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ORIGINAL ARTICLE

Inheritance of reproductive phenology traits and related QTL identification in apricot

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Abstract Reproductive phenological traits of great agronomical interest in apricot species, including flowering date, ripening date and fruit development period, were studied during 3 years in two F_1 progenies derived from the crosses 'Bergeron' × 'Currot' (B × C) and 'Goldrich' × 'Currot' (G × C). Results showed great variability and segregation in each population, confirming the polygenic nature and quantitative inheritance of all the studied traits. Genetic linkage maps were constructed combining SSR and SNP markers, using 87 markers in the 'B × C' population and 89 markers in 'G × C'. The genetic linkage maps in both progenies show the eight linkage groups (LGs) of apricot, covering a distance of 394.9 cM in 'Bergeron' and of 414.3 cM in 'Currot'. The 'Goldrich' and 'Currot' maps were of 353.5 and 422.3 cM, respectively. The average distance obtained between markers was thus 7.59 cM in 'Bergeron' and 7.53 cM in 'Currot', whereas the 'Goldrich' and 'Currot' averages were 5.6 and 7.5 cM, respectively. According to the polygenic nature of the studied phenology traits, QTLs linked to flowering date, ripening date and the fruit development period were identified during the 3 years of the study in all LGs except for LG 8. Among the QTLs identified, major QTLs for flowering and ripening date and the fruit development period were identified in LG 4, especially important in the 'G × C' population.

Keywords *Prunus armeniaca* · Breeding phenology · Flowering · Ripening · Breeding · Molecular markers · SNPlex · SSR

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Introduction

Temperate tree species (including *Prunus* species) have developed a strategy to adapt to alternating and well-differentiated seasons based on bud dormancy. This helps to protect the bud from winter cold, ensuring that flowering occurs under optimal conditions. In these fruit tree species, exposure to cold in the winter (fulfilment of chilling requirements for overcoming endodormancy) followed by a warm period (fulfilment of heat requirements) in spring is essential for flowering (Campoy et al. 2011a; Dirlewanger et al. 2012; Sánchez-Pérez et al. 2012). In addition, the fruit development period and ripening date are other two important phenology traits related to flowering date (Dirlewanger et al. 2012).

In the present century, climate change is considered to be one of the most important environmental problems. This phenomenon has produced significant increases in temperature in recent decades. According to the Intergovernmental Panel on Climate Change (http://www.ipcc.ch/) forecasts, by 2100 the average global surface temperature is expected to have

increased by between 3.7 and 4.8 °C with respect to pre-industrial levels. Accordingly, a reduction in chilling accumulation has been demonstrated in different areas (Baldocchi and Wong 2008; Luedeling et al. 2009a, b). Consequently, climate warming during the winter and spring has been responsible for several already apparent disruptions in temperate fruit trees (Hanninen and Tanio 2011; Luedeling et al. 2011). The symptoms of inadequate chilling fulfilment are delayed bud burst, reduced bud burst and uneven bud burst and flowering (Erez 2000). This situation can affect reproductive phenology in temperate fruit species and their adaptation to the climate change. This issue is especially important in *Prunus* species, which include few low-chill commercial cultivars such as apricot (*Prunus armeniaca* L.), Japanese plum (*Prunus salicina* L.) and sweet cherry (*Prunus avium* L.).

In *Prunus* species, fluctuations in flowering date are mainly due to differences in chilling and heat requirements. Nevertheless, the physiological and biochemical bases controlling flowering date are not completely understood. Different studies have revealed that chilling requirements have a major effect on flowering date compared to heat requirements in *Prunus* species such as apricot (Ruiz et al. 2007; Campoy et al. 2012), almond [*P. amygdalus* (Batsch) syn. *P. dulcis* (Miller) Webb] (Sánchez-Pérez et al. 2012), sweet cherry (Alburquerque et al. 2008; Dirlewanger et al. 2012; Castède et al. 2014, 2015) and peach [*P. persica* (L.) Batsch] (Okie and Blackburn 2008). In fact, in *Prunus* species, the late flowering varieties need more chilling units than the early flowering varieties to fulfil their chilling requirement and bloom (Andrés and Durán 1999; Ruiz et al. 2007; Olukolu et al. 2009; Campoy et al. 2011a; Socquet-Juglard et al. 2013).

On the other hand, for the fruit industry, phenological traits are extremely important. Flowering date is a consequence of chilling requirements and can be used to choose the right cultivar for a particular growing area, ensuring that the chilling requirements will be adequately satisfied and also minimising the risk of spring frost. Ripening date is probably the most important variable determining the price of fruit in early market-oriented production. A short fruit development period is an important breeding trait in combination with late flowering in order to avoid spring frost and produce fruit for the early market. A short fruit development period in combination with early flowering, on the other hand, can make it possible to produce the earliest fruit in the season, meaning higher prices for producers. Flowering date, ripening date and productivity are all traits that are strongly influenced by the fulfilment of chilling requirements (Campoy et al. 2011a).

Recent works on the genetic control of flowering date in *Prunus* have highlighted the importance of at least 3 years of study in order to achieve a precise phenotypic characterisation of phenological traits, thus accounting for year-to-year variability (Sánchez-Pérez et al. 2007a; Castède et al. 2014, 2015). This precise yet time-consuming phenotyping is usually the bottleneck for detecting QTLs (quantitative trait loci). QTLs may make it possible to increase the efficiency of classical breeding programmes through marker assisted selection (MAS) (Salazar et al. 2014). Substantial progress has been made in identifying quantitative trait loci (QTLs) for phenological traits in *Prunus* species including almond (Sánchez-Pérez et al. 2007a, 2012), apricot (Campoy et al. 2011b; Dirlewanger et al. 2012), sour cherry (*P. cerasus* L.) (Wang et al. 2000), sweet cherry (Castède et al. 2014; Dirlewanger et al. 2012) and peach (Fan et al. 2010; Dirlewanger et al. 2012). The mechanisms controlling flowering and ripening date in this species could be conserved as suggested by the co-localisation of QTLs and the identification of common genes in other *Prunus* species (Dirlewanger et al. 2012; Castède et al. 2014).

SSR (simple sequence repeat) markers have been used extensively in genetic mapping studies of several species of the *Prunus* genus and for the detection of QTLs of phenological traits by diverse authors in peach, almond, cherry and apricot (for a review, see Salazar et al. 2014). Microsatellite SSRs are characterised by high variability, relative abundance, low density mapping and a higher error rate in genotyping. SNPs (single nucleotide polymorphisms), on the other hand, are characterised by low variability (low meiosis information), greater abundance, high density mapping and a lower error rate in genotyping (Dhanapal et al. 2012; Ball et al. 2010).

The availability of the peach genome sequence (Verde et al. 2013) facilitates the characterisation of the regions with known QTLs using SNP markers not only in peach but also in related species thanks to the high synteny within the *Prunus* genus (Dondini et al. 2007; Dirlewanger et al. 2012; Klagges et al. 2013). Furthermore, the development of new technological approaches based on next-generation sequencing (NGS) has facilitated genomics-assisted breeding through marker assisted selection (MAS), genome-wide association studies, and genomic selection (GS), as reviewed by Varshney et al. (2014). In apricot, a first approach to developing SNP markers from RNA-Seq libraries has been recently described, entailing a significant decrease in the time and cost of genotyping (Salazar et al. 2015). SNP markers, due to their high abundance, allow us to cover a large extension of the genome and are ideal for genetic mapping (Ball et al. 2010).

