Cloning and Characterization of a Self-compatible *S^f* Haplotype in Almond [*Prunus dulcis* (Mill.) D.A. Webb. syn. *P. amygdalus* Batsch] to Resolve Previous Confusion in Its *S^f*-*RNase* Sequence

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Additional index words. self-incompatibility, *S* haplotype, *S*-*RNase*, *SFB*

Abstract. Most of the self-compatible (SC) cultivars of almond [*Prunus dulcis* (Mill.) D.A. Webb. syn. *P. amygdalus* Batsch] have the *S^f* haplotype. In this study, we cloned and characterized the *S* locus region of the *S^f* haplotype of SC ‘Lauranne’. The relative transcriptional orientation of *SFB^f* and *S^f*-*RNase* and the physical distance between them are similar to those of other functional self-incompatible (SI) *S* haplotypes of *Prunus*, indicating that the genomic structure of the SC *S^f* haplotype appears to be intact. Although there is no apparent mutation in the coding sequence of *SFB^f*, the *S^f*-*RNase* sequence in this study and previously reported *S^f*-*RNase* sequences show discrepancies. First, as opposed to previous indications, the ‘Lauranne’ *S^f*-*RNase* sequence encodes a histidine residue in place of a previously reported arginine residue in the conserved C2 region of *Prunus* *S*-*RNase*. Direct sequencing of the polymerase chain reaction products from the *S^f*-*RNase* of ‘Tuono’ confirmed that ‘Tuono’ *S^f*-*RNase* also encodes the histidine residue. We found another difference in the ‘Lauranne’ *S^f*-*RNase* sequence and other reported *S^f*-*RNase* sequences. Namely, ‘Lauranne’ *S^f*-*RNase* encodes a phenylalanine residue in place of a previously reported leucine residue in the conserved C5 region of *Prunus* *S*-*RNase*. This is also the case for ‘Tuono’ *S^f*-*RNase*. Expression analysis of *S^f*-*RNase* and *SFB^f* by reverse transcriptase–polymerase chain reaction showed that *S^f*-*RNase* transcripts were barely detectable in pistil, whereas *SFB^f* transcripts were accumulated at a similar level to the level that was observed with *SFB* of other functional SI *S* haplotypes of almond. We discuss the possible molecular mechanisms of SC observed with the *S^f* haplotype with special references to the expression of *S^f*-*RNase*.

Most of the fruit tree species in the genus *Prunus* (Rosaceae), including almond [*Prunus dulcis* (Mill.) D.A. Webb.], exhibit the *S*-*RNase*-based gametophytic self-incompatibility (GSI) system (de Nettancourt, 2001; Yamane and Tao, 2009). The GSI reaction in *Prunus* is controlled by the *S* locus, which contains the style-specific ribonuclease gene (*S*-*RNase*) (Tao et al., 1997; Ushijima et al., 1998) and the pollen-specific F-box protein gene (*SFB*) (Ushijima et al., 2003; Yamane

et al., 2003) for the pistil and pollen specificities, respectively. Because the pistil and pollen determinant genes have been identified, the variants of the *S*-*RNase* and *SFB* combinations are called *S* haplotypes and the variants of a given *S* locus gene are called pistil and pollen *S* alleles.

Almond cultivars are largely self-incompatible (SI) (Tufts and Philip, 1922), although self-compatible (SC) cultivars exist (Socias i Company, 1990). Grasselly and Olivier (1976) reported that several SC cultivars such as Tuono, Filippo Ceo, Occhiorosso, and Genco were found among the almond population of the Italian region of Puglia, where *P. webbii* (Spach) Vierh. grows wild. Thus, most of the SC almond selections were considered to be derived from interspe-

cific hybridization with SC *P. webbii* (Socias i Company, 2004). As a consequence, the SC *S^f* haplotype of ‘Tuono’ has been long thought to be derived from *P. webbii*. However, a recent finding of the almond SI *S³⁰* haplotype, a putative wild-type *S^f* haplotype, poses a question about this hypothesis (Bošković et al., 2007). The origin and molecular basis of SC in *S^f* haplotype is intriguing for future SC breeding programs in almond.

