

Mapping a major gene for red skin color suppression (highlighter) in peach

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Abstract Presence, absence and intensity of red skin color in peach fruit are key characters for breeding varieties adapted to different market niches and consumer preferences. Red skin color is the result of anthocyanins, and in some progenies, the suppression of red skin color has been found to be controlled by a single gene, *H/h*, where plants homozygous for the recessive allele (*h*) produce the highlighter (anthocyaninless) phenotype. In this paper we analyzed an F_2 population of 276 individuals segregating for this trait and mapped the *H* gene on a 5 cM region of linkage group 3. This region was saturated with additional markers and finally *H* was located in a genomic region of ~607 kbp containing 62 genes. Three of these genes corresponded to MYB10 transcription factors, known to control anthocyan biosynthetic pathways in peach and other species, such as cherry and apple. A codominant marker based on the sequence of one of

these genes (*PpMYB10.1*) was used to genotype the F_2 progeny and cosegregated with the trait. To further validate the marker it was tested in a collection of 87 peach cultivars. A strong association was found between the marker genotype and the intensity of red blush, with the three cultivars that have a complete absence of red blush being homozygous for the marker allele associated with the highlighter trait, confirming that it could be used as an efficient, although not diagnostic, marker to select this character in peach breeding programs.

Keywords Marker-assisted breeding · *Prunus* · Red blush · MYB10 · Anthocyanin biosynthesis

Introduction

The commercial value of peach [*Prunus persica* (L.) Batsch] fruit is highly dependent on skin color, since it contributes to the attractiveness of the fruit and therefore determines consumer acceptance (Iglesias and Echeverría 2009). High levels of red blush are usually pursued in peach breeding programs aimed at cultivars for the fresh market (Scorza and Sherman 1996; Liverani et al. 2002; Cantín et al. 2010). As a consequence, there has been a major increase in overall color of the cultivars developed in recent years (Crisosto and Costa 2008; Iglesias and Echeverría 2009; Reig et al. 2013). However, absence or

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reduction of skin color is sought for peach cultivars intended for the canning industry to avoid staining in the final product (Gradziel et al. 1993). This is also preferred in some “niche markets” where clingstone peaches have been traditionally important, as in Spain (Iglesias and Ruiz 2016). As fully yellow skin peaches are also highly appreciated for fresh consumption in other Mediterranean countries and in Central America, there are some breeding programs producing cultivars for these markets. Similarly, cultivars with different shades of white skin are valued in Asia, and have an occidental counterpart in the more recently developed white-skinned peaches (Nicotra et al. 2002), with a distinctive ivory overtone. The percentage of skin blush can determine fruit typology, as with the Spanish Calanda peaches and the Japanese Okayama peaches. In these cases, absence of red skin color is a distinctive characteristic of the cultivar, so different strategies, such as covering the fruit with paper bags, are used to avoid the development of any skin blush.

Peach skin color is determined by both, a background color, and a red over-color (or blush). The progression of coloration is linked to the stage of peach development. Skin background changes during maturation from green to yellow or white, and during this process, a red pigmentation can appear on top of this background color, very early during fruit development in some cultivars and in the last phases in others. This red over-color can develop with different intensities and patterns depending on the genotype (mottled, striped, variegated, spotted, etc.). The background is determined by the carotenoid content of the flesh and is controlled by a single locus (*Y/y*) in linkage group 1 of the peach genome (Brandi et al. 2011; Falchi et al. 2013). The red over-color is due to the accumulation of anthocyanins, the water soluble pigments responsible for the red, blue or purple pigmentation of many plant tissues (Zhang et al. 2014). The intensity and percentage of red blush in peach are defined by the genotype, but they also depend on environmental factors such as sun exposure of the fruit and temperature (Layne et al. 2001). The degree of red blush of the peach skin has usually been considered to be under the control of multiple genes and several QTLs have been identified in intra (Cantín et al. 2010; Eduardo et al. 2011; Frett et al. 2014) and inter-specific populations (Quilot et al. 2004; Donoso et al. 2016). In certain cases, the inheritance of the red skin color character has been described as a single Mendelian trait. One is the red