The idea of combining the use of SSRs and SNPs for genetic mapping was described by Dhanapal et al. (2012) in the first paper describing chilling injury susceptibility from a molecular point of view in peach. The recent modern SNP genotyping platforms, which are almost completely automated, make the error rate lower than in the case of SSRs, while a higher density of SNPs is required to have equal or greater accuracy than SSRs (Salazar et al. 2015). However, it has been shown that when the genotyping error rates are low, we can obtain accurate maps with both markers (Ball et al. 2010).

This work consists of a 3-year study of the most important reproductive phenological traits (flowering date,

ripening date and fruit development period) in two F1 apricot progenies with the same male parent, the extra-low-chill Spanish landrace 'Currot'. In addition, the identification of stable QTLs has been used to identify putative candidate genes responsible for these reproductive phenological traits.

Material and methods

Plant materials

The plant material assayed included two F1 apricot progenies of 187 and 200 seedlings from the crosses between 'Bergeron' × 'Currot' (B × C) and 'Goldrich × Currot' (G × C), respectively. The 'B × C' and 'G × C' seedlings were planted in field conditions in 2009. 'Bergeron' is a self-compatible French cultivar characterised by high chilling requirements, late flowering and late ripening. 'Goldrich' is a self-incompatible North American cultivar obtained at Washington State University (USA) from the cross 'Sunglo' × 'Perfection'. 'Goldrich' presents high chill requirements, a late flowering date and a middle ripening date. Finally, the common male parent 'Currot' is a self-compatible Spanish cultivar characterised by extra-low chill requirements, a very early flowering date and very early ripening. The cross was designed to maximise the segregation for phenology traits, as 'Currot', the common parent, provides early flowering and ripening dates in both populations.

Phenological evaluation

The phenological traits of interest (flowering date, ripening date, and fruit development period) were determined during three consecutive years (2012, 2013 and 2014) in 'B × C' and 'G × C' populations. Flowering date was evaluated every 4 days and expressed in Julian days (days after January 1st) until 50 % of the flowers were completely opened (F50). Ripening date was determined when the fruits were at the commercial maturity stage and expressed in Julian days. Finally, the fruit development period was calculated as the difference between flowering date and ripening date. Statistical analyses were performed using an SPSS 12.0 package for Windows (Chicago, USA). Differences between genotypes and years as well as genotype-year interactions were determined by analysis of variance (ANOVA). The distribution of the seedling population for each trait was represented in frequency histograms. Bivariate correlations between different traits were calculated with row data of the 3 years, using the Pearson correlation coefficient.

SSR and SNP analysis

Total genomic DNA was extracted from young expanded leaves using the CTAB procedure described by Doyle and Doyle (1987). For mapping, 99 SNPs were selected and organised using 3 SNPlex, and 39 SSRs were analysed by multiplex. The SSR markers used in this work have been developed in peach (Cipriani et al. 1999; Sosinski et al. 2000; Dirlewanger et al. 2002), apricot (Hagen et al. 2004; Messina et al. 2004) and almond (Testolin et al. 2004). SSR amplifications were performed according to multiplex PCR protocol by Hayden et al. (2008), using tag F primer labelled with FAM, VIC, NED or PET fluorescent dyes, while tag R primer was unlabelled. A volume of 2 μl of genomic DNA (concentration 10 ng/μl) was used in a 10 μl mix reaction mix. For fragment analysis, 5 μl of each PCR (with VIC, FAM and NED) were pooled with the 10 μl of PCR (with PET) to prepare a plate with a final volume of 25 μl (1:1:1:2). Finally, 4 μl of the PCR pool was mixed with 5.8 of formamide and 0.2 μl of GeneScan500 LIZ-250 size standard in an ABI Prism 3730 DNA Analyser (Applied Biosystems, MA, USA). SSR peaks were visualised using Peak Scanner 1.0 software. The SNP design and analysis was performed according to Salazar et al. (2015) from 'Rojo Pasión' and 'Z506-7' transcriptome data. 'Rojo Pasión' and 'Z506-7' were obtained from crossing 'Orange Red' × 'Currot' and have a common parent with our populations.

Genetic linkage analysis and QTL identification

The genetic linkage maps for each parent from the 'B × C′ and 'G × C' populations were constructed using JoinMap.4 (Van Ooijen 2006) with the Kosambi function. The map was produced by integrating the molecular markers that segregated in both parents only. All linkage groups (LGs) were calculated with a frequency of recombination of 0.4 and a minimum LOD value of 3. In all cases, higher LOD values were used. For each year, QTL analysis was performed with the MAPQTL software version 4 using interval mapping (IM) (parametric) and multiple QTL mapping (MQM) tests. The phenotypic and genotypic data were analysed together by first performing a test of 1000 permutations to designate a significant LOD score threshold of $\alpha_{0.05}$ for each quality trait and year (van Ooijen 2006). Multiyear analysis was performed by MultiQTL v2.6 software using 3-year data. This analysis combines all years together using a multiple environment option, which increases the accuracy of the QTL detection (Campoy et al. 2015). Automatic cofactor selection and MQM analysis were used in QTLs region repetitive for at least 2 years in order to reduce the residual variance for the effect of the other segregating QTLs. Linkage maps and QTL intervals were drawn using MapChart 2.1 software (Voorrips 2002) and LOD colour gradient by Harry Plotter (Longhi et al. 2012).

Results

Inheritance and correlations of phenological traits

Results of the flowering and ripening date, evaluated in Julian days, and the fruit development period, evaluated in days, in the parents and the two apricot F1 progenies assayed during three consecutive years are shown in Fig. 1. These results indicate a quantitative inheritance of the three phenological traits assayed in the apricot F1 progenies studied. Flowering date and ripening date showed an irregular distribution with high inter-annual variation influenced by the differences in chill accumulation and other climatic conditions. On the contrary, fruit development period showed a more balanced distribution among years (Fig. 1). In addition, some transgressive values (out of the range of parents) were also observed in the three assayed years and populations.

Flowering date showed quantitative transmission to the progenies with values in 2012, 2013 and 2014 ranging between 48 and 88 Julian days in both populations. During these 3 years, the majority of descendants showed a higher range of bloom in comparison with the parents, with 'Currot' blooming earlier than 'Bergeron' and 'Goldrich'. In addition, a small percentage of the offspring had later blooming dates than the parents (Fig. 1).

A late flowering date has been observed to have a greater influence than an early flowering date, especially in the 'B × C ′ population, probably because of the use of 'Bergeron' (an extremely late flowering cultivar) as mother. Differences in flowering date were especially observed in 2012. This was because chill accumulation was later and more intense in this year, occurring in the short time period between January 1 and February 15, which affected dormancy. On the other hand, 2013 and 2014 were more regular years in terms of chill accumulation, as evidenced by a wider segregation for all the studied traits. Differences in chill accumulation were more evident in the 'Currot' parent with earlier flowering in the years 2013 and 2014 (mid-February) with respect to 2012 (late-February). All the observed trends demonstrated that all the studied traits have a polygenic nature (Fig. 1).

