RESEARCH PAPER

Self-incompatibility (S) locus region of the mutated S6-haplotype of sour cherry (Prunus cerasus) contains a functional pollen S allele and a non-functional pistil S allele

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Received 17 March 2003; Accepted 17 July 2003

Abstract

This study characterizes the S6m-haplotype, a mutated S6-haplotype with an altered HindIII cut site, of sour cherry (Prunus cerasus). Inheritance and pollination studies of S-haplotypes from reciprocal crosses between ‘Erdi Botermo’ (EB; S6S6mS6m) and ‘Rheinische Schattenmorelle’ (RS; S6S6mS6m) revealed that the S6m-haplotype conferred unilateral incompatibility with a non-functional pistil component and a functional pollen component. Expression analyses of S6-RNase and SFB, a candidate gene for pollen-S, in the S6m-haplotype showed that SFB was transcribed in EB pollen, but S6-RNase was not transcribed in EB styles. These results were consistent with data from the inheritance and pollination studies. Inverse PCR for the flanking regions of S6-RNase in the S6- and S6m-haplotypes revealed an approximately 2600 bp insertion present at approximately 800 bp upstream of the S6-RNase in the S6m-haplotype, which is responsible for the alternation of the HindIII cut site and a possible cause of inhibition of the transcription of S6-RNase. SFB was present downstream of S6-RNase in both the S6- and S6m-haplotypes and expressed in the same way, supporting the idea that SFB is a good candidate for pollen-S in Prunus.

Key words: Pistil determinant, pollen determinant, polyploidy, Prunus avium, Rosaceae, SFB, S-RNase.

Introduction

Gametophytic self-incompatibility (GSI) is a widespread mechanism in flowering plants that prevents self-fertilization and promotes out-crossing (de Nettancourt, 1977). Most rosaceous fruit tree species exhibit the GSI system, in which the compatibility of a cross is determined by the haploid genome of the pollen and the diploid genome of the pistil (McCubbin and Kao, 2000). The specificity of the SI response is determined by the haplotypes of the polymorphic S-locus, which presumably contains at least one gene for the pistil determinant and one for the pollen determinant. The pistil determinant in three plant families, namely, Rosaceae (Sassa et al., 1992), Solanaceae (Anderson et al., 1986; McClure et al., 1989) and Scrophulariaceae (Xue et al., 1996), is known to be an S-RNase. Although the pollen determinant (pollen-S) in these families has not been identified, a very good candidate gene for the pollen-S, an S haplotype-specific F-box protein gene (SFB), has recently been found in three species of Prunus in the Rosaceae, namely P. dulcis (Ushijima et al., 2003), P. avium and P. cerasus (Yamane et al., 2003). The features of SFB, such as its pollen-specific expression, the tight linkage with S-RNase, and the high level of allelic polymorphism, are appropriate characteristics for the male determinant in the S-RNase-based GSI system, although direct evidence that SFB is the pollen-S remains to be presented.

Natural and induced mutations in SI reactions, as well as transgenic techniques (Lee et al., 1994; Murfett et al., 1994), have been utilized successfully to elucidate the molecular basis of the S-RNase-based GSI system. A stylar-part mutation found in Pyrus pyrifolia (synonym of P. serotina) has provided evidence that the S-locus of Rosaceae is bipartite with different pistil and pollen components (Sassa et al., 1997). The characterization of mutations affecting S-RNase activity and thus the stylar specificity of the SI reaction have led to the identification...
of a histidine residue essential for RNase activity in *Lycoperisicon peruvianum* (Royo et al., 1994) and *Petunia inflata* (McCubbin et al., 1997). The majority of pollen-part mutations in solanaceous plants were associated with the duplication of the *S*-locus region (Brewbaker and Natarajan, 1960; Golz et al., 1999, 2001; Pandey, 1965, 1967; van Gastel and de Nettancourt, 1975). This observation is consistent with an interesting aspect of the GSI system—that it commonly breaks down as a result of polyploidy resulting in SC individuals.

