angle (Ang_1-4, Ang_4-7, and Ang_1-7) by simple linear regression between evaluation moments.

2.3. Data analysis

Fruit quality and postharvest parameters were analyzed by the Shapiro-Wilk test and ANOVA for parametric analysis of variance components, considering genotypes and years as independent factors. Pearson coefficients and heritability in the broad sense on an average basis (H^2) were calculated. Broad sense heritability was defined as $H^2 = \sigma_G^2/(\sigma_G^2 + (\sigma_e^2/n))$, where σ_G^2 and σ_e^2 were the genotypic and residual variances, respectively, and n is the number of replicates (years) of each individual (Doligez et al., 2013). To determine the fruit softening with non-destructive methods, the firmness slopes between days 1, 4, and 7 were estimated using fruit firmness by compression. Thus, firmness slopes were calculated by linear regression model between days simulating three possible softening slopes between day 1 and day 4, day 4 to day 7, and between days 1, 4, and 7. The angle for each softening slope was calculated as the arctangent of slope value. Finally, multivariate hierarchical cluster analysis was implemented to determine softening rate groups using average linkage and Euclidian

distance to cluster F1 individuals, according to BLUP coefficients from the softening angle between years (see below). The Best Linear Unweighted Predictor (BLUP) coefficients were calculated by General Linear Models using year factor as a fixed effect and genotype factor as a random effect. All analyses, histograms and hierarchical clustering for fruit softening were carried out using INFOSTAT v16 software.

In addition, for phenology, fruit quality traits, and texture attributes (destructive method), we estimated BLUPs of the genotypic effect on each fruit quality trait, to use them as phenotypic values in QTL analysis. This estimation was performed first by constructing linear mixed-effects models for each trait, considering the repeated measurements obtained in the three seasons (up to 10 fruits per F1 individual), as well as random factors, corresponding to covariates selected on the basis on their correlation significance (p-value < 1E-6, see below) with the modeled trait. In this way, the resulting BLUP of genotypic effects for each modeled trait considered: i) the effect of traits associated to maturity and their variation inside the fruit sample, e.g., using maturity markers such as I_{AD}, color and/or firmness to model phenotypic values for a ripening-depending trait (SSC, acidity or fruit weight); ii) the seasonal variation effect, using harvest season as a random factor; iii) the pleiotropic effect of some traits over modeled traits (FDP or ripening time are pleiotropic for other fruit quality traits in *Prunus*; Eduardo et al., 2011); and iv) importantly for postharvest parameters which evolve through ripening (e.g., such as texture attributes or I_{AD}), constructed models considering the effect of time after harvest: in this way, we included texture attributes measurements at harvest and seven days after harvest as fixed factors, so the estimated predictors take into account the evolution rate of these traits through time.

To model each trait, considering the effect of genotype and season (among others), phenotypic data from three years (including phenology, fruit quality, and postharvest parameters) were analyzed by the R software (R Core Team, 2017). Linear correlation analysis was run among traits to identify candidate covariates useful as a fixed-effects factor in a mixed-effects linear model. In this way, using the "cor()" function, phenotypic correlation matrices were calculated between trait pairs, using all the available data points (complete observations, Kendall method), considering all the biological replicates during three seasons. The "corrplot()" function was used to draw correlation plots and an ad-hoc script (Fig. S1) that allowed to depict only highly significant correlations (p-value < 1E-6). Finally, for each trait, the "lmer ()" function from lme4 package was used to perform mixed-effects linear model analysis with the restricted maximum likelihood (REML) method and determine BLUP of each F1 individual, without using pedigree information (Piepho et al., 2008). Genotype and year were considered as random factors, while candidate covariates were considered fixed factors in an unbalanced design (Capistrano et al., 2005). Models were constructed iteratively including or discarding candidate covariates until the model showed an acceptable level of goodness-of-fit, considering three criteria: i) lowest AIC value, ii) normality of the residuals (visually checked in QQ plots) and iii) heteroscedasticity of the residuals (visually checked in residual plots). Significance values for random factors were determined using the "rand()" function from lmerTest package.

2.4. Saturation of the genetic linkage map by using SNP and SSR markers

A saturated genetic map was constructed by SNP and SSR markers using 151 individuals from the '98 − 99' × 'Angeleno' progeny. SNP variants were obtained from a previous GBS carried out in the same population (Salazar et al., 2017). In this work, we have constructed a new genetic map using SNP data from the 151 individuals and updating the SNP positions from peach genome v.1 (Verde et al., 2013) to peach genome v2.0 (Verde et al., 2017). In addition, to give greater consistency to the genetic maps, we genotyped a total of 41 microsatellite (Single Sequence Repeat, SSR) markers.

Total genomic DNA was extracted from 151 individuals as describe Doyle and Doyle (1987) and DNA was amplified using SSRs from different *Prunus* species including peach (Cipriani et al., 1999; Testolin et al., 2000; Sosinski et al., 2000; Dirlewanger et al., 2002) and apricot (Hagen et al., 2004; Messina et al., 2004) along different chromosomes. Primers were synthesized by Integrated DNA Technologies (IDT). PCR reactions were performed in 15 µl volume, as described by Sánchez-Pérez et al. (2006). PCR products were separated by gel electrophoresis using 3 % Metaphor® agarose (Biowittaker, Maine, USA; 0.5 × TBE buffer) stained with GelRed™ Nucleic Acid Gel Stain® (Biotium, Hatwad, CA, USA). Finally, pseudo-test cross strategy was employed to construct parental genetic linkage maps, using updated SNPs and SSRs positions in the peach genome v2 as fixed order in JoinMap v.3.0 software (Ooijen, 2006) using Kosambi mapping function with a frequency of recombination between 0.1 and 0.3. Besides, SNPs with unbalanced locus genotype frequency or with inconsistency between genome and map position were filtered (Salazar et al., 2017).

2.5. Marker trait associations and QTL identification

In order to determine genomic associations to traits we followed two ways: i) application of General Linear Models (GLM) using thousands of SNPs from GBS to search for direct single marker-trait associations, and ii) construction of a female and male genetic map to search for QTL intervals linked to phenology, fruit quality, texture attributes and postharvest parameters evolution, season by season. To obtain a consensus phenotypic data for each trait across the three evaluated seasons, we constructed different mixed-effect linear models to estimate BLUPs for each trait and the F1 individuals using the result of the sum of mean and BLUP for each trait as a phenotypic data in the marker-trait analyses. In the case of postharvest parameters that were evaluated for two seasons, only marker-trait associations and QTLs were considered season by season.

Marker-trait association analyses between SNPs and phenotypic data including postharvest parameters, were carried out by TASSEL v5 by General Linear Model (GLM). GLM function in TASSEL v5 makes an association analysis using a least-square fixed effects linear model using principal component analysis (PCA) from genotypic data joined to phenotypic traits to define consistent associations between genotypes and traits (Bradbury et al., 2007). Then, we ran marker-trait associations by GLM using Manhattan plots, for better visualization of the effect of each genomic association by chromosome.

On the other hand, QTL mapping analysis was performed with MapQTL v6 software (Ooijen, 2009), for each parent and map data. Phenotypic data employed in these analyses corresponded to the BLUP estimations described above. Interval Mapping (IM) and Kruskal-Wallis (KW) were first used as parametric and non-parametric single marker-trait analyses, respectively. We employed Permutation Test (PM) function in MapQTL v6 (10,000 permutations) to determine the genome-wide LOD significance threshold corresponding to a p-value of 0.05 and considered as significant QTLs with a LOD value higher than the threshold determined with PM. Cofactor markers that best represented QTLs from IM were used by the Automatic Cofactor Selection (ACS) function. Multiple QTL Model (MQM) was employed to determine QTLs interacting in the genetic control of each trait and to reduce the linkage-associated residual variance, and thus tighten the QTL interval. This approach helps to clarify the most significant marker-trait associations, allowing in some cases to recover different QTLs in strong linkage; this was made using the cofactor markers obtained with ACS. If a new significant QTL appeared different from cofactors, this ACS-MQM process was iterated, until any new QTL were noticed. Marker trait associations by GLM and QTL identification by IM were calculated season by season. In addition, phenotypic data obtained from the BLUP for such trait plus the median value of the three seasons were used to obtain a consensus marker-trait association by GLM and QTL identification by MQM. Finally, linkage map figures were generated using MapChart 2.5 software (Voorrips, 2002).

