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SHORT COMMUNICATION

A codominant diagnostic marker for the slow ripening trait in peach

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Abstract The peach [*Prunus persica* L. (Batsch)] slow ripening (SR) trait is a mutation preventing the normal ripening process. Individuals with this phenotype are discarded in peach breeding programs. This trait is determined by a single gene (*Sr*/*sr*), where the recessive homozygote (*sr*/*sr*) confers the SR phenotype, and has been mapped to linkage group 4 of the peach genome. A large deletion of 26.6 kb containing the sequence of a NAC transcription factor has been proposed as the causal mutation. Two dominant markers based on the sequence of this region have been assayed previously and found to be diagnostic (genotypes always predicted the phenotypes). However, their dominant nature—a null allele for the marker was associated with the *sr* allele—made it impossible to predict the individuals that carried the SR trait. Here we used resequencing information to develop a codominant molecular marker for the SR trait in peach. The marker was validated in the 'Belbinette' × 'Nectalady' F1 and the 'Venus' F2 populations, and in 27 lines, 18 of which are known to carry the *sr* allele. The marker cosegregated with the SR phenotype in all cases, allowing the discrimination of two DNA fragments of different size associated with normal-ripening alleles, in addition to a third fragment associated with the *sr* allele. The utility of this marker in peach breeding programs is discussed.

Keywords Fruit quality · Molecular marker · *Prunus persica* · Marker-assisted selection

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The slow ripening (SR) phenotype in peach is a trait controlled by a single gene (*Sr/sr).* When the recessive *sr* allele is in homozygosis, it prevents the fruit undergoing normal ripening, and the unripe fruit do not detach from the tree, remaining there even after leaf fall (Ramming 1991). *Sr* has recently been mapped by Eduardo et al. (2015) and by Nuñez-Lillo et al. (2015) in the same region of linkage group 4 (G4), where a NAC transcription factor candidate gene (ppa008301m) is located. This gene has been proposed by Pirona et al. (2013) as candidate for the related character of maturity date (MD). Eduardo et al. (2015) identified a large deletion around the ppa008301 gene in SR individuals, which was confirmed by Nuñez-Lillo et al. (2015). The deletion spanned a region of 26.6 kb between positions 11,111,981 and 11,137, 943, in peach genome v2.0 and contained two genes: ppa008301m (ANAC072) and ppa021959m (putative transposase). Based on the sequence of this region, Eduardo et al. (2015) and Nuñez-Lillo et al. (2015) each identified a marker that co-segregated with the SR phenotype in all materials tested.

Marker-assisted breeding (MAB), which requires molecular markers tightly linked to target traits, is being progressively implemented in fruit breeding programs (Ru et al. 2015). This is possible now in peach for characters such as flat versus round fruit (Picañol et al. 2013) and fruit acidity (Eduardo et al. 2015). This gives breeders better control of their genetic material, to optimize the design of crosses and to increase budget efficiency using markers for early seedling selection. SR seedlings have been identified in several peach breeding programs in the USA, Italy, Spain, France and Chile: the unmarketable fruit that is produced is discarded during the selection process. Markers are already available for MAB of SR, but they have a dominant pattern of inheritance, amplifying a DNA fragment for the normally-ripening allele(s), and resulting in no amplification of the *sr* allele, precluding its identification in heterozygosis. This is a limitation for use in plant breeding, as detection of normally-ripening plants that carry the *sr* allele is a critical feature for parent selection in breeding programs. Avoiding crosses with parents that are both carriers of *sr* results in non-SR individuals in their progenies; conversely, only crosses between parents that both carry the *sr* allele require selection with markers in their progeny. The objective of this work was the development of a codominant marker for the SR trait using information from the resequencing of an SR seedling from the 'Venus' F2 population.

The progeny of the cross 'Belbinette' × 'Nectalady' (BbxNl; $N = 113$) and 'Venus' F2 ($N = 151$) populations, plus 27 additional cultivars or selections, were used in this study. Genomic DNA from these materials was extracted from young leaves following the CTAB method described by Doyle and Doyle (1990). Primers for a new marker, PSR1, designed close to the limits of the deletion (Fig. 1) were designed using Primer 3 (www.primer3.ut.ee) (PSR1-F: GCAAAGCGATGTTCCACGAA; PSR1-R: GCGGCCAAATGGTATTCAGG). We used these primers and those of the PSR2 marker described by Eduardo et al. (2015) (PSR2-F: CCACCCACCT-CATCATTTTT; PSR2-R: CTTGCGGCACAGA-TACTGAA), located within the deletion (Fig. 1), to amplify the DNA in a single PCR reaction. The outcome of this reaction was one or two DNA fragments per individual that were treated as a single marker (PSR3). PCR reactions were performed in a 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). The PCR program conditions were: 1 min at 95 °C, 35 cycles of 15 s at 95 °C, 15 s at 51 °C and 1 min at 72 °C, with a 5 min extension step at 72 °C. PCR products were separated in 2 % agarose gels in TAE 1× buffer, stained with ethidium bromide and then visualized under a UV light. A scheme of the primers used for PSR3 amplification is given in Fig. 1.