In *Prunus*, the breakdown of GSI with polyploidy is exhibited in diploid sweet cherry (*Prunus avium*) (2n=2x=16) and tetraploid sour cherry (*P. cerasus*) (2n=4x=32). By contrast with the SI diploid sweet cherry, tetraploid sour cherry can be either SI or SC. During efforts to elucidate the genetic control of SI/SC in tetraploid sour cherry, a mutated *S*-haplotype was found with an altered *HindIII* cut site in *´erdi Botermo´ (EB; Yamane et al., 2001). This haplotype, named the *S*<sup>6m</sup>-haplotype, is potentially very useful for genetic studies of *S*-haplotype inheritance because the *S*<sup>6</sup>-haplotypes of the seed and pollen parents can be distinguished in terms of the size of the RFLP band obtained following digestion with *HindIII* and probing with an *S*<sup>3</sup>-RNase cDNA probe. For example, Hauck et al. (2002) showed that no progeny from the cross *´Rheinische Schattenmorelle´* (RS; *S*<sup>6</sup>*S*<sup>6m</sup>*S*)×EB (*S*<sup>6m</sup>*S*<sup>6</sup>)*S*<sup>6m</sup>*S*) inherited the *S*<sup>6m</sup>-haplotype.

In this study, to characterize the *S*<sup>6m</sup>-haplotype further, inverse PCR was performed to clone flanking regions of the *S*<sup>6</sup>-RNase containing the upstream cis regulatory regions of the *S*<sup>6</sup>-RNase and *SFB*<sup>6</sup>, a candidate gene for pollen-<em>S</em>-the *S*<sup>6</sup>-haplotype of sweet and sour cherries (Yamane et al., 2003). An approximately 2600 bp insertion was located at approximately 800 bp upstream of *S*<sup>6</sup>-RNase, which is responsible for the alternation of the *HindIII* cut site in the *S*<sup>6m</sup>-haplotype and a possible cause of the inhibition of the transcription of *S*<sup>6</sup>-RNase. *SFB*<sup>6</sup> was present downstream of *S*<sup>6</sup>-RNase in both the *S*<sup>6</sup>- and *S*<sup>6m</sup>-haplotypes and expressed in the same way. These data supported the idea that *SFB* was a candidate gene for pollen-*S* in *Prunus* and suggested that the *S*<sup>6m</sup>-haplotype conferred unilateral incompatibility with a non-functional pistil component and a functional pollen component, which coincided with the data from the inheritance and pollination studies. The involvement of this mutation in the evolution of SC in tetraploid sour cherry is discussed.

**Materials and methods**

**Plant material**

Two sour cherry (*P. cerasus*) cultivars, *´erdi Botermo´ (EB; *S*<sup>6</sup>*S*<sup>6m</sup>*S*) and *´Rheinische Schattenmorelle´* (RS; *S*<sup>6</sup>*S*<sup>6m</sup>*S*), and one Michigan State University sour cherry seedling selection, 2(7) (*S*<sup>6m</sup>*S*<sup>6</sup>), from a cross between RS and EB were used for this study. These sour cherry cultivars and a selection were tetraploid and grown at the Michigan State University Clarksville Horticultural Experiment Station, Clarksville, Michigan. Although three different *S*-haplotypes have been identified in EB and 2(7), it is not known at the present time if the fourth *S*-haplotype is a null-haplotype or a second copy of one of the three *S*-haplotypes already identified (Hauck et al., 2002; Yamane et al., 2001). Two diploid sweet cherry (*P. avium*) cultivars, *´Benishuho´* (BS; *S*<sup>6</sup>*) and *´Van´* (VN; *S*<sup>6</sup>), growing at Yamagata University, Japan, were also used.

**Pollination experiment**

EB was pollinated with RS at the Clarksville Horticultural Station in 2000. Nine seeds obtained were sown in the spring of 2001, and young leaves of seedlings from the nine seeds were collected in 2002. The *S*-haplotypes of the progeny obtained were determined by genomic DNA blot analysis using *HindIII* digestion and a cDNA probe specific for *S*<sup>3</sup>-RNase as described by Hauck et al. (2002).

**Pollen tube growth assay**

Pollination experiments were performed based on Yamane et al. (2001). In brief, ten emasculated flowers of EB and 2(7) were hand pollinated with BS or VN pollen when receptive in the laboratory at 25°C. The pollinated pistils were collected 72 h after pollination and immersed in fixing solution (1:3:1, by vol. chloroform:95% ethanol:glacial acetic acid) for 24 h, transferred to 100% ethanol, and stored at 4°C until used. The pistils were washed thoroughly under running tap water and incubated in 10 N NaOH for 5–6 h to soften the tissues. The pistils were then soaked in 0.1% aniline blue solution with 33 mM K<sub>3</sub>P<sub>4</sub> for 1 h. Pollen tubes were observed by ultraviolet fluorescent microscopy (BX60, Olympus, Tokyo, Japan).