We previously sequenced the partial *S^f*-*RNase* sequence that was flanked by the Pru-C2 and Pru-C5 primer sequences that were designed from the conserved C2 and C5 regions, respectively, of rosaceous *S*-*RNase* (Tao et al., 1999; Ushijima et al., 1998) and the full coding sequence for *SFB^f* (Hanada et al., 2009). No substantial differences or defects in the deduced amino acid sequence were found in the partial *S^f*-*RNase* and full-length *SFB^f* sequences. Although our full-length *SFB^f* sequence completely matches the partial *SFB^f* sequences that were reported by other research groups, partial *S^f*-*RNase* sequences that were posted on the public database by several research groups, including our group, show differences (Barckley et al., 2006; Bošković et al., 2007; Channuntapipat et al., 2001; Ma and Oliveira, 2002). Although it appeared later that the *S^f*-*RNase* sequence reported by Barckley et al. (2006) could be from misannotated *S^f*-*RNase* (synonymous to *S^b*-*RNase*) because their *S^f*-*RNase* sequence shows 100% match to *S^f*-*RNase* of Tuono (*S^f*), there are still some minor differences among the *S^f*-*RNase* sequences reported by the different research groups. This led us to thoroughly reinvestigate the *S* locus of the *S^f* haplotype in almond. We, therefore, isolated fosmid clones that contained the *S^f* locus of the SC ‘Lauranne’ to determine the full coding sequence of the *S^f*-*RNase* and the structure of the *S* locus region of the *S^f* haplotype. Furthermore, we conducted expression analysis of *S* locus genes by reverse transcriptase–polymerase chain reaction (RT-PCR) and discuss the possible molecular mechanisms of SC observed with the *S^f* haplotype with special references to the expression of *S^f*-*RNase*.

Materials and Methods

Plant materials. Young leaves, pistils, and pollen grains were collected from three almond cultivars, Lauranne (*S³S^f*), Ferragnès (*S^fS³*), and Tuono (*S^fS^f*), that were grown at the CITA de Aragón in Zaragoza, Spain. ‘Lauranne’ is from ‘Ferragnès’ × ‘Tuono’, and the *S^f* and *S³* haplotypes in ‘Lauranne’ are estimated to be from ‘Tuono’ and ‘Ferragnès’, respectively. We also used young leaf samples from ‘Tuono’ that was grown at the University of California at Davis, CA. Leaf samples were collected, frozen in liquid nitrogen, lyophilized, and stored at –20 °C with desiccant until used.

Isolation of DNA. Genomic DNA was isolated from young leaves using the Nucleon PhytoPure® plant and fungal DNA extraction kit (GE Healthcare, Piscataway, NJ) with

Received for publication 29 Jan. 2009. Accepted for publication 10 Feb. 2009.

This work was supported by a Grant-in-Aid (no. 20248004) for Scientific Research (A) to R.T.

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some modifications as described previously (Watari et al., 2007). In brief, 0.5 g of freeze-dried leaves was ground to a powder with MULTI-BEADS SHOCKER® (YASUI KIKAI, Osaka, Japan), suspended in washing buffer [0.1 M HEPES (pH 8.0), 0.1% (w/v) PVP, 2% (v/v) 2-mercaptoethanol], and mixed thoroughly. The mixture was centrifuged (6500 × g at 4 °C for 15 min) to collect the pellet; the pellet was resuspended in washing buffer and centrifuged again. This partially purified nuclear fraction was used to isolate DNA with the Nucleon PhytoPure® plant and fungal DNA extraction kit.

Cloning and characterization of the *S'* haplotype. A fosmid library was constructed from the genomic DNA of 'Lauranne' (*S³S'*) using the CopyControl Fosmid Library Production Kit (Epicentre, Madison, WI) as previously described (Ikeda et al., 2004; Ushijima et al., 2001). The library was screened with a DIG-dUTP-labeled (Roche Diagnostics, Basel, Switzerland) cDNA probe corresponding to the coding sequence between the conserved C2 and C4 regions of rosaceous *S*-RNase (Tao et al., 1999; Ushijima et al., 1998). A genomic clone that contained the *S* locus of the *S'* haplotype was selected and used for further studies. DNA sequences of both strands of the *S'*-RNase were determined by primer walking using the BigDye Terminator Version 3.1 Cycle Sequencing Kit and a 3730xl DNA analyzer (Applied Biosystems, Foster City, CA).

