

Full Length Research Paper

The sequence-related amplified polymorphism (SRAP) markers linked to the color around the stone (Cs) locus of peach fruit

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The distribution and concentration of anthocyanin, a major subgroup of the flavonoids and important determinants of peach fruit flesh color, is regulated by several genes. In the international reference *Prunus* map, the locus of flesh color around the stone (Cs) is located on linkage group LG3. This report describes the development of a sequence-related amplified polymorphism (SRAP) marker linked to major genes controlling the Cs in peach. One F1 progeny from a cross between the peach cultivars 'Chongyanghong' × 'Yanhong' segregated by fruit flesh color around the stone was used for bulked segregant analysis. Two kinds of bulked DNA were constructed and used for the polymerase chain reaction in SRAP analysis. After 88 pairs of primers were screened against the two kinds of bulked DNA, three dominant SRAP markers were selected in cooperation with the Cs bulk. One of the three markers (Me07Em02) was linked to the Cs locus at a distance of 0 cM. The SRAP markers would provide an effective tool for marker-assisted selection for the trait of anthocyanin intensity of peach flesh in peach breeding programs.

Key words: *Prunus persica* (L.) Batsch, flesh color around the stone, sequence-related amplified polymorphism (SRAP).

INTRODUCTION

Peach is an important and a model species for the study on wood fruits in the Rosaceae family and some important agronomic characters are controlled by major genes, including disease resistances, as well as flower, vegetative, or fruit quality traits. Their simple inheritance makes them obvious targets to the search of tightly linked markers for early selection. A reference map with a set of anchor markers constructed from a Texas (almond) ×

Earlygold (peach) population (T×E) is available (Dirlewanger et al., 2006) and a framework physical map for peach is also available (Zhebentyayeva et al., 2008).

The traits of red color around the stone are controlled by a single dominant gene designated as Cs/cs (Yamamoto et al., 2001). Although, the morphological traits of flesh color, fruit skin color and red color around the stone which relates to the pigment have been mapped on several peach linkage maps (Chaparro et al., 1994; Rajapakse et al., 1995; Warburton et al., 1996; Sosinski et al., 1998; Abbott et al., 1998; Yamamoto et al., 2001), the distance between the markers and the red color around the stone was not close enough for mapping gene cloning. Additional markers are needed for gene discovery under candidate regions of interest.

Sequence-related amplified polymorphism (SRAP) (Li

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Abbreviations: Cs, Color around the stone; SRAP, sequence-related amplified polymorphism.

Table 1. List of the forward and reverse primers of SRAP used in this study.

Forward primer		Reverse primer	
Name	Sequence	Name	Sequence
Me01	5'-TGAGTCCAAACCGGATA-3'	Em01	5'-GACTGCGTACGAATTAGT-3'
Me02	5'-TGAGTCCAAACCGGAGC-3'	Em02	5'-GACTGCGTACGAATTTGC-3'
Me03	5'-TGAGTCCAAACCGGAAT-3'	Em03	5'-GACTGCGTACGAATTGAC-3'
Me04	5'-TGAGTCCAAACCGGACC-3'	Em04	5'-GACTGCGTACGAATTTGA-3'
Me05	5'-TGAGTCCAAACCGGAAG-3'	Em05	5'-GACTGCGTACGAATTAAC-3'
Me06	5'-TGAGTCCAAACCGGTAA-3'	Em06	5'-GACTGCGTACGAATTGCA-3'
Me07	5'-TGAGTCCAAACCGGTCC-3'	Em07	5'-GACTGCGTACGAATTCAA-3'
Me08	5'-TGAGTCCAAACCGGTGC-3'	Em08	5'-GACTGCGTACGAATTCTG-3'
		Em09	5'-GACTGCGTACGAATTCGA-3'
		Em10	5'-GACTGCGTACGAATTCAG-3'
		Em11	5'-GACTGCGTACGAATTCCA-3'

and Quiros, 2001) is a molecular marker technique which designed for amplifying the open reading frames (ORFs) of a gene and widely utilized for constructing genetic linkage maps. In order to find DNA markers that are tightly linked to a target regions of red color around the stone, we present the construction of a genetic linkage map for the locus of *Cs* using SRAP marker combined with bulked segregant analysis (BSA) (Michelmore et al., 1991).

MATERIALS AND METHODS

Plant materials

A F₁ population derived from a cross between the peach (*Prunus persica* (L.) Batsch) cultivars 'Chongyanghong' (no red color around the stone) and 'Yanhong' (red color around the stone) was used for co-segregation analysis between the SRAP marker and fruit flesh color around the stone phenotype. The phenotype of flesh color around the stone was evaluated in mature fruits.