**RT-PCR**

Total RNA was isolated from leaves, styles with stigma at the balloon stage of development, and pollen grains from EB and RS as described elsewhere (Tao et al., 1999). One microgram of total RNA was used for first strand cDNA synthesis by SUPER SCRIPT II RT (Life Technologies, Tokyo, Japan). For *S-RNase* expression, *Pru-C2* (5′-CTA TGG CCA AGT AAT TAT TCA AAC C-3′) and *Pru-C4R* (5′-GGA TGT GGT AGC ACG GAA GCCG-3′) primers (Tao et al., 1999) were used to amplify all *S-RNase* genes, whereas *Pru-T2* (5′-TST TST TGS TTT TGG ACG ATT CAA AAC C-3′) and *S6-INV-R2* (5′-AGG TCG CTT GAT GCC ACG-3′) primers were used to amplify *S-RNase* only. Polymerase chain reaction (PCR) conditions were identical to that used by Tao et al. (1999). For *SFB* expression, *S6-exon-F1* (5′-CCA ACC TCA AAA GAT GAC ATT C-3′) and *S6-C3R* (5′-TCC TTA CAG CCT TAT AGT CG-3′) primers (Yamane et al., 2003) were used. Expression of the actin gene was used as a reference with the primers ActF1 (5′-ATG GTG AGG AGG AGA TTC AAC AC-3′) and ActR1 (5′-GGA GTG GAA AAC C-3′) primers. PCR conditions were identical to those used by Yamane et al. (2003).

**Inverse PCR and sequencing**

Total DNA was isolated from young leaves by the hexadecyltrimethylammonium bromide (CTAB) method as described by Stockinger et al. (1996). One microgram of total DNA was digested with *HindIII*, self-ligated with T4 ligase, and used as a template DNA for inverse PCR with oligonucleotide primers, *S6-INV-F2* (5′-TGG TAC CAC AAT CCG GGA TCC-3′) and *S6-INV-R2*. The PCR products were subcloned into the T-A cloning vector (pGEM-T Easy Vector System; Promega, Madison, Wisconsin) and their DNA sequences were determined using the Dye Terminator Cycle Sequencing Kit (Applied Biosystems, Tokyo, Japan) and the ABI PRISM™ 310 Genetic Analyzer (Applied Biosystems, Tokyo, Japan).
Table 1. RFLP segregation of S-haplotypes in the progeny from reciprocal crosses between ‘Erdis Botermo’ (EB) and ‘Rheinische Schattenmorelle’ (RS)

For the parental S-haplotypes, + and − indicate presence and absence, respectively. Data for EB × RS was obtained in this study while that for RS × EB was cited from Hauck et al. (2002), in which only the data for 85 progeny that could be unambiguously scored for each S-haplotype was included.

<table>
<thead>
<tr>
<th>S-haplotype</th>
<th>Parents</th>
<th>EB × RS</th>
<th>RS × EB</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Presence</td>
<td>Absence</td>
</tr>
<tr>
<td>$S^6$</td>
<td>+</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>$S^6$</td>
<td>−</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>$S^4$</td>
<td>+</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>$S^6m$</td>
<td>−</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>$S^6m$</td>
<td>+</td>
<td>3</td>
<td>6</td>
</tr>
</tbody>
</table>

Table 2. Cross-compatibility results for pollination of sour cherry pistils with sweet cherry pollen based on examination of pollen tube growth in styles 72 h after pollination

Although three different S-haplotypes have been identified in the stylar parents 2(7) and ‘Erdis Botermo’ (EB), it is not known at the present time if the fourth S-haplotype is a null-haplotype or a second copy of one of the three S-haplotypes already identified.