Regarding the ripening date of the parents used in this study, 'Currot' is the earliest variety (mid-May) while 'Goldrich' has an intermediate ripening date (first week of June). 'Bergeron' is the latest ripening parent (late June). In 'B × C' and 'G × C' populations, we can observe in general a normal distribution. However, we can also observe, similar to the case of flowering date, that histograms showed a greater grouping of descendants in 2012 than in 2013 (Fig. 1). Results also showed a greater influence of medium ('Goldrich') and late ('Bergeron')

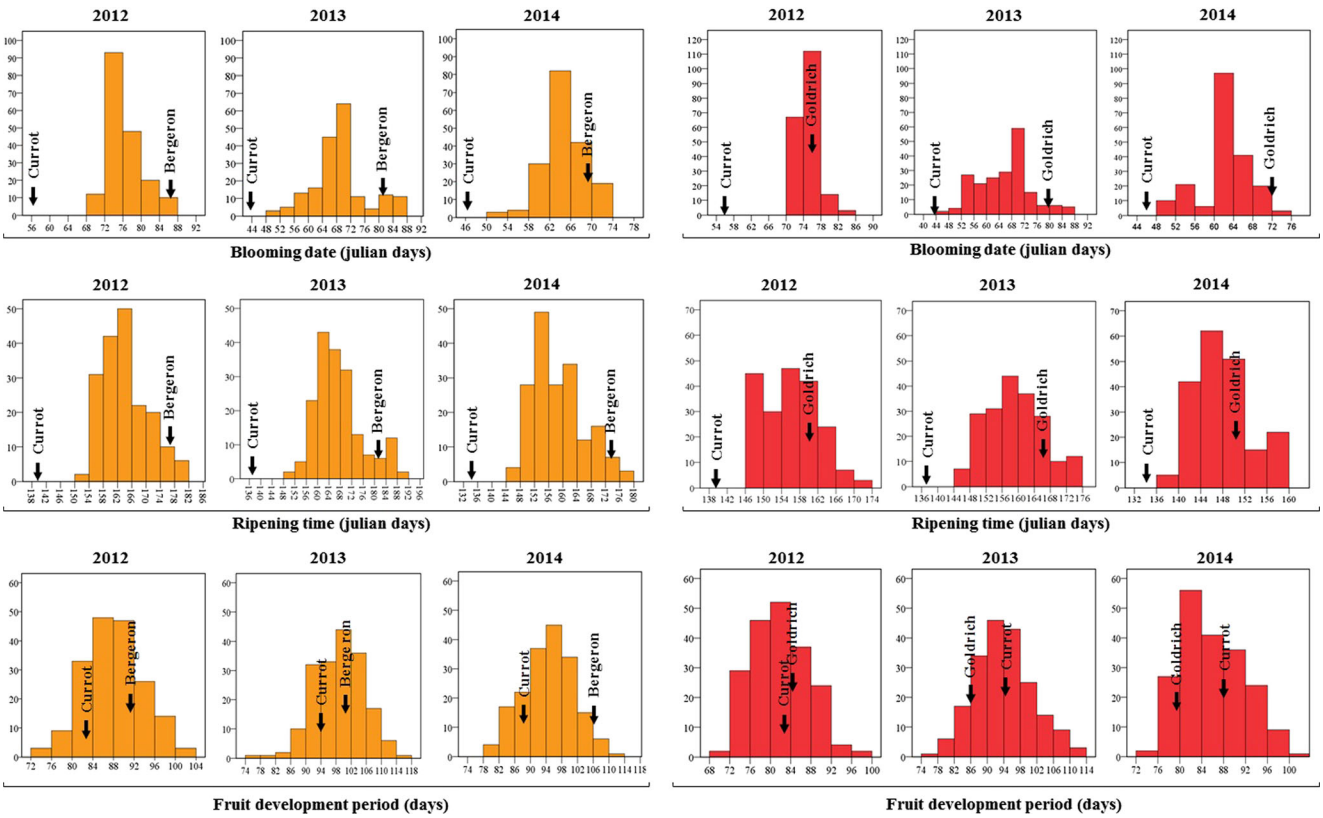

Fig. 1 Distribution of 187 seedlings of 'Bergeron' × 'Currot' (*orange bars*) and 200 seedlings of 'Goldrich' × 'Currot' (*red bars*) F1 apricot progenies for the following phenology traits: flowering date (BD), ripening date (RT) and fruit development period (FDP) for the years 2012, 2013 and 2014

ripening parents in comparison with the early ripening parent 'Currot' in both populations.

Finally, regarding fruit development period, results showed a normal distribution in both the 'B × C' and 'G × C' populations, in consonance with flowering and ripening date, with most of the descendants showing a fruit development period of between 70 and 110 days. In addition, we observed a shorter fruit development period in the year 2012. In 2013, some descendants had a larger cycle, probably due to the earlier flowering date during this year suggesting that early descendants need more time to ripening.

On the other hand, no correlations were found among most agronomic traits in apricot during the 3 years of the study. Correlations were only significant in some cases with Pearson correlation coefficient (r) values higher than 0.5 (Table 1). Despite the annual differences observed in the phenotypical evaluation of the two F1 progenies (Fig. 1), there was nevertheless high correlation among years for all phenological traits studied, especially for ripening date and fruit development period in 'B × C' (0.929**) and 'G × C' (0.868**) progenies (Table 1).

Genetic linkage analysis and QTL identification

Genetic linkage maps of 'Bergeron' × 'Currot' and 'Goldrich' × 'Currot' populations were constructed with 130 and 166 seedlings, respectively. A total of 208 molecular markers were tested, out of which 71 were microsatellites (SSRs) and 137 SNPs. In the 'B × C' population, 87 markers (37 SSRs and 50 SNPs) were mapped in both parents, while in the 'G × C' population, 90 markers were mapped (35 SSRs and 55 SNPs; Fig. 2). The genetic linkage maps in both progenies show the eight LGs of apricot, covering a distance of 394.9 cM in 'Bergeron' and of 414.3 cM in 'Currot'. The 'Goldrich' and 'Currot' maps were of 353.5 and 422.3 cM, respectively. The average distance obtained between markers was thus 7.59 cM in 'Bergeron' and 7.53 cM in 'Currot', whereas the 'Goldrich' and 'Currot' rates were 5.6 and 7.5 cM, respectively. We should also note that, as expected, the two maps from 'Currot' are almost identical because this parental was common in both populations (Fig. 2).

Figure 2 shows the molecular linkage maps constructed with JOINMAP software in the 'Bergeron' × 'Currot' (B × C) and 'Goldrich' × 'Currot' (G × C) F1 apricot progenies. This figure also shows the identification via MapQTL of the QTLs linked to flowering date, ripening date and fruit development period identified during the 3 years of the study (2012, 2013 and 2014). According to the polygenic nature of the studied phenology traits, the QTLs linked to the flowering date, ripening date and fruit development period were identified during the 3 years of the study in all LGs except for LGs 6 and 8.