versus non-red skin color, where the anthocyaninless or highlighter (*H*) phenotype is characterized by an absence of red pigmentation in the fruit, including skin, flesh and pit although other tissues (such as petals, anthers, leaf injury sites) present normal red coloration. This phenotype was first named by Beckman et al. (2005), who observed that the suppression of red skin color segregated as a single gene (*H/h*), with the recessive allele (*h*) responsible for the highlighter phenotype. A similar monogenic control was found for the opposite phenotype: the presence of red color over the entire fruit epidermis (the “full red”, FR, phenotype), where a gene (*Fr/fr*) with the homozygous recessive genotype *frfr* displaying the FR phenotype was described by Beckman and Sherman (2003). Neither the *H* nor the *Fr* genes have been mapped. Very recently, Tuan et al. (2015) investigated the relationships between a cluster of three MYB10 transcription factor genes located on chromosome 3, and concluded that one of them (*PpMYB10.1*) was involved in the accumulation of anthocyanins leading to the skin color phenotype in two peach cultivars. A marker based on the sequence of this gene was associated to skin coloration in a sample of 23 Japanese cultivars, including some with the highlighter phenotype.

With the development of tightly linked or diagnostic markers for key traits, marker-assisted breeding is being increasingly applied in fruit tree improvement (Ru et al. 2015; Eduardo et al. 2015a). For peach, these include fruit traits such as melting/non-melting (Peace et al. 2005), flat/round (Picañol et al. 2013), subacid/acid (Eduardo et al. 2014) and slow/normal ripening (Eduardo et al. 2015b). These markers are useful to design crossing plans more efficiently, or to select breeding progenies with the desired characteristics, saving time, space and labor resources. The objective of this paper was to understand the inheritance of the highlighter phenotype, to map the gene responsible for this character in the peach genome, and to develop molecular markers linked to *H* useful for parent and seedling selection in peach breeding.

Materials and methods

Plant material and phenotyping

An F₂ population of 276 individuals obtained from selfing the Spanish local cultivar ‘Sanguí d’Arbeca’

(SA), segregating for the highlighter phenotype, was used for map construction. Individuals of the F₂ population were planted in fields at the IRTA Experimental Station at Gimènells (Lleida, Spain) on their own roots, and following the technical practices of commercial orchards. All trees were phenotyped, in 2014, for fruit external color at commercial maturity (based on background skin color change and manual evaluation of firmness), and were classified as highlighter when their skin was completely anthocyanin-less, or red, when having any degree of red blush, as proposed by Beckman et al. (2005).

A broad collection of 87 peach cultivars including a diverse range of fruit types, growing in the same Experimental Station and grafted on Cadaman rootstock, was used for marker validation. Phenotyping data was already available as part of the IRTA peach variety trial network (Reig 2013). Their main fruit characteristics are given in Table 1, including the data from the percentage of fruit skin color, visually estimated at harvest, over at least 3 years.

DNA extraction, genotyping and mapping

DNA from SA, the 276 individuals of the F₂ population and the 87 peach cultivars was extracted from young leaf tissue using the Doyle and Doyle (1990) protocol in 96 well plates. Forty SSRs previously mapped along the *Prunus* genome (Online Resource 1) were assayed in the parental cultivar SA, in order to identify heterozygous markers. Initially, a set of 93 F₂ individuals was genotyped only with the SSRs heterozygous in SA located on linkage group 3 (G3), since a candidate gene for external fruit color in peach has been reported in this chromosome (Frett et al. 2014; Tuan et al. 2015). As these markers confirmed the mapping of the trait on G3, the two closest markers flanking the *H* gene were genotyped in the remaining 183 individuals and recombinant individuals were identified. Based on the peach genome sequence v2.0 (www.rosaceae.org), six new SSR markers, denoted as CPP followed by the number assigned to this SSR in the IGA SSR annotation of the peach genome v1 (<http://services.appliedgenomics.org/projects/drupomics/gbrowse/>), were developed in the region between the markers flanking *H* using the information on peach SSRs provided on the IGA peach genome v1 browser. Information on the primers used for their amplification

is given in Table 2. Recombinant individuals were genotyped for the four SSR markers of this set that were heterozygous in SA and a marker (MYB10.1) developed by Tuan et al. (2015), based on an indel polymorphism of the *PpMYB10.1* gene sequence occurring between a red and a non-red cultivar, and also located in the G3 interval. MYB10.1 was also genotyped in the collection of 87 cultivars. Primers and PCR conditions were as in Tuan et al. (2015).