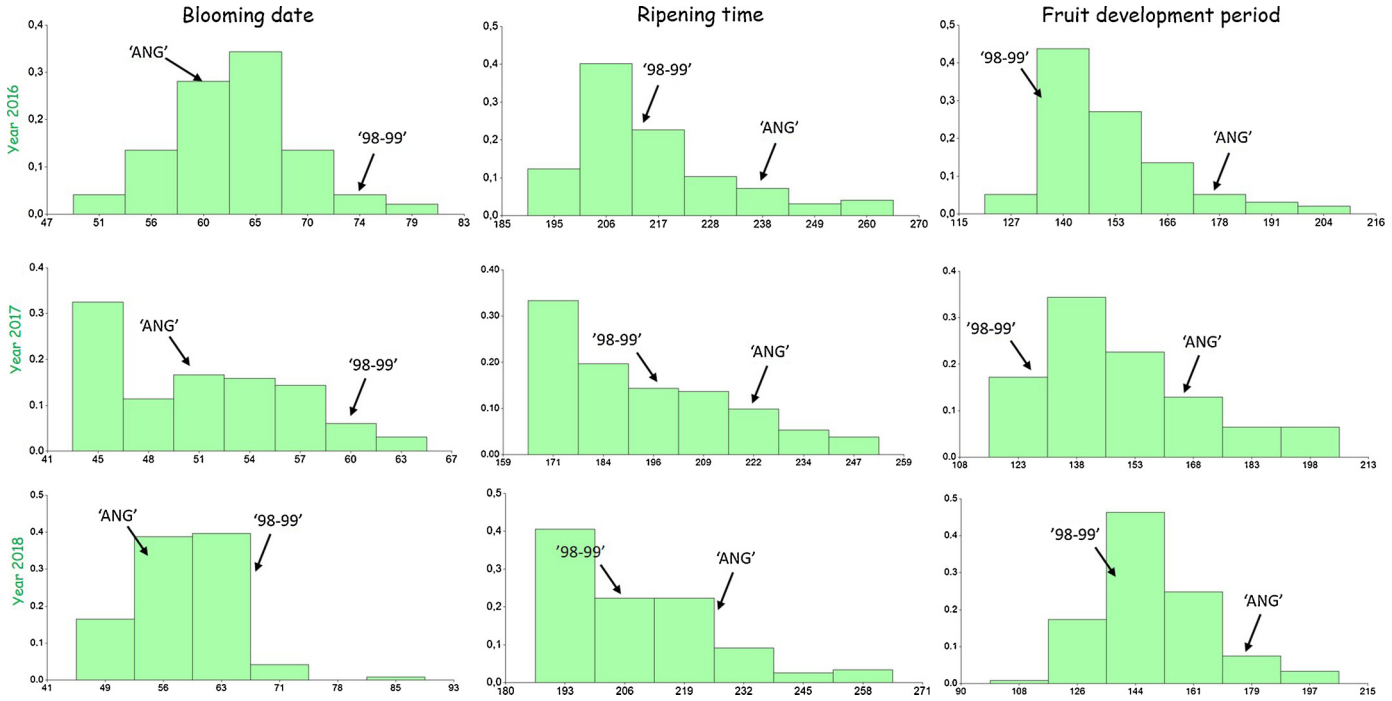

Fig. 1. Frequency histograms showing mean grouped values for assayed phenological traits in three different seasons (2016, 2017 and 2018): blooming date (Julian days), ripening time (Julian days) and fruit development period (days) evaluated in the F1 Japanese plum population' 98–99' × 'Angeleno'.

3. Results

3.1. Evaluation of phenology, fruit quality traits and texture attributes

Results of phenology and fruit quality traits evaluation showed a great variability and segregation. Frequency histograms showed a normal distribution (Figs. 1 and 2), checked with the Shapiro-Wilk test, especially SSC_1, SSC_7, and MALIC (p = 0.3970, 0.9302, and 0.89, respectively; Table S3). However, the histogram distribution of phenological traits showed positively skewed distributions, reflecting a predominance in early or medium season individuals, over late or very late (Fig. 1).

FW ranged between 16 and 118 g, showing a normal distribution, with positive skewness intensity depending on the year. SSC ranged between 11–29 % in three seasons, and there were no significant differences between day 1 and day 7, with an average of 18 % (Fig. 2 and Table S1). The MALIC level ranged between 0.6 and 2.5 %, with an average over three seasons between 1.3 and 1.5 %, and SSC/MALIC ratio was over 10 for three seasons (Fig. 2 and Table S1). As for SHP, around 50 % of individuals showed a spherical shape (3), followed by a 15–30 % of elongated (1) and a 15–20 % of slightly flattened (4) fruit (Fig. 2).

After the ANOVA (Table S4) and Kruskal-Wallis (Table S5) analysis, significant differences between seasons and genotypes were observed, except for BD. Regarding the comparison between seasons, no significant differences for FDP or SHP were observed (Table S5) showing a greater influence of the genetic component. However, the rest of the assayed traits showed differences between seasons with a greater environmental influence.

Regarding the correlations between seasons, the highest average value was 0.74 for RT (p-value < 0.0001; Table 1). Other traits such as BD, FDP, FW and SHP showed a good correlation above 0.5 (p-value < 0.0001). Broad sense heritability (H^2) reached values close to 0.9 (in the case of RT and FDP) and between 0.7 and 0.8 (in the case of SHP). This is consistent with high correlations mentioned above (Table S8). According to fruit texture attributes by destructive method, the

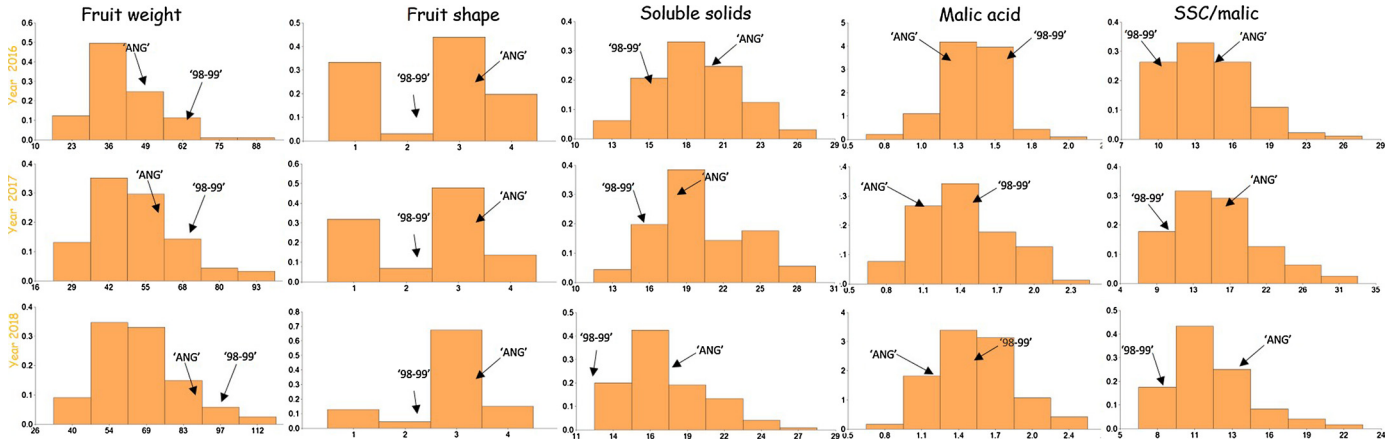

Fig. 2. Frequency histograms showing mean grouped values for the assayed fruit quality traits in three different seasons (2016, 2017 and 2018): fruit weight (g), fruit shape (1-4), soluble solids (%), malic acid (%) and SSC-malic acid ratio evaluated in the F1 Japanese plum population' 98–99' × 'Angeleno'.

Table 1
Pearson correlations between years for phenology, fruit quality traits and texture attributes in the assayed F1 Japanese plum population '98–99' × 'Angeleno'.

Traits	Years	Pearson	p-value	Traits	Years	Pearson	p-value	Traits	Years	Pearson	p-value
BD	16vs17	0.54	< 0.0001	Amax_1	16vs17	0.29	0.0097	Amax_7	16vs17	0.13	0.2865
	17vs18	0.59	< 0.0001		17vs18	0.43	< 0.0001		17vs18	0.52	< 0.0001
	16vs18	0.49	< 0.0001		16vs18	0.35	0.0008		16vs18	0.38	0.0003
RT	16vs17	0.75	< 0.0001	ATotal_1	16vs17	0.49	< 0.0001	ATotal_7	16vs17	0.39	0.0011
	17vs18	0.78	< 0.0001		17vs18	0.36	0.0007		17vs18	0.59	< 0.0001
	16vs18	0.69	< 0.0001		16vs18	0.38	0.0002		16vs18	0.44	0.0002
FDP	16vs17	0.74	< 0.0001	BYD_1	16vs17	0.51	< 0.0001	BYD_7	16vs17	0.31	0.0109
	17vs18	0.72	< 0.0001		17vs18	0.45	< 0.0001		17vs18	0.62	< 0.0001
	16vs18	0.63	< 0.0001		16vs18	0.47	< 0.0001		16vs18	0.43	0.0001
FW	16vs17	0.47	< 0.0001	DLinear_1	16vs17	0.63	< 0.0001	DLinear_7	16vs17	0.48	< 0.0001
	17vs18	0.54	< 0.0001		17vs18	0.55	< 0.0001		17vs18	0.69	< 0.0001
	16vs18	0.59	< 0.0001		16vs18	0.51	< 0.0001		16vs18	0.50	< 0.0001
SHP	16vs17	0.68	< 0.0001	FFinal_1	16vs17	0.36	0.0013	FFinal_7	16vs17	0.23	0.064
	17vs18	0.57	< 0.0001		17vs18	0.33	0.0019		17vs18	0.48	< 0.0001
	16vs18	0.59	< 0.0001		16vs18	0.30	0.0046		16vs18	0.32	0.0027
MALIC	16vs17	0.50	< 0.0001	FMAX_1	16vs17	0.46	< 0.0001	FMAX_7	16vs17	0.28	0.0199
	17vs18	0.50	< 0.0001		17vs18	0.44	< 0.0001		17vs18	0.61	< 0.0001
	16vs18	0.39	0.0002		16vs18	0.46	< 0.0001		16vs18	0.41	0.0001
SSC	16vs17	0.36	0.0011	PKS_1	16vs17	0.52	< 0.0001	PKS_7	16vs17	0.40	0.0008
	17vs18	0.41	0.0001		17vs18	0.54	< 0.0001		17vs18	0.64	< 0.0001
	16vs18	0.22	0.0379		16vs18	0.49	< 0.0001		16vs18	0.39	0.0002
SSC/MALIC	16vs17	0.37	0.0018	Young_1	16vs17	0.52	< 0.0001	Young_7	16vs17	0.38	0.0014
	17vs18	0.25	0.0317		17vs18	0.44	< 0.0001		17vs18	0.59	< 0.0001
	16vs18	0.15	0.1578		16vs18	0.45	< 0.0001		16vs18	0.45	< 0.0001