The transcriptional orientation and physical distance between *S'*-RNase and *SFB'* were determined by long PCR using an Expand Long Template PCR system (Roche Applied Science, Mannheim, Germany) as previously described (Watari et al., 2007). Each reaction consisted of 1× Expand Long Template buffer 3.5 mM of each dNTP, 2.75 mM MgCl₂, 300 nM of each primer, and 25 ng of fosmid DNA as a template, and 1 U of Expand Long Template Enzyme mix in a 15-μL reaction volume. Long PCR was performed using a program with an initial denaturing step at 94 °C for 2 min, 10 cycles of 94 °C for 10 s, 58 °C for 30 s, 68 °C for 15 min, 20 cycles of 94 °C for 10 s, 58 °C for 30 s, 68 °C for 15 min with an extension of an extra 20 s after each cycle, and final extension at 68 °C for 7 min. Three different primer combinations were used: Pru-C2 (5'-CTA TGG CCA AGT AAT TAT TCA AAC C-3') and SFB C5F (5'-TAC CAY WTM ATT GAG AAA GGT CC-3'), Pru-C2 and rtPd_SFBF_F (5'-TGA TTA CGA CTC CAA GCC AAC TC-3'), and rtPd_Sf_F (5'-CCT CCT TCG TTG CAA AGG AA-3') and SFB C5F. Primer positions are shown in Figure 1. Amplified fragments were separated by 0.5% agarose gel electrophoresis and visualized with ethidium bromide under ultraviolet light. Amplified fragments were also purified using ExoSAP-IT (USB, Cleveland, OH) and sequenced by the DTCS Quick Start kit and CEQ 8000 Genetic Analysis System (Beckman Coulter, Fullerton, CA).

DNA sequences encoding the region around the conserved C2 and C5 regions

(Fig. 2) of the *S'*-RNase of 'Tuono' were amplified by PCR using two primer sets, AS1 II (5'-TAT TTT CAA TTT GTG CAA CAA TGG-3') and PdSf_2intR (5'-TAA ATG CCA AAT AGT A-3'), and PdSf_F1 (5'-AAA GCA GCA ACT CAA AGA ATA C-3') and PdSf_R2 (5'-AAA AAG ATA AGA AAC AAA AGA C-3'), respectively (Fig. 3). All PCR reactions contained 1× ExTaq buffer, 200 μM of each dNTP, 400 nM of each primer, 50 ng of template genomic DNA, and 0.5 U of TaKaRa ExTaq polymerase (TaKaRa Bio, Ohtsu, Japan) in a 25-μL reaction volume. PCR was performed using a program with an initial denaturing step at 94 °C for 1 min, 35 cycles of 94 °C for 1 min, 58 °C for 1 min, 72 °C for 2 min, and a final extension at 72 °C for 7 min. Amplified fragments were purified by ExoSAP-IT and

sequenced using DTCS Quick Start kit and CEQ 8000 genetic Analysis System.

Reverse transcriptase-polymerase chain reaction. Total RNA was isolated from pistils and pollen grains from 'Lauranne', 'Tuono', and 'Ferragnès' by cold phenol method and first-strand cDNAs were synthesized by SuperScript III RT (Invitrogen, Carlsbad, CA) with adapter-dT primer as described (Watari et al., 2007). Gene-specific primers for *S'*-RNase, *S'*-RNase, *SFB'*, and *SFB'* were designed from *S'*-RNase (AB433984), *SFB'* (AB361036), *S^b*-RNase (AB011469), and *SFB^b* (AB092967) sequences. Almond *S^b*-RNase and *SFB^b* are synonyms of almond *S'*-RNase and *SFB'*, respectively (Channun-tapipat et al., 2001). The primer combinations used to amplify the RT-PCR products from *S'*-RNase, *S'*-RNase, *SFB'*, and *SFB'*

Fig. 1. Schematic diagrams illustrating *S'*-RNase and *SFB'* gene organization in the *S* locus region of the *S'* haplotype. The nucleotide "A" of the start codons of *S'*-RNase and *SFB'* are at +1. Gray boxes represent exons of the *S*-RNase and *SFB*. Oligonucleotide primers used to amplify the regions A, B, and C that include the intergeneric region between the *S'*-RNase and *SFB'* are indicated by open triangles with the primer names. The agarose gel image of the amplified products is shown below. Lanes A, B, and C show the polymerase chain reaction products from the regions A, B, and C, respectively.

Table 1. DNA sequences of oligonucleotide primers used for reverse transcriptase-polymerase chain reaction analysis.