For SSR genotyping, PCR reactions were carried out using a PE9700 Thermal Cycler (PE/Applied Biosystems, Foster City, CA, USA) in a total volume of 10 µl, containing 20 ng of peach genomic DNA, 1× NH₄-based reaction buffer, 1.5 mM MgCl₂, 0.5 mM dNTPs, 0.25 µM of each primer and 1 U of BIOTAQ (Bioline). Forward primers were labeled with a fluorochrome (FAM, VIC, NED or PET). PCR amplifications were under the following conditions: 1 min at 95 °C, 30 cycles of 15 s at 95 °C, 15 s at the appropriate annealing temperature, and 30 s at 72 °C, followed by a 5 min extension at 72 °C. Products were analyzed by capillary electrophoresis using the ABI/Prism 3130xl (PE/Applied Biosystems) automatic sequencer as in Aranzana et al. (2003). CPP SSRs were genotyped using the indirect tag primer labeling approach as described in Donoso et al. (2015) and were analyzed using the ABI/Prism 3130xl automatic sequencer. The conditions for MYB10.1 indel amplification were similar to those of the SSRs, but using 1 min of extension. PCR fragments were visualized in ethidium bromide agarose gels under UV light.

Genetic maps were constructed with JoinMap v.4.1 (Van Ooijen 2011) considering the *H* gene as dominant. Groups were established with a LOD 3.0 and maps were calculated with the Kosambi distance function. Linkage group nomenclature follows the *Prunus* reference map (T × E) (Dirlewanger et al. 2004). MapChart 2.1 software (Voorrips 2002) was used to draw the maps.

Results

Highlighter mapping

Scoring of the H phenotype in the 276 individuals of the F₂ population revealed 205 individuals with red blush and 71 highlighter. This is in agreement with a 3:1 segregation ($\chi^2 = 0.08$, 1 df, non-significant) as

Table 1 Genotypes of the 87 cultivars with indel MYB10.1 marker and percentage of red blush