Table 1 Pearson correlation coefficients between blooming date (BD), ripening date (RD) and fruit development period (FDP) for 187 apricot seedlings from the cross between 'Bergeron' × 'Currot' and 200 apricot seedlings from the cross between 'Goldrich' × 'Currot'

Trait_Year	BD-12	RD-12	FDP-12	BD-13	RT-13	FDP-13	BD-14	RD-14	FDP-14
				'Bergeron' × 'Currot'					
BD-12	1								
RD-12	0.510**	1							
FDP-12	−0.096	0.808**	1						
BD-13	0.765**	0.540**	0.099	1					
RD-13	0.536**	0.929**	0.705**	0.658**	1				
FDP-13	−0.212**	0.518**	0.746**	−0.333**	0.492**	1			
BD-14	0.698**	0.517**	0.116	0.778**	0.647**	−0.096	1		
RD-14	0.362**	0.838**	0.721**	0.455**	0.854**	0.537**	0.494**	1	
FDP-14	−0.023	0.628**	0.745**	0.033	0.569**	0.680**	−0.056	0.839**	1
				'Goldrich' × 'Currot'					
BD-12	1								
RD-12	0.286**	1							
FDP-12	−0.154*	0.903**	1						
BD-13	0.667**	0.400**	0.090	1					
RD-13	0.392**	0.868**	0.713**	0.613**	1				
FDP-13	−0.421**	0.369**	0.588**	−0.631**	0.226**	1			
BD-14	0.560**	0.391**	0.125	0.778**	0.583**	−0.377**	1		
RD-14	0.246**	0.813**	0.721**	0.335**	0.769**	0.349**	0.399**	1	
FDP-14	−0.252**	0.424**	0.559**	−0.352**	0.232**	0.654**	−0.496**	0.599**	1

The correlation is significant at the 0.05 (*) and 0.01 level (**)

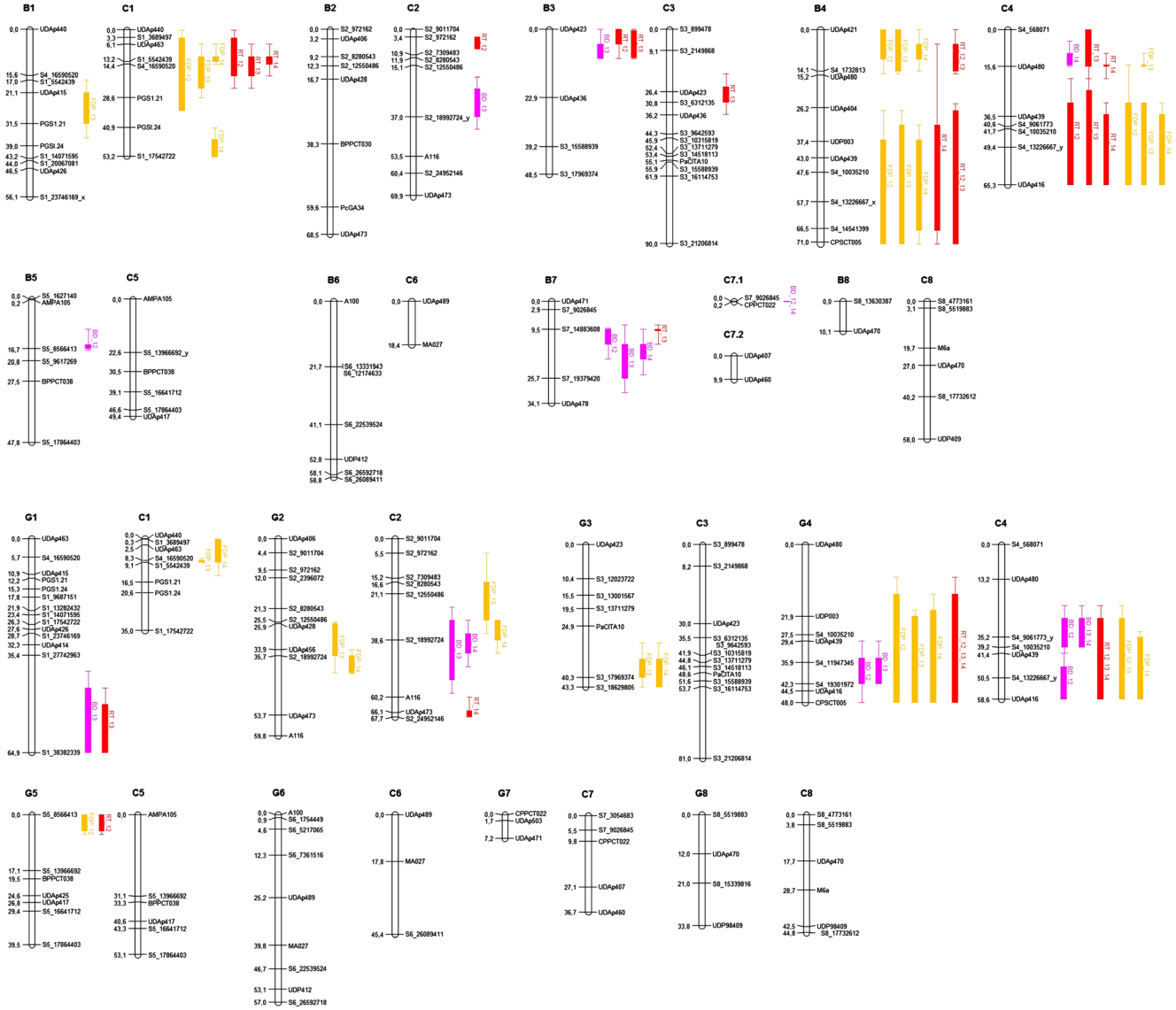

Fig. 2 Molecular linkage map constructed with JOINMAP software in 'Bergeron' × 'Currot' (B × C) and 'Goldrich' × 'Currot' (G × C) F1 apricot progenies, and the identification, using MapQTL, of the QTLs linked to flowering date (BD in *violet*), ripening date (RT in *red*) and fruit development period (FDP in *orange*) identified during the 3 years of the study (2012, 2013 and 2014)

In addition, a multiyear QTL analysis was also performed using JOINMAP 3.0 in 'Bergeron' × 'Currot' (B × C) and 'Goldrich' × 'Currot' (G × C) F1 apricot progenies. The constructed maps in this multiyear analysis we less saturated due to the integration of the different maps, eliminating the heterozygous markers in both progenitors (Fig. 3). This multiyear analysis reinforced the results obtained in the year-by-year analysis and completed these results with the identification of other significant QTLs in LGs 6 and 8. Among the QTLs identified, major QTLs for ripening date and the fruit development period were identified in LG 4, especially important in the 'G × C' population (Fig. 4).

In the case of the flowering date analysis, different QTLs were identified in different years on linkage groups LG 1, LG 2, LG 3, LG 4 and LG 7 (Fig. 2). Multiyear analysis completed these results with the identification of other significant QTLs in LGs 6 and 8 (Fig. 3). Flowering date is a trait showing high inter-annual variations, probably because of chill and heat accumulation differences between years. Such variations reinforce the need to perform QTL studies over a period of several years to avoid environmental effects. In the progenies tested, we detected different QTLs in all LGs (Tables S1–S5; supplemental material).