<table>
<thead>
<tr>
<th>Style parent (S-haplotypes)</th>
<th>Pollen parent (S-haplotypes)</th>
<th>Benishuho ($S^6S^6$)</th>
<th>Van ($S^6S^6$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2(7) ($S^6S^6S^6$) EB ($S^6S^6m$)</td>
<td>Incompatible</td>
<td>Compatible</td>
<td>Compatible</td>
</tr>
<tr>
<td>2(7) ($S^6S^6m$)</td>
<td>Incompatible</td>
<td>Compatible</td>
<td>Compatible</td>
</tr>
</tbody>
</table>

Results and discussion

Inheritance and pollination studies reveal a dysfunctional pistil S-allele in the $S^{6m}$-haplotype

The S-haplotype combinations of EB and RS are $S^6S^6$, $S^6mS^6$, and $S^6mS^6$, respectively (Yamane et al., 2001). Although EB is tetraploid and should have one additional S-haplotype, the additional S-haplotype supposedly present in its genome remains unidentified by RFLP analysis with the probe specific for S-RNase (Hauck et al., 2002; Yamane et al., 2001). The alphabetical S-haplotype were assigned to putative S-haplotypes whose function for self-incompatibility is unknown (Hauck et al., 2002; Yamane et al., 2001). The $S^{6m}$-haplotype is a mutated $S^6$-haplotype with an altered HindIII cut site that could be detected by genomic DNA blot analysis although the coding sequence of its S-RNase was unaffected and identical to that of the $S^6$-haplotype (Hauck et al., 2002; Yamane et al., 2001). As shown in Table 1, the $S^{6m}$-haplotype was inherited in the EB × RS progeny, whereas no progeny inherited the $S^{6m}$-haplotype in the reciprocal cross of RS × EB. By contrast with the $S^{6m}$-haplotype, the $S^6$-haplotype was inherited in the progeny from both of the reciprocal crosses between RS and EB. These data indicated that the $S^{6m}$-haplotype could be a mutated S-haplotype that confers a unilateral incompatibility in which the pollen component is functional, but the stylar component is non-functional. To confirm that the stylar component in the $S^{6m}$-haplotype was dysfunctional, pollen tube growth tests were performed with sweet cherry pollen. Sweet cherry is a diploid species closely related to tetraploid sour cherry and shares several S-haplotypes with sour cherry. To confirm the ability of sweet cherry pollen tubes to grow in the sour cherry pistils (Hauck et al., 2002), 2(7) ($S^6S^6$) and EB ($S^6S^6m$) pistils were pollinated with pollen of VN ($S^6S^6$), which contains none of the S-haplotypes found in either of the two sour cherry cultivars. VN ($S^6S^6$) pollen tubes were able to grow down the full length of the 2(7) ($S^6S^6S^6$) and EB ($S^6S^6mS^6$) styles (Table 2). When BS ($S^6S^6$) pollen was pollinated onto EB ($S^6S^6mS^6$) and 2(7) ($S^6S^6S^6$), dysfunction of the pollen inhibition, attributed to the $S^{6m}$-haplotype of EB, was apparent. BS ($S^6S^6$) pollen tubes were able to grow down the full length of the EB ($S^6S^6S^6$) styles (Fig. 1), while they were arrested at the upper part of the 2(7) ($S^6S^6S^6$) styles (Fig. 1). Since no progeny was shown to have the $S^{6m}$-haplotype in RS × EB (Table 1; Hauck et al., 2002), the BS pollen tubes that grew down the full length of the EB styles could be from $S^6$ pollen, but not from $S^6m$ pollen. The data from the inheritance and pollination studies could suggest that the S-RNase in the $S^{6m}$-haplotype was unable to arrest the $S^6$ pollen.

S$^6$-RNase is not transcribed but SFB$^6$ is transcribed in the $S^{6m}$-haplotype