Cultivar	G	Y	S	M	Indel MYB10.1 genotype	Blush (%)	Cultivar	G	Y	S	M	Indel MYB10.1 genotype	Blush (%)
Ghiaccio1	P	W	R	SH	2/2	0	NG4 720	N	Y	R	M	1/1	60–90
P01F002A110	P	W	R	M	2/2	0	ASF 04-93	P	W	F	M	1/1	70–90
Summer sun	P	Y	R	N	2/2	0	ASF 05-01	N	Y	R	M	1/1	70–90
Ghiaccio2	P	W	R	SH	2/2	0–5	ASF 99-02	N	Y	R	M	1/1	70–90
IFF0813	N	Y	R	M	2/2	0–5	Early top	N	Y	R	M	1/1	70–90
Fercluse	P	Y	R	N	2/2	0–10	EP 93.06	P	W	F	M	1/1	70–90
Maria dorata	N	Y	R	N	2/2	0–10	Fire top	N	Y	R	M	1/1	70–90
Feraude	P	Y	R	N	2/2	0–10	Garaco	N	W	R	M	1/1	70–90
Feriot	P	Y	R	N	1/2	0–10	Magique	N	W	R	M	1/1	70–90
Romea	P	Y	R	N	1/2	0–20	Latefair	N	Y	R	M	1/1	70–90
Hesse	P	Y	R	N	1/2	0–25	Surprise	P	W	R	M	1/1	70–90
Catherina	P	Y	R	N	1/2	0–30	ASF 01-03	N	Y	R	M	1/1	80–90
P1284	P	Y	R	N	1/2	30–50	ASF 02-27	N	W	R	M	1/1	80–90
Sangui d'Arbeca	P	Y	R	N	1/2	30–60	ASF 06-51	P	W	R	M	1/1	80–90
ASF 04-94	P	W	F	M	1/2	50–80	ASF 06-88	N	W	F	M	1/1	80–90
ASF 05-08	N	Y	R	M	1/2	70–80	Oriola	N	Y	F	M	1/1	80–90
Agabés	P	Y	R	N	1/1	0–10	Sweet moon	P	W	R	M	1/1	80–90
Jesca	P	Y	R	N	1/1	10–20	Sweet star	P	W	R	M	1/1	80–90
IFF0331	P	W	R	SH	1/1	30–60	Azurite	P	Y	R	M	1/1	80–100
IFF0800	N	W	R	M	1/1	40–60	Extreme July	P	Y	R	M	1/1	80–100
Amiga	N	Y	R	M	1/1	40–70	Garcica	N	W	R	M	1/1	80–100
Fairlane	N	Y	R	M	1/1	40–70	Gartairo	N	Y	R	M	1/1	80–100
IFF1180	P	W	F	M	1/1	40–70	Grenat	P	Y	R	M	1/1	80–100
Martí	P	Y	R	N	1/1	40–70	Honey fire	N	Y	R	M	1/1	80–100
UFOS	P	Y	F	M	1/1	40–80	Honey royale	N	Y	R	M	1/1	80–100
UF0-9	P	W	F	M	1/1	40–80	Onyx	P	W	R	M	1/1	80–100
Bigbel	N	W	R	M	1/1	50–70	PG31312	N	Y	R	M	1/1	80–100
IFF1182	N	W	R	M	1/1	50–70	Rich lady	P	Y	R	M	1/1	80–100
M110	P	Y	R	M	1/1	50–70	Rose diamond	N	Y	R	M	1/1	80–100
Alice	N	Y	R	M	1/1	50–80	UF04	P	W	F	M	1/1	80–100
NG187	N	Y	R	M	1/1	50–80	ASF 00-01	N	Y	R	M	1/1	90–100
ASF 03-81	P	W	F	M	1/1	60–80	ASF 06-24	N	Y	R	M	1/1	90–100
ASF 04-71	N	W	F	M	1/1	60–80	ASF 06-71	N	W	F	M	1/1	90–100
ASF 06-90	P	W	F	M	1/1	60–80	ASF 07-78	N	W	F	M	1/1	90–100
Crimson lady	P	Y	R	M	1/1	60–80	Big bang	N	Y	R	M	1/1	90–100
IFF0962	P	Y	R	M	1/1	60–80	Big top	N	Y	R	M	1/1	90–100
IFF1190	P	Y	R	M	1/1	60–80	Garde ta	N	Y	R	M	1/1	90–100
IFF1233	P	Y	R	M	1/1	60–80	Luciana	N	Y	R	M	1/1	90–100
UF07	P	Y	F	M	1/1	60–80	M-104	P	Y	R	M	1/1	90–100
ASF 07-26	N	W	R	M	1/1	60–90	Mesembrine	N	W	F	M	1/1	90–100
Big sun	P	Y	R	M	1/1	60–90	Platibelle	P	W	F	M	1/1	90–100

Table 1 continued

Cultivar	G	Y	S	M	Indel MYB10.1 genotype	Blush (%)	Cultivar	G	Y	S	M	Indel MYB10.1 genotype	Blush (%)
EP 94.20	P	Y	R	M	1/1	60–90	Platfirst	P	W	F	M	1/1	90–100
Extreme red	N	Y	R	M	1/1	60–90	Platifun	P	W	F	M	1/1	90–100
Extreme sweet	N	W	R	M	1/1	60–90							

Cultivars with a range of blush down to 0% had blush only on the sun-exposed part of the fruit and no blush on the non-exposed part or on fruit inside the canopy. The two IndelMYB10.1 alleles are called 1 and 2

G peach/nectarine, Y yellow flesh/white flesh, S flat/round, M melting/nonmelting/stony hard

Table 2 Primers of the new six SSRs used to fine map the highlighter trait

Primer name	Primer sequence	Scaffold	Position (bp)	SA genotype
CPP12410-F	AAATGATTGATTTGTGTGTTCCA	3	17934495	272/276
CPP12410-R	AACCCACACACCCCAGTTTA			
CPP12449-F	GGCTTATGTGTGGGAGAGGA	3	18171092	–
CPP12449-R	AGCTACACCAAGCATTCAAGG			
CPP12479-F	TTTCACGACTCCCTCTTGCT	3	18379600	263/267
CPP12479-R	CCACGACAAGACAACCTCAA			
CPP12533-F	TGCATGATTGCATTCTATCC	3	18707597	225/234
CPP12533-R	AGTTGTTGGGTGCACAATGA			
CPP12566-F	TGAGCCTCATCAGTCGTCAT	3	18893608	265/267
CPP12566-R	CTTCATGAGCAGCAGAGCAG			
CPP12602-F	ATTGGAAATTGGCTTCCACA	3	19054085	–
CPP12602-R	CCACTCAATTCTTTGCCATC			