DNA extraction

Total genomic DNA was extracted from fresh young leaves of each individual at May using a modified method described by Paterson et al. (1993) without adding the ascorbic acid and mercaptoethanol in the DNA extraction buffer or nuclei lysis buffer. Working solutions of genomic DNA at 30 ng/μl in Tris-EDTA (TE) buffer were prepared for SRAP analyses. Two kinds of DNA bulks were constructed by mixing the same volume of DNA of 20 individuals with red flesh color around the stone (B1) or 20 individuals without flesh color around the stone (B2), respectively. 138 individuals from the F₁ progenies crossed between the 'Chongyanghong' and 'Yanhong' were selected for segregation analysis.

Screening of SRAP markers linked to the locus of *Cs*

Eight forward primers and 11 reverse primers of SRAP according to Li and Quiros (2001) were designed and synthesized by SBS Genetech Co., Ltd. (Beijing, China PR) (Table 1). The PCR reactions were carried out in a volume of 25 μl using the same proportions of the assay of Li and Quiros (2001) modified with the

annealing temperature as follows: the first five cycles at 42°C, then raised to 52°C for another 30 cycles. The primers of SRAP were named according to the combination of name of forward and reverse primer, respectively. The PCR results were separated by 6% polyacrylamide gel electrophoresis (PAGE) and viewed with the silver staining. Amplified fragments presented only in the pool of flesh color around the stone were considered to be putative markers. The selected SRAPs were evaluated at the co-segregation between the putative marker and the flesh color around the stone phenotype of 138 individuals of the F₁ progenies.

Linkage analysis of the SRAP markers

Linkage analysis was performed with combined SRAP datasets for the F₁ progenies using MapMaker/3.0 for PC (Lander et al., 1987) with a minimum LOD score of 3.0, maximum θ of 0.50, and Kosambi mapping function was used to convert recombination units into genetic distances. Genetic linkage map drawing was performed with the software of MapDraw (Liu and Meng, 2003).

RESULTS

The investigation of peach fruit flesh color around the stone phenotype

The F₁ progenies were segregated by fruit flesh color around the stone. There are 109 individuals with flesh color around the stone in 138 F₁ progenies. Segregation of flesh color around the stone and no color around the stone in this cross fitted to the ratio of 3:1 based on the Chi-square test, which suggested that the red color flesh around the stone was controlled by a single dominant gene. This result is same as that of Rajapakse et al. (1995).

Polymorphic primers screening in the two bulks and two F₁ progenies

A total of 88 pairs of SRAP primers (combination of eight forward primers and 11 reverse primers) were tested between the two bulks and two F₁ progenies to evaluate

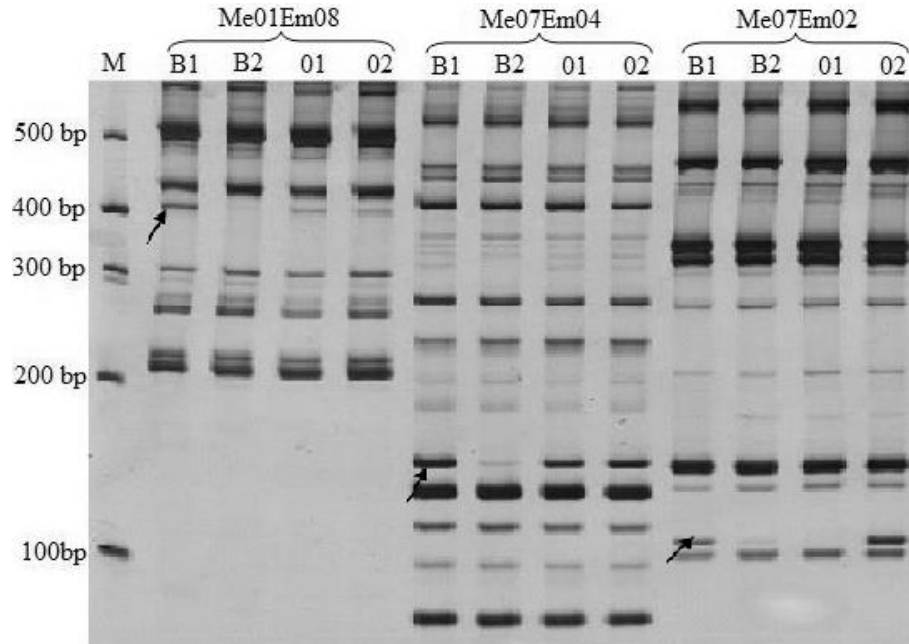


Figure 1. The PCR products amplified between the two bulks (B₁ and B₂) and two F₁ progenies (01 and 02) were separated by 6% PAGE and stained with 2% of silver. Lane M, Marker; the arrowhead indicates the polymorphic bands. PAGE, Polyacrylamide gel electrophoresis.