BD: blooming date; RT: ripening time; FDP: fruit development period; FW: fruit weight; SHP: fruit shape; MALIC: malic acid content; SSC: soluble solids content; Amax: maximum stress force; ATotal: total force area; BYD: bioyield; DLinear: force linear distance; FFinal: final force; FMAX: maximum stress force; PKS: micro-rotures counting; Young: elasticity module. At harvest (_1) and seven days after harvest (_7).

Pearson correlation between seasons was in general lower than 0.5, indicating either; i) the difficulty in the measurement of such parameters to detect significant genetic effects, or ii) a low genetic determinism.

Despite the low Pearson correlation between years for texture attributes measured with a destructive method, a high correlation was observed by year. However, the trait defined as the number of micro-fractures in the stress area (PKS) during the penetration of the plunger in the fruit flesh was the most remote in the PCA (Fig. S2)

3.2. Postharvest parameters

Most of the postharvest parameters, including LW, SI_{AD}, FI_{AD}, and Fmax showed a normal distribution according to the Shapiro-Wilk test and histograms (Fig. 3 and Table S6). The average LW reached 2 % and ranged between 1–8 %, SI_{AD} ranged between 0.79 and 1.12, and firmness as non-destructive method scored between 8 and 15 N for days 1 and 7 respectively (Table S2). According to ANOVA, we can highlight that SI_{AD}_1 showed significant differences among genotypes but not between seasons, as well as SLP_1-7 and Ang_1-7 or SLP_4-7 and Ang_1-7 showed differences between genotypes but not between seasons at p-value < 0.06, which are indicating different softening rates among individuals, with a low influence of the environment (Table S7).

Regarding Pearson coefficients between seasons, the most important correlation was for SI_{AD}_1-7(%), reaching 0.71 values (Table 2), indicating on each season similar chlorophyll degradation. Moreover, the most important correlation between chlorophyll degradation and softening rate was between SI_{AD}_1-7 and Fmax_1-7 in 2017 with a correlation over 0.5, that high softening rates could be related to faster chlorophyll degradation (Table 2). Consequently, in the hierarchical clustering for fruit softening, according to fruit softening angle between days 1 and 4, we can observe two clusters for softening rate, with red branches representing those individuals with high fruit softening rate as '98–99' female parent (49.26°), and blue branches corresponding to those individuals with a low softening rate as 'Angeleno' male parent (25.19°) (Fig. 4).

3.3. Genetic linkage mapping

We mapped a total of 1207 SNPs, 554 for '98 − 99' female parent and 654 SNPs for 'Angeleno' male parent, in the new version of the '98 − 99' × 'Angeleno' linkage map. Regarding the assayed SSRs, a total of 19 molecular markers were mapped in '98 − 99' and 21 in 'Angeleno' (Fig. 5). The resulting maps, including SSRs and SNPs, reached total genome coverage of 557.32 and 576.51 cM, with average density values of 0.97 and 0.85 cM between adjacent markers for female and male parents, respectively, indicating high saturation genetic maps (Table S9). About marker segregation type, 233 markers (18.7 %) segregated strictly for the female parent (lmxll), 435 markers (34.9 %) for the male parent (nnxnp), while 579 markers (46.4 %) were common for both parents, indicating greater heterozygosity in the case of 'Angeleno'. In general, SSRs mapped equivalent positions in the peach genome v2.1. Some examples would be UDAp456 in the LG2 was mapped between S2_18726351 and S2_19259628 (Phytozome = Pp02:19271290), PaCITA10 in the LG3 between S3_19616500 and S3_20448107 (Phytozome = Pp03:19499228) or UDAp439 in the LG4 between S4_8330704 and S4_10075270 (Phytozome = Pp04:10213007).

3.4. Marker trait associations and QTL identification for phenology and fruit quality traits

Regarding marker-trait associations and QTL analysis, several phenology and fruit quality traits have been successfully correlated to different genomic regions (Tables S10, S12 and S13). As for marker-trait associations by General Linear Model (GLM), the most significant associations were found in the LG4 and LG2 for fruit development period (FDP) and fruit weight (FW), respectively, as displayed on Manhattan plots (Fig. 6). In addition, significant QTLs for phenology traits as blooming date (BD), ripening time (RT) and fruit development period (FDP) were identified as well as for fruit quality such as fruit weight (FW), polar diameter (POL), malic acid (MALIC) or soluble solids content (SSC) were also identified.

Additionally, with MQM strategy and BLUP values as phenotypic data, we could identify 18 and 11 QTLs from '98 − 99' and 'Angeleno',

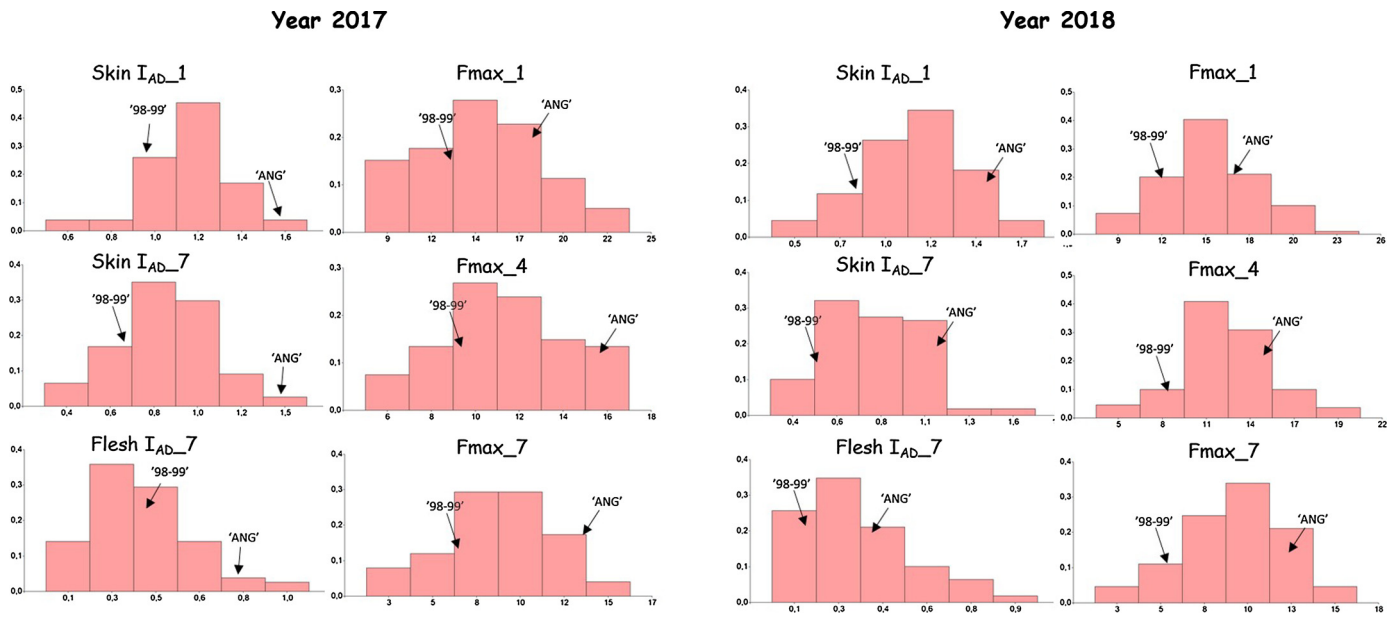

Fig. 3. Frequency histograms showing mean grouped values for the assayed postharvest parameters (non-destructive methods) in two different seasons (2017 and 2018): skin chlorophyll index for day 1 and 7 (SI_{AD}_1 or 7), flesh chlorophyll index for day 7 (FI_{AD}_7), firmness by compression for days 1, 4 and 7 (Fmax_1-4-7) evaluated in the F1 Japanese plum population' 98–99' × 'Angeleno'.