Genomic positions are according to the *Prunus* genome v2.0

expected if the highlighter character was determined by a single gene (*H/h*), where the dominant *H* allele confers the red blush character and the *hh* genotype corresponds to the highlighter phenotype. We also attempted to score the percentage of external red color, but expression of skin and flesh red color in this population evolved in the latest stages of fruit ripening (Fig. 1) impeding fully reliable phenotyping.

From the 40 SSRs distributed along the *Prunus* genome tested in the parental line SA, 24 were heterozygous, six of which mapped to G3, where the *PpMYB10.1* gene is located. These six SSRs were genotyped in a subset of 93 individuals and the *H* gene was linked to them, cosegregating with MA039a and between markers UDAp-496 and PaCITA10, spanning an interval of 5 cM (Fig. 2). The remaining 183 F₂ individuals were genotyped only for PaCITA10 and MA039a, which were the two closest flanking markers to the mapping position of

H. In the whole F₂, we identified 13 recombinant individuals between these markers. Six new SSRs were developed between MA039a and PaCITA10 (Table 2), four of which were heterozygous in SA, and so screened in the 13 recombinant individuals. Based on this information, the *H* gene was finally located between the markers MA039a (with physical position 17,772,071 bp) and CPP12479 (18,379,600 bp), while marker CPP12410 (17,934,495 bp) cosegregated with *H*. The size of the region between MA039a and CPP12479 was 607.579 bp, containing 62 annotated genes, according to the *Prunus* genome v2.0. As expected, three of these genes were the MYB10 transcription factors identified by Tuan et al. (2015). The MYB10.1 indel marker was also genotyped in the 13 recombinant individuals and cosegregated with the *H* gene and the CPP12410 marker. Genotypes of the recombinant individuals are given in Table 3.

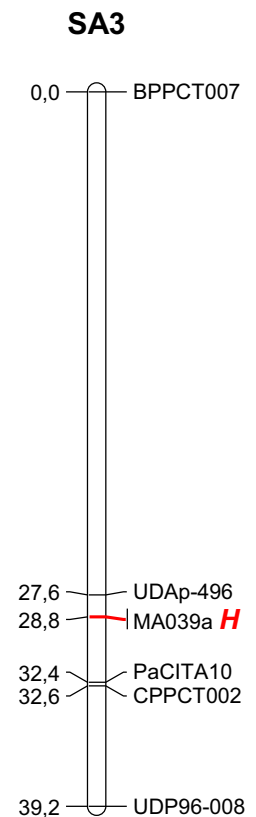


Fig. 1 Fruit skin color segregation within the F_2 SA population. Highlighter fruit (a) were uniformly yellow and maintained their yellow color throughout the ripening stages. Fruit with red blush trait were as in (b) or (c), difficult to distinguish between due to the rapid evolution when overripe. (Color figure online)

MYB10.1 Indel association with fruit skin color in a collection of cultivars

The MYB10.1 Indel marker was scored in a collection of 87 cultivars for validation (Table 1; Fig. 3). This marker presented two alleles of 426 and 609 bp, corresponding to the alleles MYB10.1-2 and MYB10.1-1 (Tuan et al. 2015), respectively, where MYB10.1-2 is associated to the *h* allele and MYB10.1-1 to the *H* allele. All but the three cultivars ‘Summer Sun’, ‘IFF0813’ and ‘Ghiaccio1’, had a more or less intense fruit red blush, including the ten cultivars having blush only in the sun-exposed part of the fruit, with a range of blush down to 0% (Table 1). The eight cultivars homozygous for the MYB10.1-2 allele had a low average of red blush (3.3%), including the three that had no red blush at all, and five (‘Ghiaccio2’, ‘IFF0813’, ‘Fercluse’, ‘Maria Dorata’