For S-RNase expression analysis, RT-PCR of stylar RNA of RS and EB was performed. In addition to RT-PCR, PCR was also performed with genomic DNA to confirm that the $S^{6m}$-haplotype contains coding sequence for S-RNase (Yamane et al., 2001). As shown in Fig. 2, both the EB and RS genomes had the S-RNase sequence flanked by Pru-T2 and S6-INV-R2 primer sequences. Furthermore, amplification with the primers corresponding to the conserved regions of sweet cherry S-RNases, Pru-C2 and Pru-C4R, was observed in both cultivars, suggesting that the S-RNase was transcribed in their styles (Fig. 2). Different sized bands obtained with PCR from genomic DNA and RT-PCR confirmed that amplification was from cDNA. Following RT-PCR using the S$^6$-RNase gene-specific primers, Pru-T2 and S6-INV-R2, amplification was observed in RS, but not in EB (Fig. 2), indicating that the S$^6$-RNase was not transcribed in the style of EB while it was transcribed in that of RS, suggesting that S$^6$-RNase in the $S^{6m}$-haplotype is not transcribed. To characterize the expression of the $S^{6m}$-haplotype further, RT-PCR was performed for SFB$^6$ with total RNA from RS and EB pollen. SFB$^6$ is an S haplotype-specific F-box protein gene for the S$^6$-haplotype that has been recently discovered in the S-locus region of the S$^6$-haplotype of RS and sweet...
cherry ‘Satonishiki (S^3 S^6; Yamane et al., 2003). SFB^6 was located in very close proximity, about 380 bp downstream, of the S^6-RNase and believed to be a very good candidate for pollen determinant in the S^6-haplotype of cherries (Yamane et al., 2003). RT-PCR with SFB^6-specific primers revealed pollen-specific transcription of SFB^6 in both RS and EB pollen (Fig. 3). Since the pollen component of the S^6m-haplotype of EB was supposed to be functional based on the inheritance and pollination studies (Table 1; Hauck et al., 2002; Yamane et al., 2001), this result is consistent with SFB being a very good candidate for the pollen determinant of GSI in Prunus (Ushijima et al., 2003; Yamane et al., 2003).

**Figure 1.** Pollen tube growth of ‘Benishuho (S^6 S^6)’ pollen in sour cherry pistils. (A) The pistil of ‘Erdi Botermo’ (EB; S^4 S^6 S^m S^a), 72 h after pollination. (B) The pistil of MSU seedling 2(7) (S^6 S^m S^a), 72 h after pollination. The upper two photographs show the stigmas and upper styles while the lower photographs show the base of the styles. The blight signals were from pollen tubes, but the faint signals were from the fibres of the pistils. BS pollen tubes grew down the full length of the EB style while they were arrested in the upper part of the 2(7) style.

**Figure 2.** PCR and RT-PCR analyses with the primers from conserved regions of the S-RNase of Prunus, Pru-C2 and Pru-C4R, and the primers for S^6-RNase specific amplification, Pru-T2 and S6-INV-R2. Total DNA and total RNA from styles with stigmas of ‘Erdi Botermo’ (EB) (S^4 S^6 S^m S^a) and ‘Rheinische Schattennormelle’ (RS) (S^6 S^a S^m S^b S^c) were used. M: 123 bp DNA ladder.

**Insertion of 2600 bp is present in the S'-flanking region of the S^6-RNase in the S^6m-haplotype**

To investigate the nature of the mutation in the S^6m-haplotype, the S-locus regions of the S^6- and S^6m-haplotypes were compared. Bands of approximately 5 kb and 8 kb were amplified by inverse PCR from the S^6- and S^6m-haplotypes, respectively. These results are consistent with the different size fragments obtained from RFLP analysis with digestion by HindIII and the probe that was specific for S^6-RNase (Tao et al., 1999; Yamane et al., 2001). Sequencing analysis revealed an approximately
seemed to be a transposable element-like insert because
the insert fragment was flanked by 12 bp repetitive
sequences (CTAAAAGATAGG). Transposable element
insertion is quite possible in the S-locus region because the
S-locus regions of Lycopersicon, Antirrhinum and almond
are reported to be very rich in retrotransposons (Lai et al.,
2002; Ushijima et al., 2003; Royo et al., 1996). The
S-locus region of Brassica that exhibits sporophytic SI is
also known to be rich in retrotransposon sequences (Cui
et al., 1999; Suzuki et al., 1999).