Table 2
Summary of the most significant Pearson correlations for postharvest parameters using non-destructive methods in the assayed F1 Japanese plum population '98–99' × 'Angeleno'.

Pearson correlations between years			p-value
Trait	Year 1 /Year 2	Pearson	
LW_1-7(%)	2017vs2018	0.51	< 0.0001
SI_{AD}_7	2017vs2018	0.63	< 0.0001
SI_{AD}_1-7(%)	2017vs2018	0.71	< 0.0001
Fmax_4	2017vs2018	0.52	< 0.0001
Fmax_7	2017vs2018	0.52	< 0.0001
Fmax_1-4(%)	2017vs2018	0.51	< 0.0001
Fmax_4-7(%)	2017vs2018	0.51	< 0.0001
Fmax_1-7(%)	2017vs2018	0.59	< 0.0001
Pearson correlations between traits			**p-value**
Trait 1	Trait 2	Pearson	
SI_{AD}_1-7(%)_17	Fmax_1-7_17	0.54	< 0.0001
SI_{AD}_1-7(%)_18	Fmax_1-7_18	0.46	< 0.0001
SI_{AD}_1-7(%)_17	Fmax_4-7_17	0.51	< 0.0001
SI_{AD}_1-7(%)_18	Fmax_4-7_18	0.46	< 0.0001

LW: loss of weight; SI_{AD}: skin chlorophyll index; Fmax: maximum force by compression; SI_{AD}(%): skin chlorophyll index difference between days; Fmax (%): maximum force difference between days. At harvest (_1), four days after harvest (_4) and seven days after harvest (_7).

respectively. Regarding blooming date (BD), QTLs were identified in LG1, LG6, and LG7, explaining together a 46 % of the trait variance in the studied F1 progeny, with significance values (LOD) higher than 5.2. In '98 − 99' percentages of explanation variance (PEVs) were close to 15 % for LG1 (cofactor 1_35496339, additive effect of 2.4 days) and 12 % for LG6 (cofactor 6_20045379, additive effect of 1.9 days; Fig. 7 and Table S19). For 'Angeleno' in the other hand, a BD QTL was identified in the LG7 with a PEV of 19.1 % (cofactor 7_11131142, additive effect of 2.2 days; Fig. 7 and Table S20).

Despite that RT and FDP QTLs were very significant, and since RT is the result of the sum of BD and FDP, we decided to consider only QTLs for FDP. Therefore, the most significant FDP QTL was detected only in the LG4 of 'Angeleno' (Fig. 7; Table S20), since the PEV reached values over 30 % (cofactor 4_10985897, additive effect: + 9.5 days). In addition, this QTL interval was confirmed season by season by Interval Mapping, reaching a PEV value close to 50 % in 2018 (Table S13). The most significant SSR marker close to the peak QTL was UDAp439 which was significant for all seasons, explaining a high PEV value, representing a phenotypic difference of about 20 days between "hh"/"hk" and "kk" genotypes over three years: this suggests that the 'h' allele could be dominant for earlier RT and/or shorter FDP (Tables S16 and S17).

Among the detected fruit quality QTLs, we can highlight the strong association of FW in the LG2 of '98 − 99' where 2_18489481 was the most significant cofactor reaching 24 % of PEV and an additive effect of 5.5 g (Fig. 8; Table S19). Interestingly, the closest SSR to this QTL was UDAp456, where individuals bearing "f' allele had fruits with around 13 g more than other genotypes (Table S18). In 'Angeleno', a minor but significant QTL was detected on LG7 (cofactor 7_20071259), with a PEV of 11.8 % and an additive effect of 4.2 g (Table S20).

QTLs related to SHP were identified by IM in the LG8 across three seasons, from both parents, around position 21 Mbp of the reference genome (Tables S12 and S13). More precisely, POL QTLs were located in the LG8 and LG7, being 8_21147873 and 7_18835377 as cofactors and reaching PEV values of 31.7 % and 26.7 %, for female and male parents, respectively, (Tables S19 and S20). Besides, we suggest that the genetic background of FW and POL traits have common genetic determinants in the LG7 (Table S20).

In the case of MALIC and SSC QTLs, no important QTLs were identified by IM season by season. However, after MQM analysis of BLUP values, some genomic associations between these traits and LGs were detected (Tables S19 and S20). Thus, MALIC QTL was detected in the LG3 for both parents, being PaCITA10 SSR marker and 3_16183344 SNP marker as cofactors and reaching around 15 % of PEV. For SSC, the QTLs were identified in the LGs 4 and 5 of female and male parents, respectively, with lower but significant PEV values (9.4–12.7 % and LOD > 4.1). Finally, despite the low PEV of the QTLs for MALIC and SSC, BLUPs could help to locate important fruit quality traits with high polygenic nature, intra-individual variability, and environmental influence.

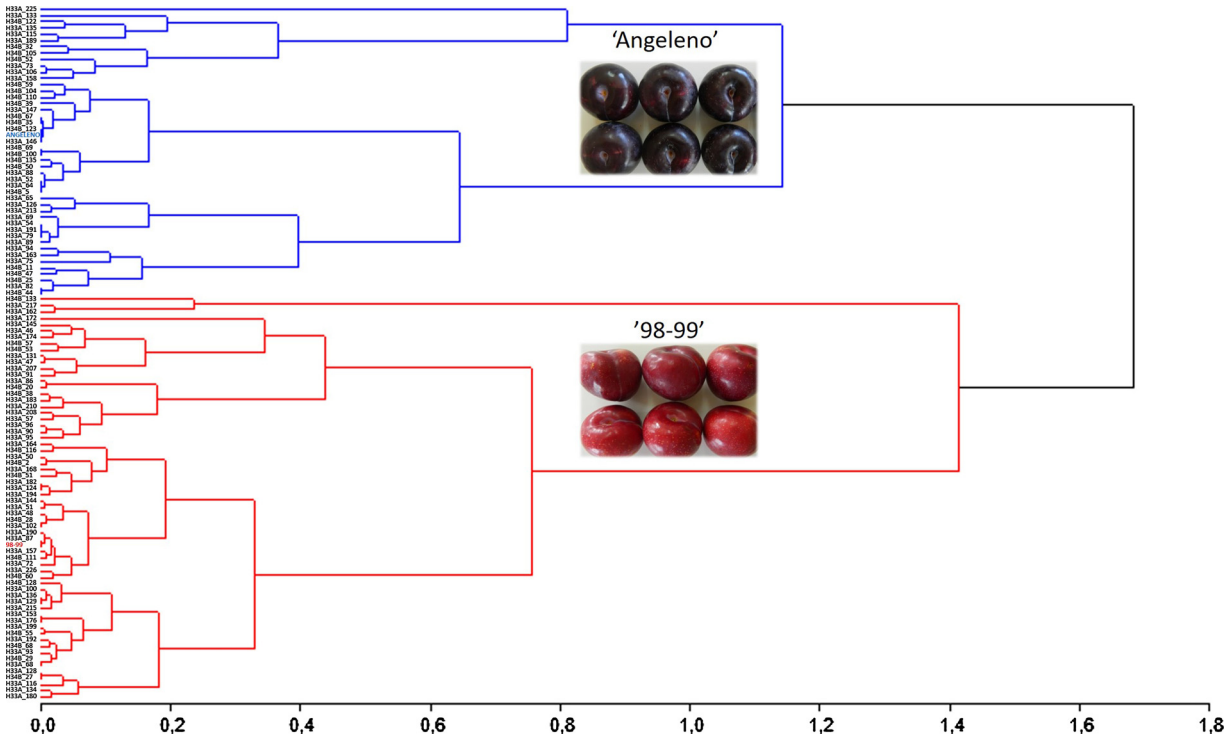

Fig. 4. Hierarchical clustering for fruit softening as angle by non-destructive method between day 1 and 4 of the F1 Japanese plum population'98–99' × 'Angeleno' using BLUPs coefficients between years. Softening angles ranging from 15° (H33A-225) to 40° (H34B-111) are in blue (slow softening) and softening angles ranging from 43° (H33A-116) to 58° (H33A-116) are in red (rapid softening).

3.5. Marker trait associations and QTL identification for postharvest parameters and texture attributes evolution

As for postharvest parameters (non-destructive methods), we applied General Linear Model (GLM) to search for marker-trait associations season by season. We observed several significant markers for loss of weight (LW), skin chlorophyll index between day 1 and 7 (SI_{AD}_1 − 7), flesh chlorophyll index at day 7 (FI_{AD}_7), maximum force (Fmax) for different combination days as well as fruit softening as slope (SLP) or angle (Ang) as non-destructive methods (Table S11). On the other hand, using IM in different season data, all of them were inconsistent between years showing a very low PEV (Tables S14 and S15). However, if we analyze the fruit texture evolution obtained from destructive methods, significant genomic associations for bioyield (BYD) or Young elasticity module (Young) were found in LGs 4 and 5, as shown in Manhattan plots (Fig. 6).