In the 'B × C' population, different flowering date QTLs were identified in all LGs except LG 6, but the main QTLs were found in the LGs 4 and 7 of 'Bergeron' and in the LGs 1, 2, 3, 4 and 8 of 'Currot' (Fig. 2). By multiyear analysis, a LOD value above 10 was estimated for all these QTLs (Fig. 3 and Table S5). However, the most significant QTL values for 'Bergeron' and 'Currot' were identified in LG 7 and LG 2,

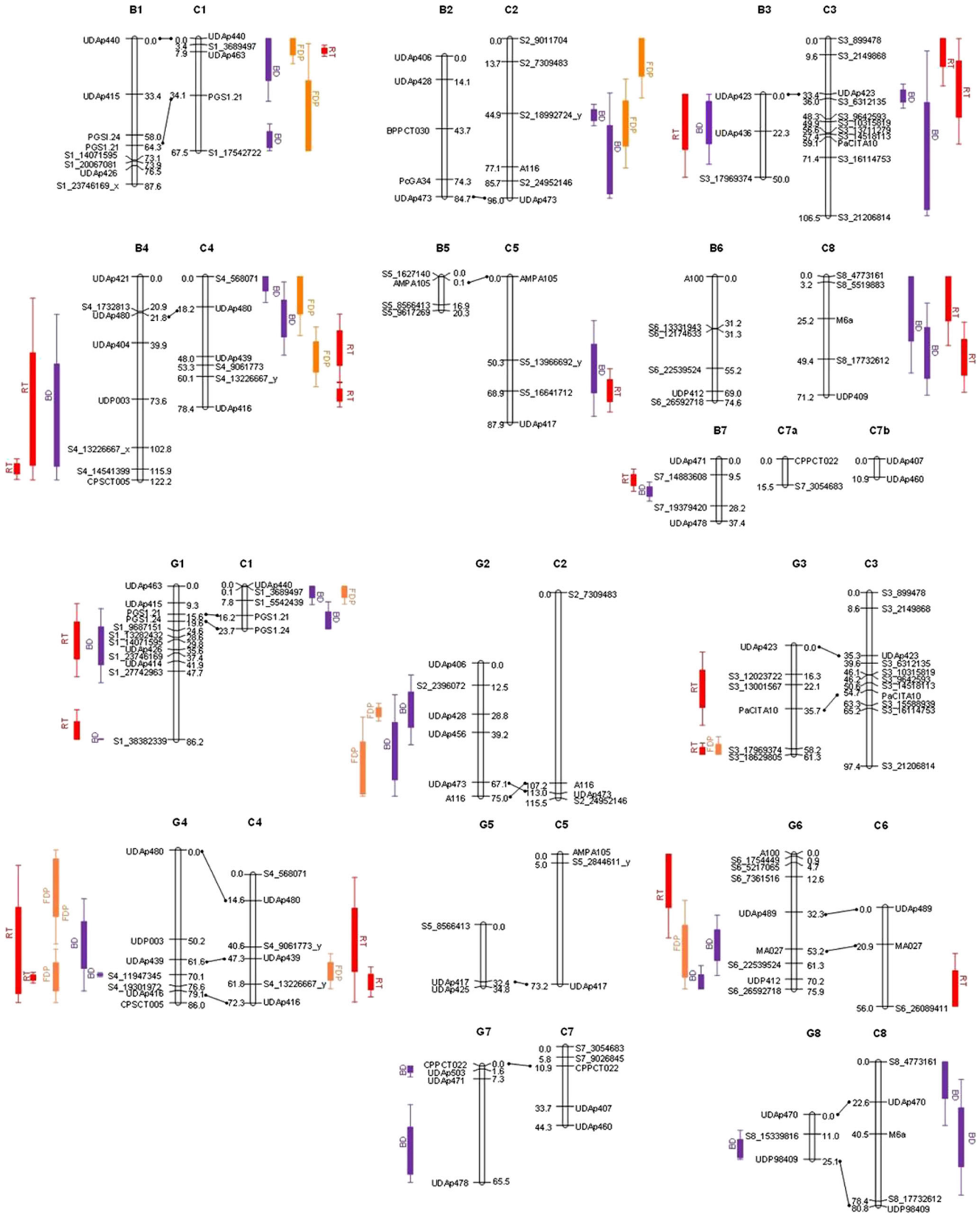

Fig. 3 Molecular linkage maps constructed with JOINMAP 3.0 software and multiyear QTL analysis in 'Bergeron' × 'Currot' (B × C) and 'Goldrich' × 'Currot' (G × C) F1 apricot progenies for flowering date (BD in *violet*), ripening date (RT in *red*) and fruit development period (FDP in *orange*)