Fig. 2 Genetic map of the ‘Sanguí d’Arbeca’ (SA) linkage group 3, where the *H* gene was located



and ‘Feraude’) with less than 10% (Table 2). Only two cultivars had less than 10% blush and were not homozygous for the MYB10.1-2 allele (‘Ferlot’, heterozygous and ‘Agabés’ homozygous for the MYB10.1-1 allele). The percentage of blush in the eight individuals heterozygous for MYB10.1 differed, with an average of 33%; four had under 30% blush (‘Ferlot’, ‘Romea’, ‘Hesse’ and ‘Catherine’) and four between 30 and 80% (‘P1284’, ‘Sanguí d’Arbeca’, ‘ASF 04-94’, ‘ASF 05-08’). Of the 71 homozygous cultivars for the MYB10.1-1 allele, all had red blush, and, except for ‘Agabés’ and ‘Jesca’, had more than 30% blush with an average of 77%. Photographs of fruit from two cultivars for each genotypic class are shown in Fig. 4.

Discussion

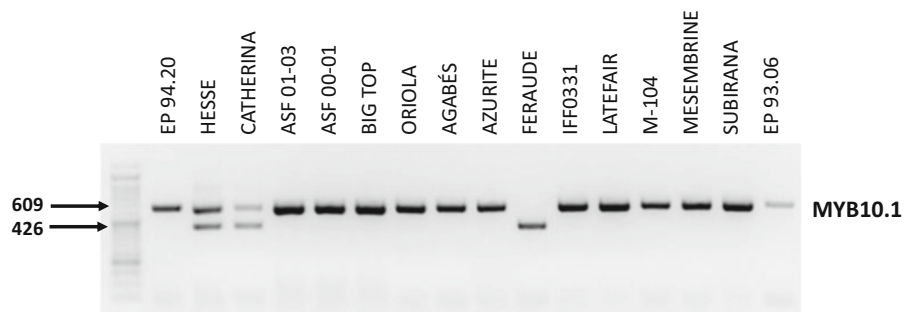
The presence of a single gene controlling the highlighter phenotype was hypothesized by Beckman et al. (2005), based on segregation analysis of several peach

Table 3 Genotypes of molecular markers and the *H/h* gene of the recombinant individuals in the F₂ SA population

Markers	Physical position v2.0	F ₂ Plant number													
		3	137	200	233	31	241	40	31	105	267	99	202	255	
MA039a	3:17772071	A	A	A	A	A	A	A	H	H	H	H	H	H	
CPP12410	3:17934495	A	A	–	A	A	A	A	A	H	H	H	H	H	
<i>H</i>	–	A	A	A	A	A	A	A	A	C	C	C	C	C	
IndelMYB10.1	3:18221203	A	A	A	A	A	A	A	A	H	H	H	H	H	
CPP12479	3:18379600	A	–	–	A	A	A	H	A	H	H	–	H	H	
CPP12533	3:18707597	A	A	A	A	H	H	H	A	A	A	H	H	–	
CPP12566	3:18893608	A	H	H	H	H	H	H	A	A	A	A	H	–	
PaCITA10	3:19499241	H	H	H	H	H	H	H	A	A	A	A	A	A	

Molecular markers were coded as “A” and “B” for the two homozygote states and “H” for the heterozygote. Missing data were indicated with a dash. *H* is a dominant gene, and was coded as *A* for the yellow fruits (corresponding to the double recessive *hh* genotype) and *C* for red fruits (including the two genotypic classes with the dominant allele, *Hh* and *HH*)

Fig. 3 Amplification pattern of the MYB101 indel marker in several peach cultivars. The 426 and 609 bp bands are alleles MYB10.1-2 and MYB10.1-1 associated to the *h* and *H* alleles, respectively



populations. In this work, the *H* gene was mapped to G3 of the peach genome, and a marker based on the sequence of the *PpMYB10.1* gene (Tuan et al. 2015) cosegregated with the trait. MYB transcription factors have been shown to be responsible for anthocyanic coloration of fruit and other plant organs in various species (Zhang et al. 2014) and this region contains three of these genes, although Tuan et al. (2015) have demonstrated that only one of them (*PpMYB10.1*) is involved in determining fruit skin color. Several genes and QTLs determining red color have been found by other authors in this same position: Frett et al. (2014) identified a major QTL for peach red blush explaining a high proportion ($R^2 = 60\text{--}88\%$) of the phenotypic variance, Donoso et al. (2016), using almond \times peach progenies, described a major gene determining anthocyanic anther color (*Ag/ag*) and two QTLs, one for fruit flesh color ($R^2 = 51\%$), and another for fruit skin red color, with ($R^2 = 37\text{--}58\%$), Yamamoto et al. (2005) mapped a gene (*Cs/cs*) for red color around the