For these traits, MQM analysis employed BLUP predictors. These values were obtained from mixed-effects linear models that included data of these traits in two postharvest moments (1 and 7 days), and thus give an estimation of the change in these values after seven days on each individual. This strategy allowed us to confirm significant QTLs for the evolution of some texture attributes as total area (Atot), bioyield (BYD), maximum force (Fmax), Young elasticity (Young), and breaking peaks (PKS) (Fig. 9 and tables S19 and S20). The most important genomic associations were identified in the LGs 4 and 5 of both parents. For instance, a BYD-related QTL was found in the LG4 of 'Angeleno' reaching a PEV close to 20 % for 'Angeleno' (the slow firmness loss parent); other QTLs such as Atotal, Fmax, PKS or Young were mapped in the LGs 4 or 5 ranging between 10 and 15 % of PEV (Tables S19 and S20). Specially BYD and Fmax are collocating with UDAp439 in the LG4 in both parents. However, MQM results indicated diverse positions of these QTLs in the LG4, between '98 − 99' (~13.167 Mbp) and 'Angeleno' (~7.4–8.3 Mbp); this could be suggesting that i) FDP QTL (around position 10.9 Mbp of 'Angeleno') is pleiotropic to fruit softening-related traits, or ii) fruit softening traits are near to FDP QTL in the LG4 sequence. Finally, some QTLs related to chlorophyll degradation were identified in the female parent for LGs 5 and 6, including skin and flesh chlorophyll degradation (SI_{AD} and FI_{AD}).

4. Discussion

4.1. Evaluation of phenology, fruit quality traits and texture attributes

In the evaluation of most phenology and fruit quality traits, we observed a wide variability range, and some of the evaluated traits showed a normal distribution, especially SSC and MALIC, confirming the polygenic nature and quantitative inheritance of these traits as reported previously in other *Prunus* species as apricots (García-Gómez et al., 2019). Other phenological traits as RT, however, showed bimodal or trimodal distributions, as reported in other *Prunus* such as peach (Eduardo et al., 2011; Pirona et al., 2013) or plum (Salazar et al., 2017) indicating an oligogenic nature.

In addition, most of the evaluated traits showed significant differences between years, suggesting that environmental orchard conditions should affect phenology and fruit quality traits, as reported in peach by Minas et al. (2018). Other phenotypic studies in peach (Eduardo et al., 2011; Serra et al., 2017) and apricot (Ruiz et al., 2010; Salazar et al., 2013) showed exceptionally high inter-annual correlations for harvest date and, in a lesser extent for fruit weight. Thus, the high correlation of FW between years in our progeny reveals a robust genetic effect over the environmental influence. Moreover, RT and FDP showed very high broad-sense heritability (> 0.8), while SHP did with moderate heritability (0.7 − 0.8), making these traits suitable candidates for genetic improvement through Molecular Assisted Selection (MAS). However, FW, SSC, and MALIC showed a lower heritability ranging between 0.5 and 0.6. In sweet cherry (Piaskowski et al., 2018), high broad-sense heritabilities were observed for maturity and fruit weight while Cirilli et al. (2016) in peach revealed moderate to high broad-sense heritability for SSC.

Fruit texture attributes showed a low correlation between seasons,

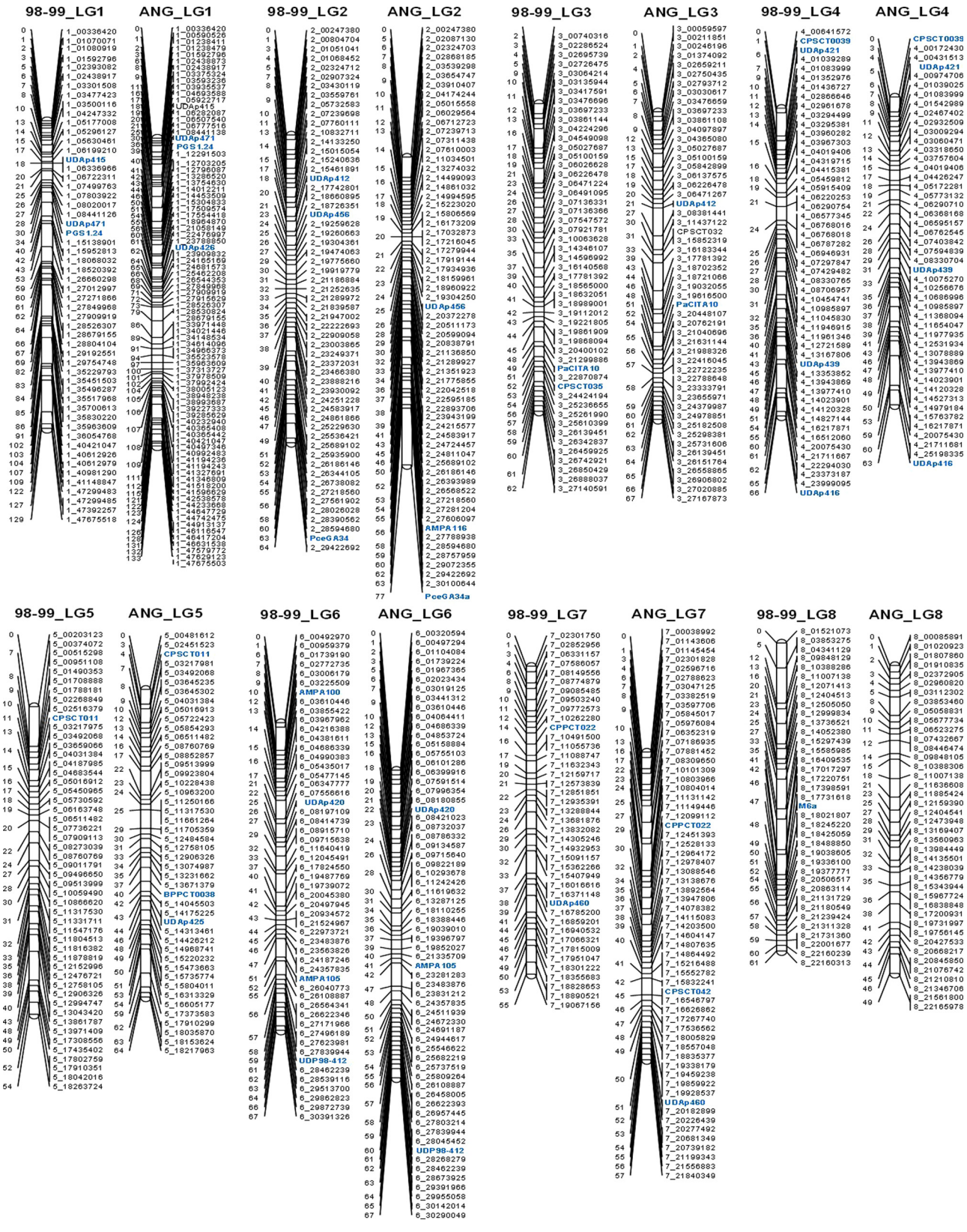

Fig. 5. Genetic linkage maps for each parent in the F1 Japanese plum population' 98–99' × 'Angeleno' (ANG). In black the SNPs positions according to peach genome v2.1 and in blue the new mapped SSRs.

but they showed a high correlation among them except the PKS trait. These results agree with previously reported results by Contador et al. (2016), that defined Young and Fmax as some of the best softening patterns of peach cultivars during its shelf-life period while PKS trait stayed away from these traits in the PCA. This fact also confirms a different firmness pattern related to the number of microfractures in the fleshy stress area.