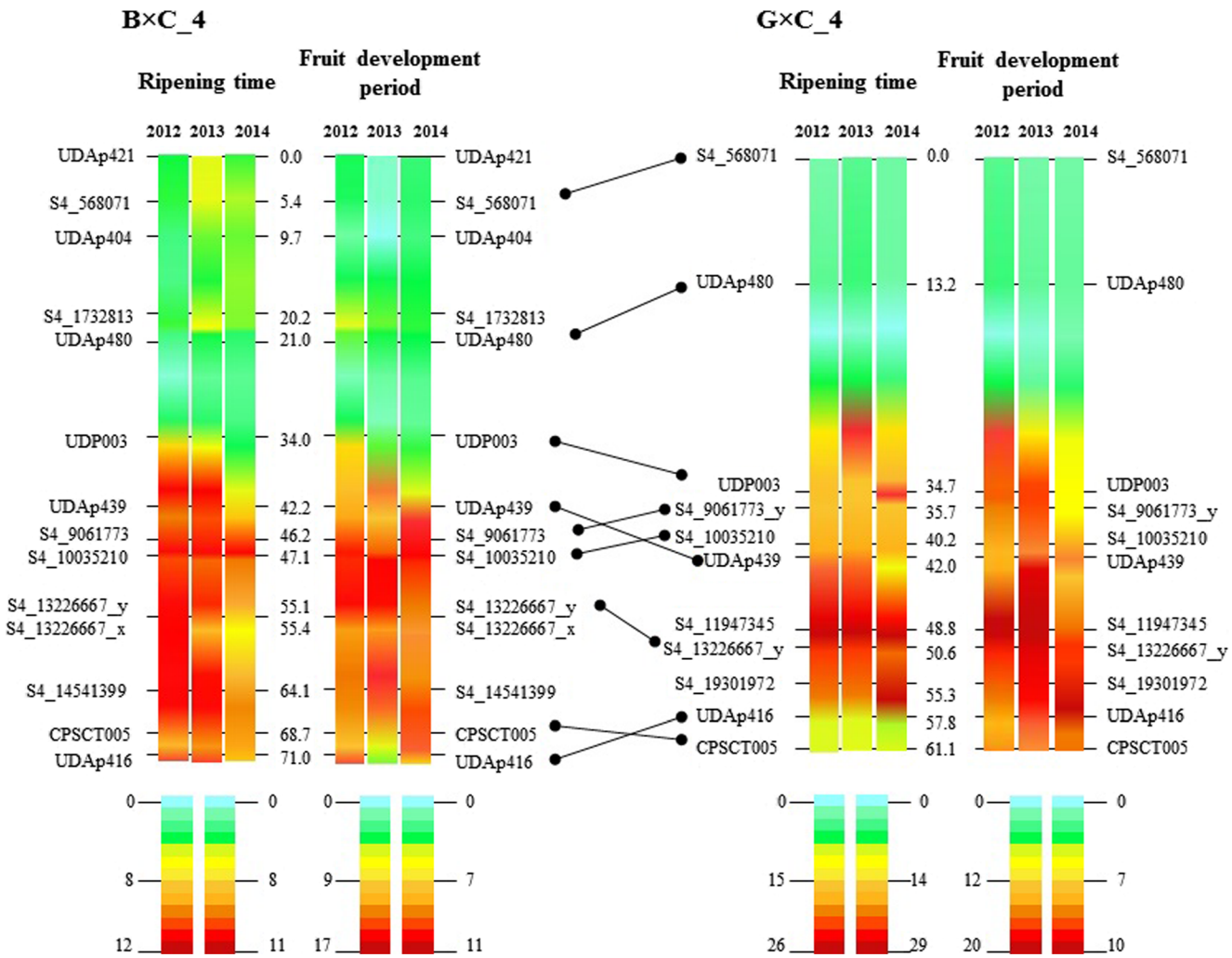

Fig. 4 LOD gradient scores by interval mapping analysis for ripening date and fruit development period in an integrated LG 4 map of 'B × C' (*left*) and 'G × C' (*right*) apricot populations

reaching LOD scores of 15.73 and 21.52, respectively, with a percentage of variance of around 20 %. The SNP markers S7_14883608 and S7_19379420 were the markers closest to the QTL peaks in 'Bergeron'. In addition, the flowering date QTL for LG 7 of 'Bergeron' was confirmed by MQM analyses in all 3 years by selecting S7_14883608 and S7_19379420 as cofactors (Table S1; supplemental material). In LG 2 of 'Currot', the marker closest to the QTL was S2_18992724 (Figs. 2 and 3). These abovementioned QTLs were highly significant in all 3 years of the study as well as downstream of the middle region of LG 2 of 'Currot'.

Regarding the 'G × C' population, flowering date QTLs were detected in LGs 1, 2, 4, 6, 7 and 8 (Fig. 2). In 'Goldrich', two major QTLs, *ft4.1* and *ft4.2*, were detected in LG 4, reaching a LOD value of 50 and a percentage of phenotypic explanation (PEV) of 32.10 % by multiyear analysis (Table S5). Furthermore, the S4_1194734 and S4_10035210 markers were selected as cofactors for 'Goldrich' and 'Currot', and the QTL interval was more reduced and accurate than IM analysis around 10 and 8 cM, respectively (Tables S3 and S4; supplemental material).

LG 1 of 'Goldrich' also shows two important QTLs for flowering date with a LOD value of around 20 (Table S5). Another important QTL was detected at the top of LG 7 of 'Goldrich', close to the CPPCT022 marker, co-localising with a QTL for flowering date in peach (Fan et al. 2010). In 'Currot', another QTL for this trait was localised at the beginning of LG 1 and LG 8, each accounting for more than 9 % of the PEV.

We also analysed ripening date and fruit development period traits together because they are closely interrelated as we have seen above for the correlations between them (Table 1 and Tables S6–S21; supplemental material). Different QTLs linked to ripening date and fruit development period were identified in different years on linkage groups LG 1, LG 2 and LG 4 (Fig. 2). Multiyear analysis completed these results with the identification of other significant QTLs in LGs 5 and 6 (Fig. 3). In 'Bergeron', we can highlight a major QTL for ripening date in LG 4 with a maximum LOD value of 14.66

and a PEV of 17.40 % downstream of the UDP003 marker. Another QTL was found in LG 3 close to the SSR marker UDAp423 (Table S12; supplemental material).

In 'Currot', the most significant QTL for this progeny was closer to UDAp439 marker reaching a LOD value of 38.77 and a PEV above 30 % (Table 2). The main QTL for ripening date in LG 4 was also detected in 'Goldrich' (reaching a maximum LOD value of 55 with a PEV of around 30 % in the same region of 'Bergeron'), between UDP003 and S4_11947345, and in 'Currot' (with a LOD value of 40.49 for ripening date and a PEV of close to 40 % in 'G × C' and a LOD value of 35.93 for fruit development period and a PEV value of 49 % in 'B × C').

In LG 2 of 'Currot', the marker closest to the QTL was S2_18992724 (Figs. 2 and 3). These abovementioned QTLs were highly significant in all 3 years of the study as well as downstream of the middle region of LG 2 of 'Currot'.

In 'B × C', LOD values for ripening date were lower than those for fruit development period, probably because of the significant influence of long fruit development periods. The nearest markers to major ripening date and fruit development period QTLs in LG 4 are UDP003, UDAp439, S4_11947345 and S4_13226667 (Figs. 2, 3 and 4). Furthermore, all QTLs described above by multiyear analysis were verified year by year by IM and MQM analyses, selecting S4_10035210, S4_11947345 and UDAp439 markers as ripening date cofactors in both populations, which helps reduce the QTL interval below 10 cM (Tables S8–S11; supplemental material).

With the aim to better define this QTL region, we have shown further details of integrated LG 4 maps. A total of 14 markers were mapped on the L G4 of the 'B × C' population with a mean distance of 5.07 cM and covering an area of 71 cM (Fig. 4). In Fig. 3, we can see that the highest LOD for both traits is in a region between the markers S4_9061773, S4_10035210 and S4_13226667 (covering 9 cM approximately). A total of 11 molecular markers were mapped on the LG4 of 'G × C' with a mean distance of 5.55 cM and covering 61.1 cM, and the QTL seems to be identified by the markers S4_11947345 and S4_13226667_y (a region of 2 cM or 1,200,000 bp; Fig. 3).

In the 'B × C' map, it is important to highlight that there are no gaps above 15 cM. Furthermore, in both maps, the gaps are of less than 8 cM in the QTL region, which provides higher accuracy for QTL analysis. In addition, five molecular markers were selected in this QTL region for both populations: UDAp439 to S4 13226667_x in 'B × C' and S4_9061773_y to S4_13226667_y in 'G × C'. This was done to carry out an automatic cofactor selection in order to reduce the effect from other segregating QTLs, obtaining the following as the most accurate cofactors: S4_9061773 and S4_11947345 for the 'B × C' and 'G × C' populations, respectively (Tables S22 and S23, supplemental material). From these cofactors, a MQM mapping analysis was carried out, which showed these markers to be the most significant loci in the QTL interval (Table S24; supplemental material).