The PSR3 marker was genotyped in the Bb × Nl and V × V populations that segregate for the SR phenotype. We identified three bands of differentsizes: a, 1184 bp; b, ~1000 bp; and c, 871 bp. 'Belbinette' was bc, 'Nectalady' was ac and 'Venus' was bc. Bb × Nl segregated for four classes (ac, bc, ab and c) and 'Venus' F2 for three classes (a, c and ac), as shown in Fig. 2. The results were in full agreement with those of the markers PSR2 (Eduardo et al. 2015) and CBV_ANACO027 (Nuñez-Lillo et al. 2015) and mapped to the same positions. All SR phenotypes corresponded to individuals having only the c band (29 out of 113 in BbxNl and 33 out of 151 in V × V), and none of the normally-ripening plants had this band alone. Overall, these results confirm our expectations, where *srsr* (c) individuals were only amplified with PSR1 primers, *SrSr* (a, b or ab) individuals were amplified only with PSR2 primers and *Srsr* had both

Fig. 1 Position of the primers used for the amplification of the PSR3 marker in the region of the *Sr* locus on linkage group 4 of the peach genome. PSR1 primers were designed close to the borders of the deletion and PSR2 primers were in the 5′ region of the ppa008301m gene. *Dashed lines* represent the SR deletion. *White boxes* indicate the genes and *black arrows* the positions of the primers used in this study

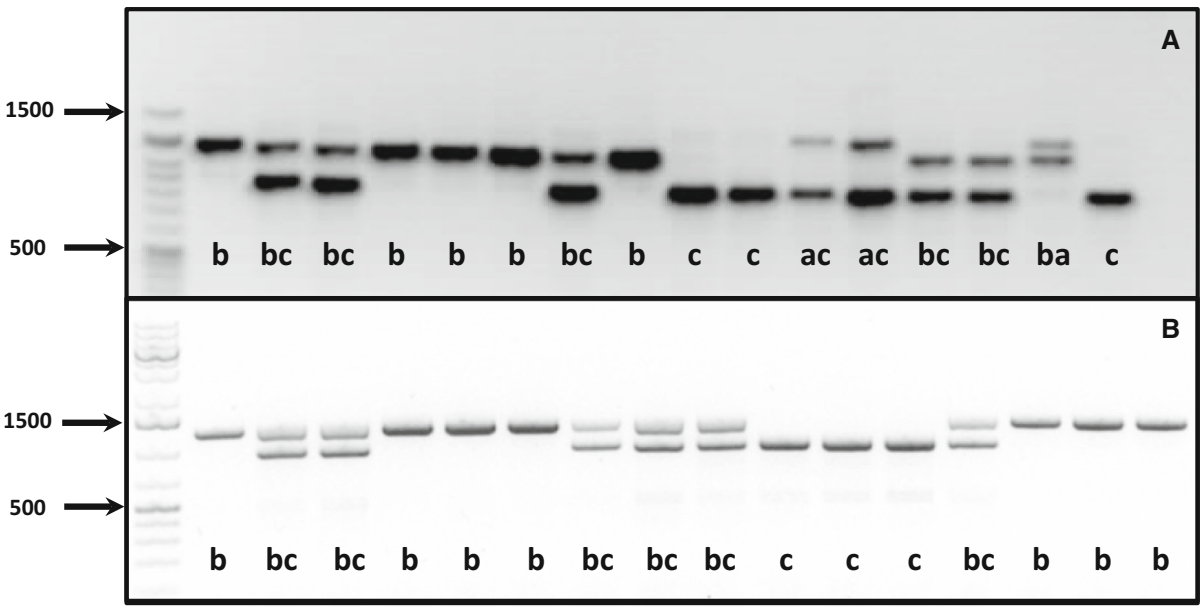

Fig. 2 Example of PCR products of the PSR3 marker for **a** eight cultivars with normal ripening behavior and eight individuals from the Bb × Nl population, and **b** seven cultivars with normal ripening and nine siblings from the progeny of the V × V population. All c individuals had the SR phenotype and those with band c and another band carry the *sr* allele

bands (ac or bc). The presence of two alleles associated with plants that ripen normally suggests that PSR1 may also be able to identify different *Sr* alleles (a and b).

To further validate the marker, and based on information from IRTA and Universidad de Chile peach breeding (UchPB) programs, we genotyped nine normal ripening cultivars (Stark Red Gold, Dr Davis, NH-053, Elegant Lady, ASF01.81, ASF02.23, ASF03.85, ASF04.26, ASF05.03) and 18 cultivars that were expected to carry the *sr* allele because SR individuals had been observed in their progenies (Sweet September, Maria Delicia, Buen Lindo, Nectaross, Nectarexquise, Maria Dolce, Late Red Jim, Flamekist, Early Red Jim, ASF02.55, ASF03.62, ASF03.64, ASF04.14, ASF04.27, ASF04.30, ASF05.15, ASF05.25 and ASF06.20). These 18 cultivars had the bc genotype, confirming the ability of this marker to discriminate between the individuals carrying the *sr* allele. Consequently, the information provided by the PSR3 marker would allow peach breeders to design crosses avoiding the presence of SR individuals in the progenies, or if a cross between two cultivars carrying the SR allele was necessary, MAS could be used to discard SR individuals before planting in the field.

A major gene/QTL for maturity date (*MD*/*md*) (Pirona et al. 2013; Eduardo et al. 2015) and a QTL for the chilling injury symptoms of fruit flesh mealiness

and bleeding (Martínez-García et al. 2013) have also been identified on the *Sr* genomic region. This could be due to pleiotropic effects of a single causal gene or to the effects of other genetic factors located in this region. Eduardo et al. (2015) suggested that the *sr* allele could be one of the alleles of the *MD* locus, and that its presence in high frequency in some breeding programs suggested that the *sr* allele in heterozygosis could confer desirable properties of postharvest behavior when in heterozygosis. Further genomic characterization of the *MD* locus would pave the way to develop molecular tools to predict maturity date and important features related to fruit postharvest behavior.

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