Primer name	Sequence
Pru-T2	5'-TSTTSTTGSTTTTGCTTCTTC-3'
rtPd_SbR	5'-TCTCTTCAGTTTGACTCGCATTTT-3'
rtPd_SfR	5'-GTCGTCGCTTGGCTCTTAGG-3'
Pd_SFBb_F1	5'-CACCAAAGTTTTGAATGTCAGGTT-3'
Pd_SFBb_R1	5'-CACTAGGATATAGGTCAAGAAGCA-3'
Pd_SFBf_F1	5'-CACCCAAATTTGAACGAAAAGAC-3'
Pd_SFBf_R1	5'-TTCCTCCTCACAAGAATAAACTT-3'
ActF1	5'-ATGGTGAGGATATTCAACCC-3'
ActR1	5'-CTTCCTGTGGACAATGGATGG-3'

Table 2. Reported *S'* and *S*³⁰ RNases from *P. dulcis* and *P. webbii*.

Haplotype	Species	Cultivar	SI/SC	EMBL accession number	Reference	Abbreviation in Figure 2
<i>S'</i>	<i>P. dulcis</i>	Lauranne	SC	AB433984	This work	Pd_LAU
<i>S'</i>	<i>P. dulcis</i>	IRTA12-2	SC	AY291117	Channuntapipat et al., 2001	Pd_12-2
<i>S'</i>	<i>P. dulcis</i>	Tuono	SC	AF157009	Ma and Oliveira, 2002	Pd_TUO1
<i>S'</i>	<i>P. dulcis</i>	Tuono	SC	AM690356	Bošković et al., 2007	Pd_TUO2
<i>S</i> ³⁰	<i>P. dulcis</i>	Cinquanta Vignali	SI	AM690357	Bošković et al., 2007	Pd_CM
<i>S'</i>	<i>P. webbii</i>	Castel del Monte-2	SC	AM690358	Bošković et al., 2007	Pw_CM2
<i>S'</i>	<i>P. webbii</i>	Unknown	Unknown	DQ993666	Unpublished ²	Pw_Sf

²The sequence was only deposited in EMBL database.

SI = self-incompatible; SC = self-compatible.

Fig. 2. Deduced amino acid sequence alignment of the reported SC *S'*-RNases and the SI *S*³⁰-RNase from *P. dulcis* and *P. webbii*. The deduced amino acid sequences were aligned using Clustal W. Conserved regions C1, C2, C3, RC4, and C5 and the rosaceous hypervariable region are boxed (Ushijima et al., 1998). The positions of the conserved histidine residues essential for catalytic activity are marked with open triangles. An open circle shows the phenylalanine residue that is different from the previously reported *S'*-RNase sequences. Gaps are marked by dashes. Asterisks indicate positions that have a single, fully conserved residue. Abbreviations and EMBL accession numbers are as shown in Table 2.

were Pru-T2 and rtPd_SbR, Pru-T2 and rtPd_SfR, Pd_SFBb_F1 and Pd_SFBb_R1, and Pd_SFBf_F1 and Pd_SFBf_R1, respectively (Table 1). As references, RT-PCR for actin gene was also conducted using the ActF1 and ActR1 primers. The PCR mixture contained 1× *ExTaq* buffer, 200 μM each of dNTPs, 400 nM each of primers, 20 ng of template cDNA, and 0.5 U of TaKaRa *ExTaq* polymerase (TaKaRa Bio). PCR for *S*-RNase was performed using a program of 30 cycles of 94 °C for 30 s, 56 °C for 30 s, and 72 °C for 1 min 30 s with an initial denaturation step at 94 °C for 3 min and a final extension at 72 °C for 7 min. PCR for *SFB* was performed using a program of 30 cycles of 94 °C for 30 s, 53 °C for 30 s, and 72 °C for 1 min 30 s with an initial denaturation step at 94 °C for 3 min and a final extension at 72 °C for 7 min. PCR condition for actin is exactly the same as that described in Yamane et al. (2003). Amplified PCR products were separated by electrophoresis on 1.5% agarose gels, stained with ethidium bromide, and visualized under ultraviolet light.