Table 2 Mean values for ripening date (RD) and fruit development period (FDP) year by year for at least three genotype classes in two molecular markers (SSR and SNP) of the linkage group 4 in 'Bergeron × 'Currot' and 'Goldrich' × 'Currot' progenies

Marker	Genotype	*N*	RD-12	RD-13	RD-14	RD average	FDP-12	FDP-13	FDP-14	FDP average
'Bergeron' × 'Currot'										
UDAp439	145-137pb (fg)	28	159.21a	161.64a	155.03a	158.63	83.53a	95.14a	91.14a	89.94
	152-137pb (eg)	33	159.54a	161.00a	154.30a	158.28	84.18a	95.21a	91.54a	90.31
	152-152pb (ee)	17	163.58b	165.58b	155.23a	161.47	89.35b	101.41b	93.05a	94.61
	145-152pb (ef)	34	164.79b	167.14b	159.38b	163.77	90.29b	101.41b	96.91b	96.21
S4_10035210	TT (kk)	28	158.85a	161.57a	154.66a	158.37	83.07a	94.67a	90.55a	89.44
	GT (hk)	74	162.35b	164.33ab	157.418a	161.37	87.43b	98.43b	94.67b	93.51
	GG (hh)	26	164.00b	165.96b	155.07a	161.68	89.57b	101.92c	93.34ab	94.95
'Goldrich' × 'Currot'										
UDAp439	146-137pb (ad)	47	151.14a	153.14a	143.65a	149.32	77.23a	89.27a	82.78a	83.10
	127-137pb (bd)	20	154.85b	157.30b	148.52b	153.56	79.20a	91.70a	85.10ab	85.34
	146-152pb (ac)	37	156.10b	158.62b	146.51ab	153.75	82.97a	97.24b	87.56b	89.26
	127-152pb (bc)	24	160.83c	165.75c	152.08c	159.56	82.33a	96.12b	87.00b	88.49
S4_10035210	TT (kk)	64	151.51a	153.60a	143.87a	149.66	77.75a	90.44a	83.09a	83.76
	GT (hk)	65	155.78b	158.10b	147.24b	153.71	81.48b	94.13b	86.36b	87.33
	GG (hh)	35	160.71c	165.68c	151.77c	159.39	85.88c	97.11b	87.20b	90.07

Genotype: numbers in the UDAp439 marker indicate the size of the alleles in base pairs, and letters for the S4_10035210 SNP marker indicate the specific polymorphic base. The segregation code by JoinMap4 is in brackets. RT-FDP columns: letters (a, b, c) indicate significant differences between means detected by Tukey's test at $p < 0.05$

N number of seedlings

Moreover, to more clearly show the correlation between certain phenotypic and genotypic classes, Tukey's test was carried out. The S4_10035210 SNP marker placed downstream of UDAp439 shows three different genotypic classes (TT, GT and GG), each corresponding to an average ripening date of 149, 153 and 159, respectively (Table 2).

Discussion

Inheritance and correlations of phenological traits

Flowering date is considered to be both quantitatively inherited in most fruit tree species (Anderson and Seeley 1993) and highly heritable (Couranjou 1995). Other authors studying flowering date as well as ripening date and fruit development period have confirmed that phenological traits are quantitatively inherited in *Prunus*, such as Sánchez-Pérez et al. (2007b) with respect to flowering in an almond progeny; Dirlewanger et al. (2012) in peach, apricot and cherry progenies; Socquet-Juglard et al. (2013) and Salazar et al. (2013) in apricot progenies; and Castède et al. (2014) in a study of genetic determinism of flowering date in cherry progenies.

The large number of seedlings showing lower or higher values than their parents suggests the influence of the whole genetic background of the parents on the transmission of phenology traits, which should be taken into consideration when designing inter-variety crosses. This situation has been also described in other quantitative traits in apricot related to fruit quality (Salazar et al. 2013).

Results also showed a greater influence of medium ('Goldrich') and late ('Bergeron') ripening parents in comparison with the early ripening parent 'Currot' in both populations. Dates are close to those described by Ruiz and Egea (2008) for the cultivars 'Currot', 'Goldrich' and 'Bergeron'. Ripening dates are close to those described by Ruiz and Egea (2008) for the cultivars 'Currot', 'Goldrich', and 'Bergeron'. Regarding fruit development period, results showed that the early descendant needed more time to ripen. These results were also observed by Ruiz and Egea (2008) and Salazar et al. (2013).

The positive correlation between ripening date and fruit development period indicates that later maturity dates are related to a longer fruit development period, which has been confirmed by other authors such as Etienne et al. 2002, in the peach progeny 'Ferjalou Jalusia' × 'Fantasia', and Salazar et al. (2013), in the apricot progeny 'Z701–1' × 'Palsteyn'. Moreover, flowering date and ripening date showed a positive correlation and in 2013 reached values of 0.66** and 0.61** in 'B × C' and 'G × C' progenies, respectively. In this year, chill units were more balanced, leading to an earlier flowering date than in the other years. In 2013, however, we obtained an inverse correlation between flowering date and fruit development period in both populations, of −0.33** and −0.63** for 'B × C' and 'G × C', respectively. This indicates that early flowering may lengthen the fruit development period (Salazar et al. 2013).

These results have been confirmed by other authors who have also detected important correlations among certain phenological traits such as bud break date, flowering date, flower density, fallen buds, ripening date, fruit development period and productivity in both peach (Badenes et al. 1998; Dirlewanger et al. 1999; Etienne et al. 2002; Quilot et al. 2004; Eduardo et al. 2011) and apricot (Ruiz et al. 2010; Salazar et al. 2013). Other correlations between ripening date and fruit quality traits have also been described in different apricot progenies, such as acidity by Badenes et al. (1998), soluble solids by Ruiz et al. (2010) and Salazar et al. (2013), and ethylene content by Ruiz et al. (2010), where autocatalytic ethylene production implies a change in the growth stage to senescence, which promotes increased respiration and fruit maturation (Seymour et al. 1993; Wills 1998).

Genetic linkage analysis and QTL identification

Regarding the distribution of markers in both maps, the location of SSRs corresponds in general to that described by other authors, except for UDAp423, mapped in LG 5 in apricot (Campoy et al. 2011b). The majority of the SNPs were mapped in the design position in the peach genome v1.0. The putative discrepancies in the SNP positions are associated with misassembled pieces of the peach genome v1.0 (Verde et al. 2013) that have been refined in the current version, peach genome v2.1, and that have already been highlighted in genetic studies of other *Prunus* species such as sweet cherry (Klagges et al. 2013). For example, S4_16590520, which should be located at the end of LG 4, was mapped to the top of LG 1, and S2_9011704 was mapped at the beginning of LG 2, upstream to S2_972162, in both populations. We should also note that, as expected, the two maps from 'Currot' are almost identical because this parental was common in both populations.

As expected, the 'Currot' maps were the longest ones because the SNPs had been previously designed from the RNA-Seq data of 'Z506-7' and 'Rojo Pasión' (both derived from 'Orange Red' × 'Currot'; Salazar et al. 2015). In spite of the high efficiency of these markers for the discrimination of a panel of 37 apricot accessions, the percentage of those useful for mapping in both populations was about 50 %. The absence of SNPs detected in 'Bergeron' or 'Goldrich' explains the lower coverage in the related maps with respect to 'Currot'. Unfortunately, this percentage was not enough to avoid some gaps (i.e. LG1 'Goldrich') in the maps and to ensure optimal coverage for all the linkage groups.