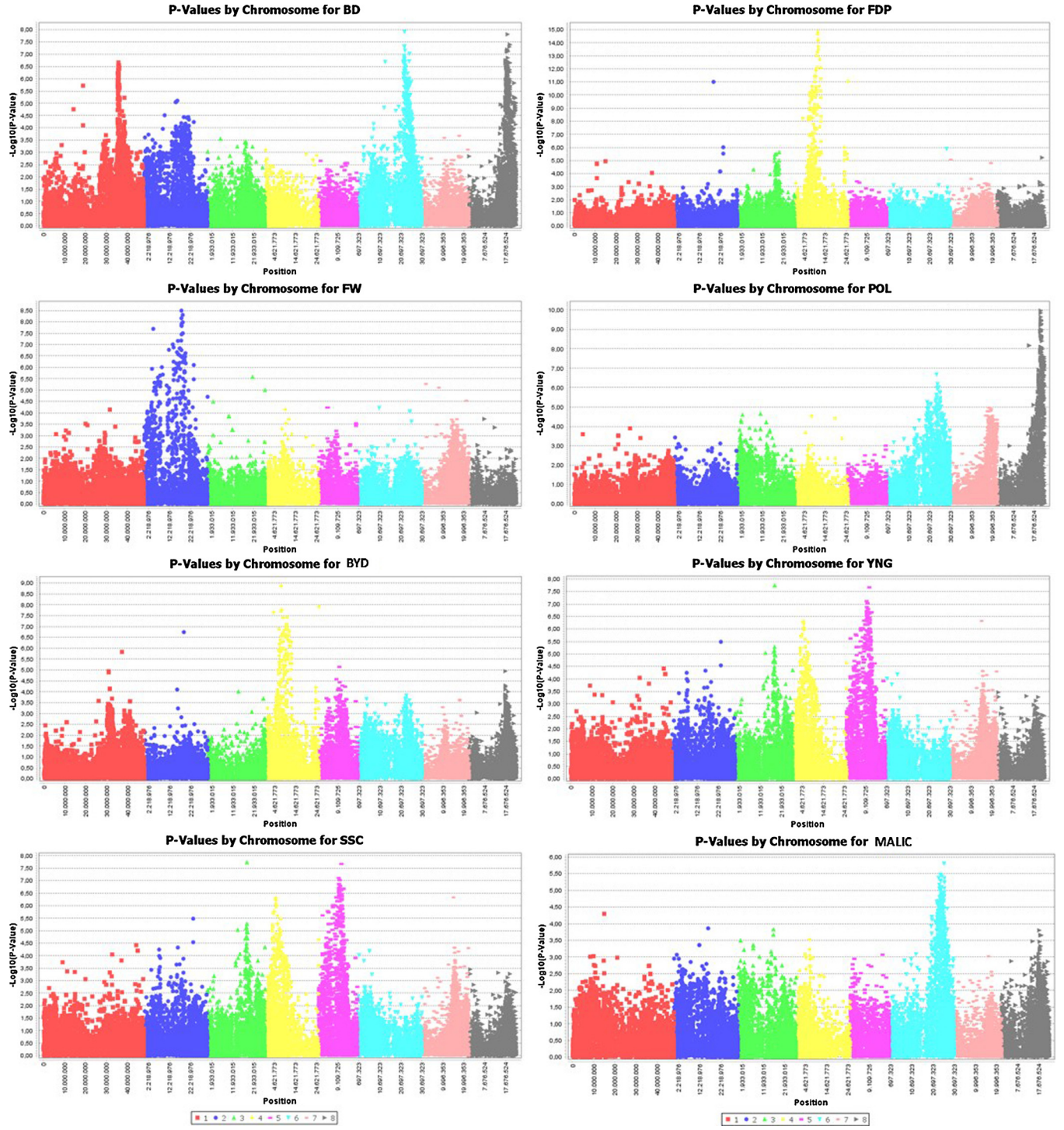

Fig. 6. Manhattan plots generated by TASSEL v5 using General Linear Model (GLM) for blooming date (BD), fruit development period (FDP), fruit weight (FW), polar diameter (POL), Bioyield (BYD), elasticity module (YNG), soluble solids content (SSC) and malic acid (MALIC) using BLUPs coefficients between years.

4.2. Postharvest parameters

Most of the postharvest parameters showed a normal distribution, which is explained by the polygenic nature of these traits, as reported in apple (Ben Sadok et al., 2015). Respect to LW, we considered the loss weight during seven days at 20 °C and, although we observed differences between genotypes (p-value < 0.0001), a more extended trial in cold storage conditions should be necessary to find higher dehydration differences between genotypes (Karaman et al., 2013). In addition, we observed a low but significant correlation between softening rate and chlorophyll degradation, showing that fruit softening rate is related to chlorophyll degradation, as reported by Guyer et al. (2014) in different green plant tissues during leaf senescence and fruit ripening.

Fruit softening slope showed significant differences between genotypes, which could be indicating differences in the shelf-life period between siblings, especially when the trait is measured between days 1 and 4. Besides, minor differences between seasons suggested linear regression as an approximate model to standardize fruit softening rate between days 1, 4, and 7.

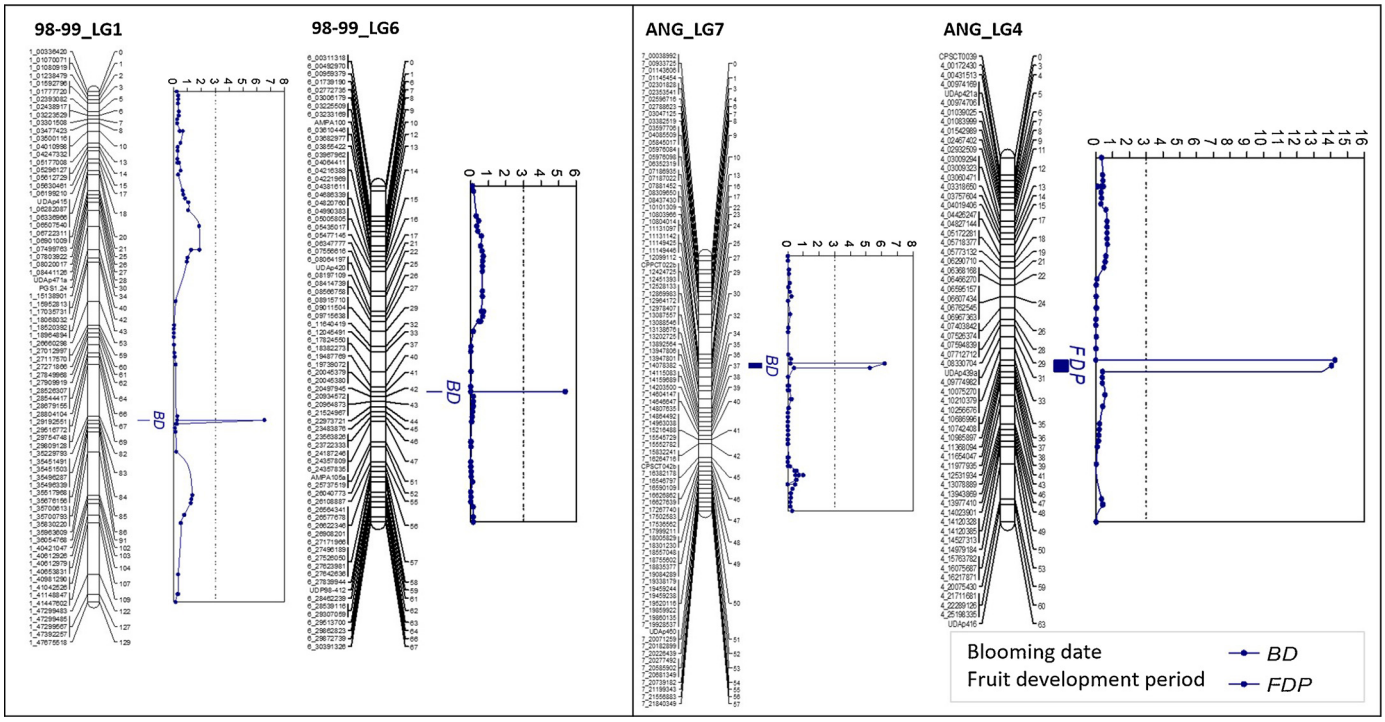

Fig. 7. Multiple QTL mapping (MQM) analysis for phenology traits including blooming date and fruit development period in '98–99' (left) and 'Angeleno' (right) parents.