Results

Several positive clones were obtained from the fosmid library constructed from 'Lauranne' (*S*³*S'*). With *Prunus S*-RNase-specific (Tao et al., 1999) and *SFB*-specific

(Yamane et al., 2003) PCRs, we selected several clones that contained full-length *S'*-RNase and *SFB* sequences. Long PCR conducted using one of the fosmid clones yielded three different length of fragments (A:7.6k, B:7.3k, C:6.3k) with three primer pairs, respectively (Fig. 1). Based on the length of amplified products, the physical distance between *S'*-RNase and *SFB* was estimated to be ≈6 kb with opposite relative transcriptional orientations. The coding region of *S'*-RNase was completely sequenced, and the deduced amino acid sequence was aligned with other reported *S'*-RNase and *S*³⁰-RNase sequences (Table 2; Fig. 2). The five conserved regions (C1, C2, C3, RC4, and C5) and a rosaceous hypervariable region (Ushijima et al., 1998) were present in the *S'*-RNase sequence predicted from the genomic clone for *S'*-RNase. To date, several different sequences of *S'*-RNase from different almond cultivars and *P. webbii* accessions have been reported (Bošković et al., 2007; Channuntapipat et al., 2001; Ma and Oliveira, 2002). Furthermore, *S*³⁰-RNase was recently identified in the SI *S*³⁰ haplotype, and its sequence indicated that it was the wild-type *S'*-RNase (Bošković et al., 2007) (Fig. 2). Compared with these sequences, the 'Lauranne' *S'*-RNase sequence showed several differences. First, the 'Lauranne' *S'*-RNase sequence had a conserved histidine residue in the C2 region

and there was no indication of replacement of the histidine residue with an arginine residue. Direct sequencing of the PCR products from the *S'*-RNase of 'Tuono' genomic DNA also indicated no replacement of the conserved histidine residue in the C2 region of the two 'Tuono' clones from CITA in Spain and the University of California at Davis in the United States (Fig. 3). We found another difference in the 'Lauranne' *S'*-RNase sequence and other reported *S'*-RNase sequences. Namely, the 'Lauranne' *S'*-RNase encodes a phenylalanine residue in place of a previously reported leucine residue in the C5 region. DNA sequencing of the PCR fragment amplified from 'Tuono' genomic DNA revealed that the two clones of 'Tuono' *S'*-RNase also encoded a phenylalanine residue in place of a leucine residue (Fig. 3).

Gene-specific primers for *SFB*, *S'*-RNase, *SFB*, and *S'*-RNase that were developed in this study (Table 1) could specifically amplify the respective genes among the three target *S* haplotypes, *S'*, *S*³, and *S'* of 'Lauranne' (*S*³*S'*), 'Tuono' (*S*³*S'*), and 'Ferragnès' (*S*³*S'*) (Fig. 4). In the expression analysis for *SFB*, *SFB*, fragments were specifically amplified from the genomic DNA and pollen cDNA of 'Ferragnès' and 'Tuono', whereas no amplification was observed either with the genomic DNA or pollen cDNA of 'Lauranne'. Similarly, *SFB* fragment was amplified from the genomic DNA and pollen cDNA of 'Tuono' and 'Lauranne', whereas no amplification was observed either with the genomic DNA or pollen cDNA of 'Ferragnès'. As expected, *SFB* showed pollen-specific gene expression and no amplification was observed with stylar cDNA of all three cultivars tested. In terms of the size of PCR products, there is no defect in *SFB*. In the expression analysis for *S*-RNase, *S'*-RNase fragments were amplified from the genomic DNA and style cDNA of 'Ferragnès' and 'Tuono', whereas no amplification was observed either from the genomic DNA or style cDNA of 'Lauranne'. As expected, *S*-RNase shows pistil-specific expression, and no amplification was observed from pollen cDNA. *S'*-RNase fragment was amplified from the genomic DNA of 'Tuono' and 'Lauranne', whereas no amplification was observed from stylar cDNA of 'Tuono' and 'Lauranne' under our PCR condition. It appeared that *S'*-RNase transcripts were not accumulated at the detectable level by our RT-PCR conditions in the styles of 'Lauranne' and 'Tuono'.

Fig. 3. Direct polymerase chain reaction (PCR) sequencing of the C2 (I) and C5 (II) regions of the *S'-RNase* of 'Tuono'. The determined 'Tuono' sequences are identical to the sequences of the respective portions of the *S'-RNase* of 'Lauranne'. Coding region and intron sequences are indicated by bold type and italics, respectively. Deduced amino acid sequences are shown under the nucleotide sequences. Boxed sequences indicate conserved regions of *Prunus* S-RNase. PCR primer name and directions are indicated by arrows.