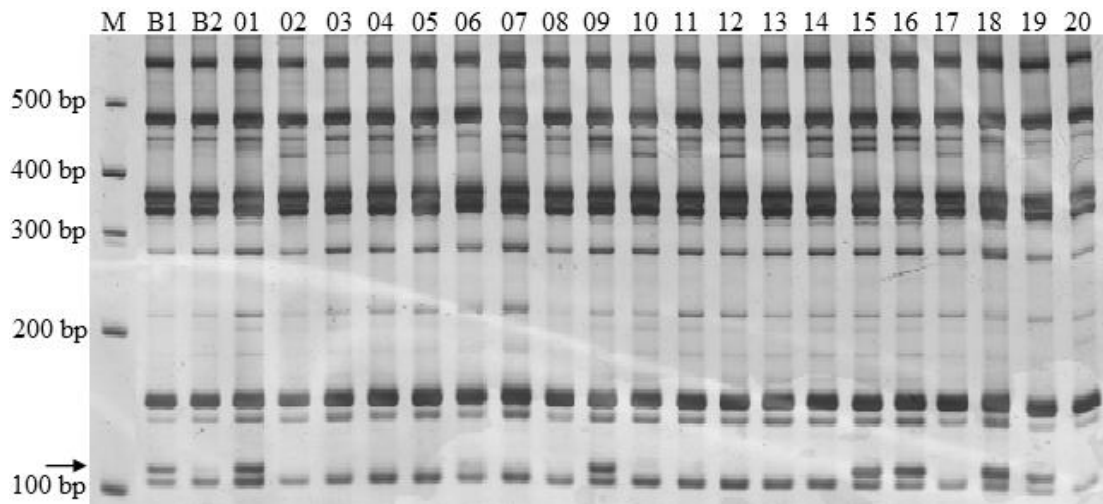


Figure 2. Segregation of amplified primer with Me07Em02 in the two bulks (B₁ and B₂) and 20 F₁ individuals (from Lanes 01 to 20) separated by 6% PAGE and stained with 2% of silver. Lane M, Marker; the arrowhead indicates the polymorphic bands. PAGE, Polyacrylamide gel electrophoresis.

their linkage to the Cs phenotype. Three polymorphic primers of SRAP marker, Me01Em08, Me07Em02 and Me07Em04 were obtained (Figure 1).

Linkage analysis of the SRAP markers with the color around the stone phenotype

Amplification of the three polymorphic primers between

the 138 F₁ individuals also represented polymorphism. The partial pattern amplified with primer Me07Em02 between the B₁, B₂ and 20 individuals is shown in Figure 2.

Linkage analysis performed with combined SRAP datasets for the F₁ progenies indicated that the three markers were linked to the Cs locus at a distance of 30.0, 0, and 18.1 cM, respectively (Figure 3).

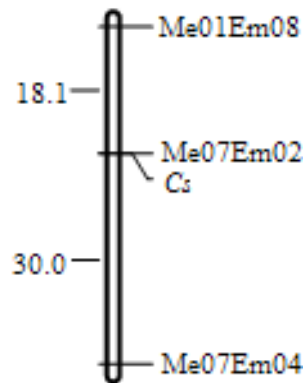


Figure 3. The genetic distance of the three markers located in the *Cs* locus.

DISCUSSION

The result of the *Cs* trait segregation in our F1 population was same as the result obtained by Yamamoto in the F2 population maybe due to genetic background of parental in the locus of *Cs* in our research. The pigmentation intensity and patterning are determined by *MYB*-regulatory factor which controlled the genes expression of flavonoid biosynthesis in different time and special (Schwinn et al., 2006). The distribution and content of anthocyanin in peach fruit (skin color, flesh color and flesh color around the stone) maybe also regulated by translating factors, because these three traits are located on different linkage group (Dirlewanger et al., 2004). The peach genome data derived from the 'Lovell' haploid (*peach v1.0*) has been made available on April 1st, 2010 at the Genome Database for Rosaceae <http://www.rosaceae.org/peach/genome>, and the single nucleotide polymorphisms (SNPs) from Roche 454 and Illumina/Solexa have also been deposited in the NCBI SNP database (Ahmad et al., 2011). Based on the fact that the SRAP marker targets coding sequences in the genome, the SRAP marker of Me07Em02 linked to the *Cs* locus at the distance of 0cM make it to be a potential probe for cloning the gene or transcription factor controlling the flesh color around the stone.

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