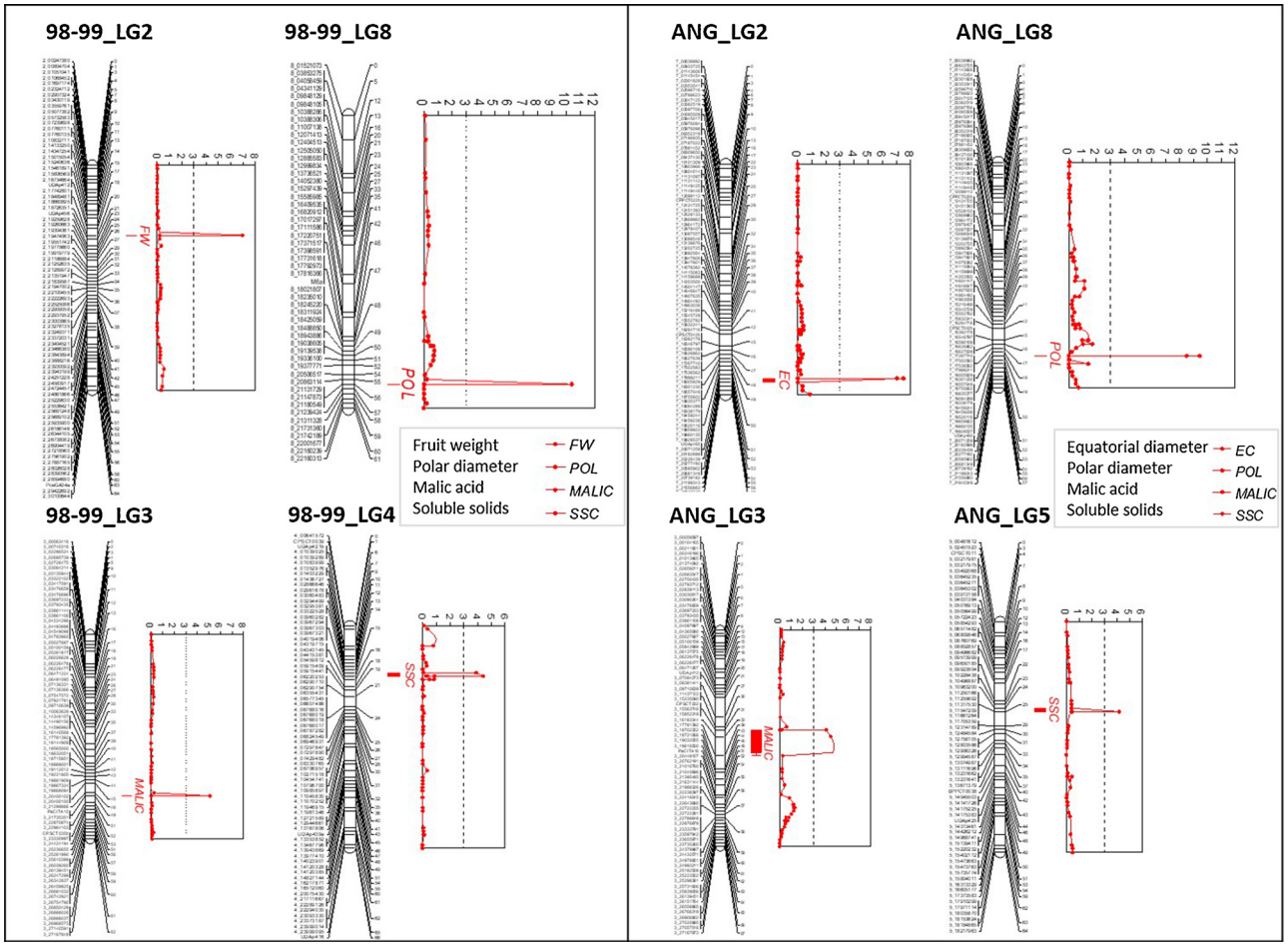

Fig. 8. Multiple QTL mapping (MQM) analysis for fruit quality traits including fruit weight, polar diameter, equatorial diameter, malic acid and soluble solids content in '98–99' (left) and 'Angeleno' (right) parents.

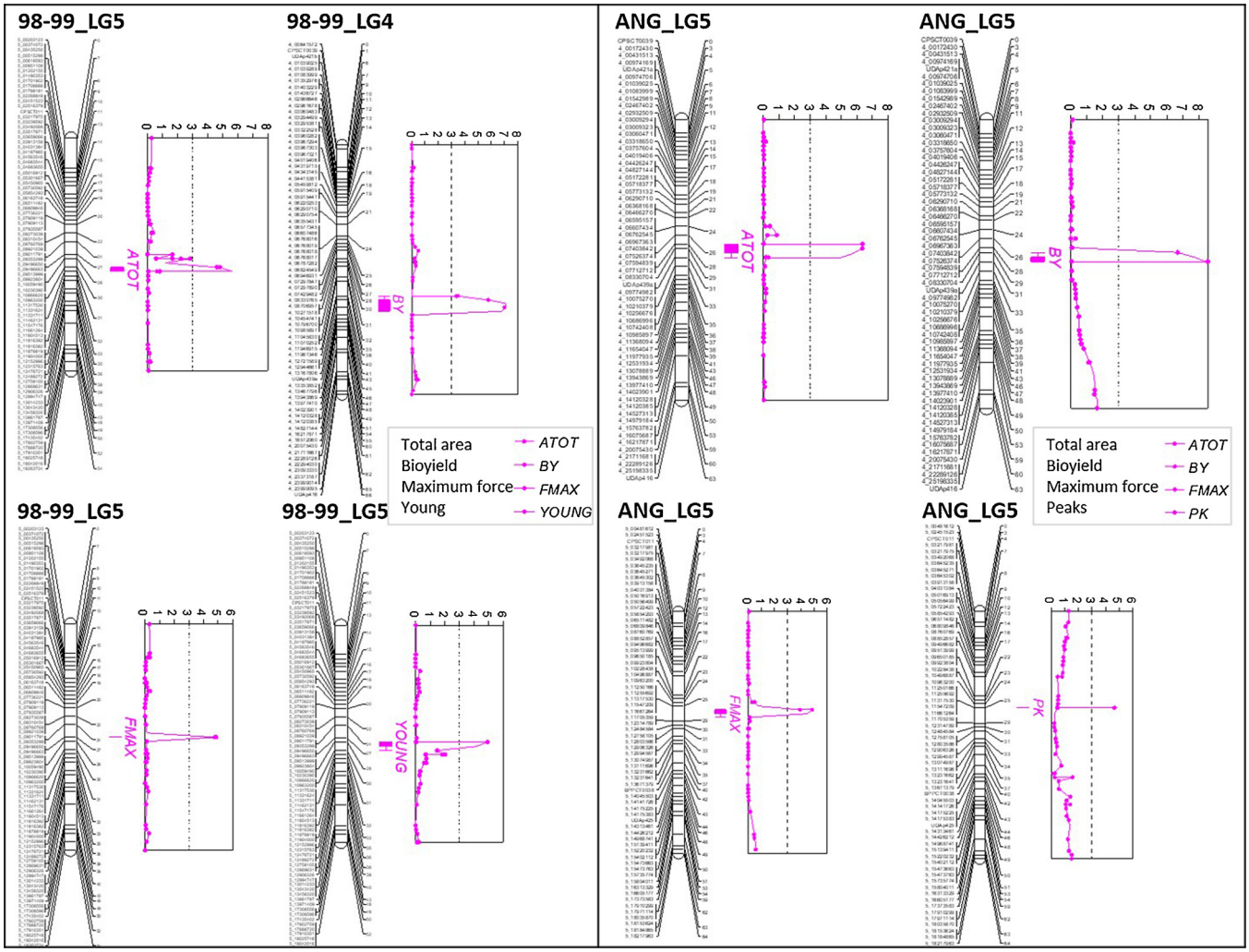

Fig. 9. Multiple QTL mapping (MQM) analysis for fruit texture attributes evolution (destructive method) including total area, bioyield, maximum force and peaks in '98-99' (left) and 'Angeleno' (right) parents.

4.3. Genetic linkage mapping

The genetic map constructed in this work has served to update the SNP positions using Peach genome v2.1 as the reference genome and to add new SSR markers from different *Prunus* species. In most cases, the mapped SSRs matched equivalent positions in the peach genome v2.1 (Goodstein et al., 2012). In other cases, we found some discrepancies between genome and map positions, possibly because to deduce the genome position of SSR markers. We ran BLAST of reported primer sequence against peach genome v2.1 database, instead of using a *P. salicina* genome (not available); thus, a possible lack of microsynteny between peach and Japanese plum genome can be originating these discrepancies.

In the previous genetic map (Salazar et al., 2017), despite the lower number of markers, we obtained more genome coverage with 688.81 and 647.04 cM. However, these differences can be explained because more markers have been mapped in the current map, including SSRs, which are increasing map saturation, but they are decreasing the genetic recombination obtaining a lower coverage in cM. In this context, genome re-sequencing is being widely used through GBS technology, which has been demonstrated as a useful tool for genetic characterization and genetic mapping in stone fruit species, such as *P. persica* (Yu et al., 2018), *P. domestica* (Zhebentyayeva et al., 2019), *P. salicina* (Salazar et al., 2017; Carrasco et al., 2018) or *P. avium* (Guajardo et al., 2015). Therefore, genetic mapping by GBS is the first step for massive marker-trait associations and QTL identification, which is necessary to deep into useful molecular marker design for MAS.

4.4. Marker trait association and QTL identification

In this work, we performed genetic mapping of quantitative traits employing several strategies on the same dataset: single marker analysis by GLM (Fig. 6 and Tables S10 and S11), interval mapping and single-marker analysis by Kruskal-Wallis non-parametric method (Tables S12 to S15) or multiple QTL mapping (MQM) using BLUPs for genotypic effect as phenotypic data (Tables S19 and S20). The latter strategy has been successfully used in the genetic analysis of complex traits in different species and types of populations, such as the QTL analysis of tree architecture development in an apple F1 progeny (Segura and Costes, 2009) or genome-wide association study (GWAS) of micronutrient content in *Aegilops tauschii* (Arora et al., 2019). In these studies, the authors have successfully detected QTLs from BLUPs, to consider the influence of pleiotropic and environmental factors that could influence the traits of interest. In the case of postharvest traits, where repeated measurements were available over time (1 and 7 days after harvest), BLUP represented the genotypic component of the change in the trait through time.*4.4.1 Phenology and fruit quality traits*

We obtained marker-trait associations by GLM and QTLs by MQM analysis linked to BD, with a high dependence from the inter-season variations, which explain the QTL identification along different

chromosomes in different years. Nevertheless, to phenotype separately the mechanisms involved in blooming time such as chilling requirements and breaking dormancy would be an exciting approach to dissect in a more precise way the genetics underlying the mechanisms related to BD (Kitamura et al., 2018; Castède et al., 2015; Bielenberg et al., 2015).