stone in a peach intraspecific F₂ population, and Sooriyapathirana et al. (2010) identified a major QTL for skin and flesh red color in cherry. In apple, the *MdMYB10* gene, located at the homologous region of peach G3 in the apple genome (Illa et al. 2011; Jung et al. 2012) is responsible for the red flesh color of the fruit (Espley et al. 2009). These results show that *MYB* genes of this region are strong candidates for being involved in anthocyanin coloration of various fruits and organs of stone and pip fruit.

The pattern of variability of the highlighter character, in a collection of 87 cultivars of peach with different intensities of skin red blush, and the variability of the MYB10.1 indel marker associated with the highlighter gene, indicates three essential features: (a) all the genotypes with highlighter phenotype had the MYB10.1-2/MYB10.1-2 genotype; (b) there was a correlation between the intensity of the red blush and the genotype of the MYB10.1 marker, where individuals homozygous for the alleles



Fig. 4 Images of fruit skin red blush in some of the varieties studied with different genotypes for the indel MYB10.1 marker (1/1, 1/2, 2/2). (Color figure online)

associated with the *h* and *H* alleles had the lowest and highest red blush scores, respectively, with heterozygous being intermediate, and (c) the prediction value of the skin color phenotype based on the MYB10.1 marker was partial, meaning that some of the cultivars studied had phenotypes different to those predicted, as is the case of four cultivars that had the MYB10.1-2 marker in homozygosis and were not highlighter, or the two cultivars, ‘Agabés’ and ‘Jesca’, that were homozygous for the MYB10.1-1 allele and had a low intensity of red blush. Tuan et al. (2015), using a collection of 23 Japanese cultivars, found that those (three) with the highlighter phenotype were homozygous for the MYB10.1-2 allele, and those (13) with red color were all MYB10.1-1 homozygotes, while the intermediate classes (pale red) had all the MYB10.1-1 allele, three in homozygosis and four in heterozygosis with MYB10.1-2. Our results, using a larger sample of cultivars from a different origin, were similar, with the exception that some MYB10.1-2 homozygotes had the pale red phenotype.

The results of (c) may be explained by the existence of factors in addition to *H* accounting for the variability of skin red blush. The penetrance of *H* may be incomplete or depend on other genes, possibly of the anthocyanin metabolic pathway or regulators of these genes. This is supported by the fact that potential regulators of anthocyanin biosynthesis are located at other positions on the peach genome (Rahim et al. 2014; Zhou et al. 2014), coinciding with the positions of the minor QTLs for skin color detected by Frett et al. (2014) on chromosomes 4 and 7, and by Donoso et al. (2016) on chromosomes 1 and 4. Another possible cause of the results observed is that alleles other than *H* and *h* may be segregating in the materials tested. For example, ‘Agabés’ and ‘Jesca’, two old non-melting cultivars from the Spanish region of Calanda, are the only homozygous cultivars for MYB10.1-1 with a low blush. They may contain an allele of the *H* gene not identified by the MYB10.1 indel marker that reduces the expression of red color. Sequencing of MYB10.1 in these cultivars could help

to explain this situation. A similar pattern has already been observed in peach yellow versus white flesh color, where three different mutations causing the same phenotype have been identified (Falchi et al. 2013).

The presence of another single recessive gene (*Fr*) producing a full red blush phenotype was also proposed by Beckman and Sherman (2003), analyzing the segregations of several progenies. It would be very interesting to check if *Fr* and *H* are the same gene. If this was the case, molecular markers able to identify the different haplotypes could predict the phenotype of the fruit skin color from fully yellow to fully red. Given that *PpMYB10.1* seems a clear candidate for the variability observed, additional markers based on its sequence may provide diagnostic tools for marker-assisted selection of parents and progeny in peach. In this work, we provide data showing that the indel MYB10.1 is an efficient, although not diagnostic, marker to select for intensity of red blush in peach segregating progenies.

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