Discussion

SC in SI *Prunus* species is usually conferred by mutated dysfunctional *S* haplotypes (Hauck et al., 2006; Tao et al., 2007; Tsukamoto et al., 2006; Ushijima et al., 2004), although SC associated with the pollen-part general factor has been reported in sweet cherry (Wünsch and Hormaza, 2004) and apricot (Vilanova et al., 2006). In almond, the *S'* haplotype is thought to be a mutated SC *S* haplotype because cosegregation of the SC and the *S'* haplotype has been described (Bošković et al., 1999). The *S'* haplotype could be a stylar part mutant, because RNase activity gel assays indicated that the *S'-RNase* of the *S'* haplotype encoded no RNase activity. This conjecture is further confirmed by a pollination study conducted by Bošković et al. (2007) showing that *S'* pollen tube growth was rejected in the style of the *S*³⁰ haplotype, a putative wild type of the *S'* haplotype.

Bošković et al. (2007) compared almond *S'-RNase* and *S*³⁰-*RNase* sequences and found a replacement of the conserved histidine residue in the C2 region of *S'-RNase* with an arginine residue to which they ascribed the SC

observed with *S'-RNase*. Although this argument is quite reasonable because the replaced histidine residue is one of the two histidine residues essential for T2/S-type RNase activity, our sequencing results pose a question regarding this argument. As opposed to the previous studies, we found a conserved histidine residue in the *S'-RNase* sequences from 'Lauranne' and the two clones of 'Tuono'. Although it is unknown why this kind of discrepancy occurred, our finding calls into question the previously reported molecular basis of SC in the *S'* haplotype (Bošković et al., 2007).

There is another discrepancy in the C5 region between the previously reported *S'-RNase* and our *S'-RNase* sequences. Namely, our *S'-RNase* sequences encode a phenylalanine residue in place of the previously reported leucine residue in the C5 region. This replacement in the C5 region was not described in the previous studies, probably because this region is used as a primer to clone the *S'-RNase* fragment and the sequence was mistakenly reported. Although most *Prunus* S-RNases have a leucine residue at this position in the C5 region, we

propose that this replacement does not affect the functionality of the *S'-RNase* because the fully functional *S*²-*RNase* of the *S*² haplotype of apricot have a phenylalanine residue at that position (Romero et al., 2004).

Expression analysis by RT-PCR indicated that *S'-RNase* transcripts were not accumulated at the detectable level in style in our PCR condition. To confirm there were no *S'-RNase* transcripts in the style, we isolated total RNA and conducted RT-PCR several times. On one occasion, a trace level of transcripts was detected from 'Tuono' style when the PCR cycles were increased to 35 cycles (data not shown). It may be possible, therefore, that transcription of *S'-RNase* was not completely shut down but the transcripts are present at a very low level in the style. Because the transcriptional level of *Prunus* S-RNase is very high and usually more amplification products were obtained from stylar cDNA than genomic DNA, we conclude that the transcription level of *S'-RNase* is not enough for a SI reaction. This could be supported by the fact that no *S'-RNase* activity was found in the RNase activity gel (Bošković et al., 1999), although our data do not support Bošković

Fig. 4. Expression analysis of *SFB*¹, *SFB*¹, *S'*-*RNase*, *S'*-*RNase*, and actin gene of almond. Polymerase chain reaction (PCR) was performed with cDNA from pollen (P), style (S), and genomic DNA (G) of 'Ferragnès' (FER), 'Tuono' (TUO), and 'Lauranne' (LAU). Because there is no intron sequence in the coding region of *SFB*, PCR products from *SFB* from genomic DNA show the same size as those from cDNA. Because PCR for *S'-RNase* was designed to amplify the region containing an intron, amplified products from cDNA and genomic DNA showed different sizes. Arrow indicates the position where PCR products from cDNA for *S'-RNase* should be located.

et al. (2007), in which no *S'-RNase* activity was ascribed to the replacement of the histidine residue with the arginine residue in the C2 region. Although it is unlikely that the replacement found in the coding region for the C5 region in *S'-RNase* affects its transcription, it is intriguing to investigate if *S³⁰-RNase* has this replacement in the C5 region.

Further comparisons of the *S'* haplotype and the original SI *S³⁰* haplotype could clarify the molecular basis of SC with the *S'* haplotype and could lead to the development of DNA markers to distinguish them for future SC breeding in almond. However, because it has been suggested that the possession of the *S'* haplotype does not necessarily result in SC, possibly because of inbreeding depression (Alonso and Socias i Company, 2005), early detection of SC seedlings should take this inbreeding effect into consideration.

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