Harvest time-related traits, RT and FDP, were associated to stable QTLs for three consecutive seasons and in the BLUP estimates, in the LG4 as have been previously reported in other *Prunus* species such as peach, apricot and cherry (Pirona et al., 2013; Salazar et al., 2013; Nuñez-Lillo et al., 2015, 2019; García-Gómez et al., 2019), as well as in Japanese plum in few individuals of the current progeny (Salazar et al., 2017). RT and FDP QTLs were confirmed in the male parent ('Angeleno') for three seasons using a progeny constituted by over one hundred individuals. In addition, UDAp439 was the nearest SSR to peak QTL colocalizing with an intronic region of Prupe.4G172400 while the SNP cofactor 4_10985897 co-located with Prupe.4G185300 in an exonic region. Nowadays, the MD in peach has been described as a quantitatively inherited trait, and different NAC-type genes are related with most of the explained variance for maturity date and crop senescence (Pirona et al., 2013; Podzimska-Sroka et al., 2015), as well as other QTLs with minor effects for the trait (Hernández-Mora et al., 2017). Recent studies are trying to design cost-efficient markers to predict MD in stone fruit breeding programs, as reported by Balogh et al., 2018.

Fruit weight is controlled by many loci (Aranzana et al., 2019), a reason to be considered a challenging target for MAS. However, the high correlation between years and the reliable QTL identification in the LG2 of the female parent ('98 − 99') for all years (PEV over 20 %) make this trait affordable for MAS. Therefore, this result is showing a strong influence of the genetic background for this complex trait despite inter-season environment variations. The SNP cofactor 2_18489481 and the nearest SSR UDAp456 are colocalizing with an intergenic region in the peach genome, where unknown regulation mechanisms could be involved in the fruit development. As García -Gómez et al. (2017) reported, the intronic and intergenic regions are more abundant than exonic regions (gene-coding). These unknown regulation mechanisms in the intergenic regions could be due to some epigenetic mechanisms such as DNA methylation which could be affecting fruit development process as it seems to indicate in *Prunus avium* where floral buds exposed to differential chilling hours could be guided by non-coding RNAs (Rothkegel et al., 2017).

Intermittent QTLs between years and LGs have been identified on other *Prunus* species such as peach where Fresnedo-Ramírez et al. (2015) detected QTLs for fruit diameter in LGs 5, 6, and 7 while for fruit weight in the LGs 2, 5 and 6. In this study, QTLs related to fruit shape as POL were identified in the LG7 and LG8, while a minor QTL for FW was identified in the LG7, which it is revealing that the longitudinal fruit growth and fruit weight could partially have common genetic determinants in the LG7.

In addition, minor QTLs for MALIC and SSC were identified by IM. However, we could find more significant QTLs in the LG3 for MALIC and LGs 4 and 5 for SSC by MQM. This approach has been demonstrated to be very useful to unmask fruit quality QTLs controlled by many genes and highly influenced by inter-season variations in other genetic mapping studies regarding complex traits (Pacheco et al., 2014). In other open-pollinated *Prunus* species such as cherry and peach, FlexQTL™ software has been proved as a very powerful tool, determining major QTLs for SSC in LG2 and LG4 while QTLs for titratable acidity were mapped in LGs 2, 4 and 6 (Zhao et al., 2014; Hernández-Mora et al., 2017). However, this software is based on data from different progenies in a common pedigree, where many sources of genetic variation can be participating in the overall variation of the trait under study, which is not the case of this research (one bi-parental cross).

4.4.1. Postharvest parameters and texture attributes

Genomic associations between different postharvest parameters, using GLM and IM, were identified season by season. In postharvest management, fruit dehydration is a crucial factor since it produces a loss of fruit mass, producing an economic repercussion. In this study, an essential QTL for LW between days 1 and 7 after harvest have been identified in the LG5, which suppose an interesting postharvest QTL because no genomic relations have been reported in other *Prunus* species for this trait. However, these differences are not very dramatic values for the industry, which suggests that assays longer than seven days, including cold storage at 0 °C combining shelf-life periods at 20 °C, must be made to confirm a useful LW QTL for postharvest.

Skin chlorophyll degradation along time (SI_{AD}_1-7), could be a useful trait associated with postharvest life. Regarding this trait, a QTL was mapped in the LG4, similar QTLs have been previously reported (Salazar et al., 2017). Therefore, SI_{AD} QTLs for LG4 were confirmed, indicating that many biological pathways are related to fruit ripening in LG4 in several *Prunus* species including ripening date (Eduardo et al., 2011; Dirlewanger et al., 2012; Pirona et al., 2013; Salazar et al., 2016) or skin chlorophyll degradation (Salazar et al., 2017). Regarding QTLs linked to softening rates (non-destructive methods by compression), when we considered the slope (SLP) or angle (Ang) of fruit softening we identified QTLs in the LG5 in similar positions that LW QTL, being 'Angeleno' related to low softening rate, opposed to '98 − 99'. Thus, softening rate and fruit dehydration could be regulated by the same upstream genes, being these traits related to other works such as Kumar et al. (2018). Nevertheless, the fruit softening QTLs showed to be intermittent between seasons, perhaps due to the difficulty of standardizing the harvest time each year. However, MQM using BLUPs and considering the evolution of fruit texture attributes between days 1 and 7, was useful to confirm QTLs of Atotal, FMAX, Young or PKS in LGs 4 and 5. Two QTLs for firmness loss were similarly detected in the same LGs in peach by Serra et al. (2017) in an F1 progeny from two crosses having 'Big Top' (a slow softening nectarine cultivar) as the female parent, and 'Armking' and 'Nectaross' (traditional melting flesh nectarine cultivars) as male parents. In this study, the QTL of LG5 in the slow softening cultivar explained between an 11 and 24 %, depending on the season; instead, QTLs from LG4 co-located with FDP QTLs and explained between a 31 and 57 % of phenotypic variance.

Eduardo et al. (2015) mapped a major gene for slow ripening in peach, near to the previously reported maturity date gene in the same region of LG4. These sources, together with our results, suggest that flesh softening and other ripening-associated processes (e.g. chlorophyll degradation) could have common regulatory elements that are, to some extent, maintained across *Prunus* genus. Other QTLs have been discovered using novel texture parameters that allow differentiating slow softening from melting flesh in peach, such as the Texture Dynamics index (TD) (Ciacciulli et al., 2017a) allowing the mapping QTLs in LG8 and LG1 by GWAS and single-marker approaches (Ciacciulli et al., 2018b). In the future, this strategy could be useful to mine the genetic determinants controlling texture attributes in other *Prunus* species, such as Japanese plum.

Finally, despite the low effect of the identified QTLs for postharvest parameters, the obtained information is of great interest in the context in which no studies about cluster genotypes for softening rate related to a specific genomic region have been published in *P. salicina*. This kind of approach should be completed with the evaluation of more seasons and progenies, in order to find more consistent relationships between genomic regions and postharvest parameters to understand the elements of the ripening process related with fruit softening and to elucidate if it would be possible the molecular assisted selection by molecular markers in these complex polygenic traits (Ben Sadok et al., 2015).

5. Conclusions

In the current Japanese plum breeding programs, the most important challenges are related to integrating reliable data acquisition and analysis of phenology, fruit quality, texture attributes, and evolution of postharvest parameters, as well as to develop efficient strategies for Molecular Assisted Selection. In this species, a saturated genetic map was reconstructed using SNPs and SSRs allowing us to identify relevant marker-trait associations linked to phenology and fruit quality traits as blooming date, ripening time, fruit development period, fruit weight, polar diameter, soluble solids, and malic acid content as well as for the evolution of fruit texture attributes measured by destructive methods as bioyield and Young elasticity. In addition, some of these associations were confirmed by IM season by season, especially for fruit development period and fruit weight. Moreover, some SSRs as UDAp439 and UDAp456 are narrowly linked to fruit development period and fruit weight in LGs 4 and 2, respectively. In the case of fruit softening using non-destructive methods, results showed that two seasons of phenotyping are not enough to generate an adequate model to map softening QTLs. However, some fruit softening QTLs by non-destructive methods are coinciding with QTLs of fruit texture evolution as a destructive method in the LGs 4 and 5. In conclusion, we may claim that this work is providing valuable information for MAS in *Prunus salicina* in the current and future breeding programs.

CRediT authorship contribution statement

Juan Alfonso Salazar: Data curation, Formal analysis, Writing and funding acquisition. **Igor Pacheco**: Data curation, Formal analysis, Writing and Supervision. **Patricio Zapata**: Resources. **Paulina Shinya**: Methodology. **David Ruiz**: Supervision. **Pedro Martínez-Gómez**: Supervision, Review & editing. **R. Infante**: Resources, Supervision, Review & editing.

Declaration of Competing Interest

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

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.postharvbio.2020.111292.

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