It is therefore desirable in the future to also have certain tools for SNP genotyping available for apricot, such as the SNP chips that are already available in other Rosaceous species such as apple (8000 SNPs; Chagné et al. 2012), pear (1000 SNPs; Montanari et al. 2013), peach (9000 SNPs,

Verde et al. 2012) and cherry (6000 SNPs; Peace et al. 2012). These tools have made it possible to design very dense maps in these aforementioned species (Antanaviciute et al. 2012; Chagné et al. 2012 in apple; Montanari et al. 2013 in pear; Eduardo et al. 2013; Martínez-García et al. 2013; Pirona et al. 2013 in peach; Klagges et al. 2013 in cherry). Nevertheless, the mapped SNPs already make it possible to anchor the available maps with the peach genome sequences and to identify several QTLs linked to phenology traits in the 'B × C' and 'G × C' populations. Moreover, multiyear QTL analysis made it possible to identify additional QTLs that had not been previously detected by the year-by-year QTL analysis approach. This confirms the accuracy of multiyear analysis by MultiQTL in apricot, as was already reported in sweet cherry (Castède et al. 2014, 2015).

These results are in agreement with the detection of flowering date QTLs in almost all the LGs of the different *Prunus* species by other authors: in LGs 1 and 5 (Quilot et al. 2004), LG 2 (Dirlewanger et al. 1999) and LG 4 (Quarta et al. 2000; Dirlewanger et al. 2012) in peach; in LG 7 in peach and almond (Fan et al. 2010; Sánchez-Pérez et al. 2012); in LGs 1 and 2 (Wang et al. 2000; Dirlewanger et al. 2012; Castède et al. 2014), LG 4 and LG 8 (Dirlewanger et al. 2012; Castède et al. 2014) in cherry; and LG 5 in apricot (Campoy et al. 2011b). In addition, Olukolu et al. (2009) identified 12 Chilling Requirement (CR) QTLs on LGs 1, 2, 5, 6, 7 and 8 in two high-density apricot maps. These authors reported that this trait highly correlates with flowering date, in agreement with reports in panels of apricot cultivars (Ruiz et al. 2007; Campoy et al. 2010).

The SNP marker S2_18992724y is located approximately in the same position as a *ParSOC1* gene that has already been described as being linked to chilling requirements in apricot (Olukolu et al. 2009; Trainin et al. 2013). Furthermore, relatively high correlation has been observed between chilling requirements and specific *ParSOC1* alleles (Trainin et al. 2013).

The QTL *ft4.1* was close to the UDP003 position. This marker co-localises a major QTL for flowering date in almond, explaining more than 50 % of the variance in a 3-year study (Sánchez-Pérez et al. 2007a). We blasted the UDP003 sequence from NCBI in IGA server and positioned it in silico at 8,757,583 bp in the peach genome v1.0 (Verde et al. 2013). This position also co-localises with a major QTL recently found in sweet cherry at ~8 Mb (in the peach genome v1.0), explaining 36 % of the mean variance over 6 years (Castède et al. 2014). Dirlewanger et al. (2012) also identified major QTLs in LG 4 in apricot and sweet cherry progenies. The nearest marker to the *ft4.2* peak seems to be S4_11947345, but the QTL region is quite wide and also includes the markers S4_19301972, UDAp416 and CPSCT005 at the end of the LG. In addition, a major QTL for flowering date in the LG 4 of 'Goldrich' was also detected by Dirlewanger et al. (2012), using a 'Goldrich' × 'Moniqui' apricot progeny. The UDP003 SSR marker was also previously associated with flowering date in apricot by Campoy et al. (2010).

The obtained results confirm the major QTL in LG 4 linked to flowering and ripening date found close to the UDAp439 marker in 'Goldrich' × 'Moniqui' and 'Lito' × 'BO 81604311' apricot progenies (Dirlewanger et al. 2012). Many other authors have also described significant QTLs in this LG within the *Prunus* genus in peach (Dirlewanger et al. 1999; Dirlewanger et al. 2012; Etienne et al. 2002; Quilot et al. 2004; Cantín et al. 2010; Eduardo et al. 2011; Pirona et al. 2013), in almond (Sánchez-Pérez et al. 2007a) and in cherry (Wang et al. 2000). In apricot, this QTL for fruit development period and ripening date was located between the markers UDA021 and UDAp439 for three consecutive years in the population 'Z701-1' × 'Palsteyn' (Salazar et al. 2013). The QTLs identified for flowering date, ripening date and fruit development period are of particular interest due to their importance in molecular assisted selection (MAS) when it comes to obtaining molecular markers in order to select early or late seedlings, thus allowing us to extend the calendar in apricot production. The high influence of LG 4 in ripening date for each parent and year probably indicates the presence of major genes in this position, as has also been indicated in peach (Dirlewanger et al. 2012; Pirona et al. 2013), apricot and cherry (Dirlewanger et al. 2012). To the best of our knowledge, this is the first QTL study for the fruit development period in apricot, indicating that this QTL is involved in the control of ripening date through the control of the timing of fruit development.

Using two F2 populations of peach, 'Contender' × 'Ambra' and 'Weeping Nj' × 'Bounty', Pirona et al. (2013) localised the MD locus between 10.97 and 11.19 Mb in a similar region of apricot. In this region, a total of 25 candidate genes were found. Among these candidate genes, the most important were ppa007577m and ppa008301m, both of which are predicted to encode NAC transcription factors (TFs). NACs constitute one of the largest plant TF families and are key regulators of developmental programmes and stress response (Pirona et al. 2013). In addition, more recently, Nuñez-Lillo et al. (2015) have found a QTL in the same region of LG 4 of peach linked to maturity date, explaining around 80 % of the variability.

Genetic linkage analysis could bring about the most success in this research line in terms of the search for the genes involved in fruit ripening in the apricot species, especially in LG 4. Ripening date determines an early or late harvest, so it is therefore highly important in the search for new selections to enlarge the production period. Our strategy is thus oriented towards finding specific regions of the genome that provide us with further information regarding the major genes controlling the ripening date trait. This will allow us to design specific molecular markers for assisted selection in nursery conditions. For this purpose, it will be necessary in the future to increase the number of individuals to look for recombinants in this QTL region and to saturate the ripening date QTL region in

LG 4 to a greater extent through a fine mapping approach. Also, increasing the number of years of phenotypic evaluation would increase the accuracy of QTL detection thanks to multi-environment analysis. In this way, we could obtain greater QTL significance values to select more specific regions where we can design different combinations of molecular markers and evaluate whether the presence or absence of certain alleles corresponds to the phenotypic class.

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Data archiving statement The progenitors used in the generation of progenies are registered in the Plant Variety Database (PLUTO; http://www.upov.int/pluto/en) belonging to the International Union for the Protection of New Varieties of Plants (UPOV) http://www.upov.int. The apricot cultivars and progenies in the study belong to the germplasm collection and breeding programmes of CEBAS-CSIC, which includes some breeding research material whose QTL data is available in the Genome Database for Rosaceae (GDR, http://www.rosaceae.org).

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