Some nucleotide substitutions were found in the flank-
ing regions of the S\(^6\)-RNase genes of the S\(^6\)- and S\(^6m\)-
haplotypes; however, the 2600 bp insertion was the only
major difference that could be found between them. This
suggests that a cis regulatory element is present around the
region where the insertion was present. In Solanum
tuberosum, promoter analysis indicated that 200 bp of
the 5\(^\prime\)-flanking region of S\(^2\)-RNase was sufficient for its
stylar transcription and revealed conserved sequences,
named Motif I and Motif III (Ficker et al., 1998), at the
position -200 to -100 of two S-RNase genes. Since a Motif
I-like sequence was also found in a similar position of
rosaceous S-RNases (Ishizaka et al., 2003; Norioka et al.,
2001), the cis-regulatory element for S-RNase transcrip-
tion in the style was estimated to be present within the
region 200 bp upstream of S-RNase genes. However, if the
insertion in the S\(^6m\)-haplotype upstream of the Motif I-like
sequence inhibits transcription of S\(^6\)-RNase, there could be
unknown cis regulatory element(s) present at around -797
of the S\(^6\)-RNase in addition to the Motif I-like sequence
found at about -140 of the S\(^6\)-RNase.

The stylar loss-of-function, due to the lack of S\(^6\)-RNase
transcription but the presence of SFB\(^6\) transcripts in pollen,
was consistent with the unilateral incompatibility reaction
observed with the S\(^6m\)-haplotype. RS pollen with the S\(^6\)-
haplotype was capable of fertilizing EB eggs because S\(^6\)-
RNase was absent in the pistil of EB. By contrast, growth
of EB pollen with the S\(^6m\)-haplotype was arrested in the
pistil of RS because the pollen-S allele of the S\(^6m\)-
haplotype was not affected by the transposable element-
like sequence insertion. Thus the pollen tube growth could
be arrested due to the recognition of the functional S\(^6\) pollen
determinant by the S\(^6\)-RNases in the RS styles. However,
since the results from the pollination experiments (Fig. 1;
Tables 1, 2) can also be explained by the loss-of-function of the so-called ‘modifier gene’ that is
necessary for SI reaction, such as HT in Solanaceae
(McClure et al., 1999), the possibility that the involvement
of mutation, other than that of the S\(^6\)-RNase gene, cannot
be ruled out. It has been reported that the SC cultivated
tomato (Lycopersicon esculentum) has mutations both in
S-RNase and HT (Kondo et al., 2002).

The S\(^6m\)-haplotype is a naturally occurring S-RNase
loss-of-function mutant that presumably contributes to the
SC phenotype of EB. EB is a hybrid selection from a cross
between the SI sour cherry landrace cultivar ‘Pandy 38’
and the SC sour cherry cultivar ‘Nagy Angol’ that was
made with the goal of breeding SC sour cherry cultivars
(Apostol and Iezzoni, 1992). Since the SI ‘Pandy 38’ has the
S-haplotype S\(^6\)S\(^d\)S\(^d\) (Yamane et al., 2001), it is
suspected that the S\(^6m\)-haplotype is derived from the pollen
parent, ‘Nagy Angol’. The fully functional S\(^6\)-haplotype is
a common S-haplotype in diploid sweet cherry, one of the
progenitor species of the tetraploid sour cherry. Yet no SC
mutants of the S\(^6\)-haplotype have ever been reported in
diploid sweet cherry. It is possible that the tetraploid nature of sour
cherry enables this species to tolerate inbreeding depress-
ion to the extent that SC mutations at the S-locus can persist in the population.

In conclusion, this study describes the possibility of
the prevention of S-RNase transcription by a 2600 bp

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**Fig. 3.** Expression of S\(^6\)-RNase, SFB\(^6\) and the gene for actin in
‘Rheinische Schattenmorelle’ (RS; S\(^6\)S\(^d\)S\(^d\)) and ‘Erdi Botermo’ (EB;
S\(^6m\)S\(^d\)S\(^d\)). RT-PCR was performed with total RNA from pollen, leaves,
and pistils of the two cultivars. See text for details.

**Fig. 4.** Schematic representation of the structures of the S-haplotype regions of S\(^6\) and S\(^6m\) that were cloned by inverse PCR. The position of the
‘A’ of the putative ATG codon of S\(^6\)-RNase is taken as +1. Motif IB-like: the region homologous to the potato S-RNase promoter conserved in
three sweet cherry S-RNase genes (Ishizaka et al., 2003).
insertion, which could lead to a stylar part mutant in an S-RNase-based GSI reaction. Furthermore, it appeared that this insertion had no effect on the expression of SFB, which supports the idea that SFB is a candidate gene for pollen-S in *Prunus*. Further insight into the genetic control of SI/SC in sour cherry could provide clues to the molecular mechanism of the SI reaction in *Prunus* and the impact of polyploidy on the evolution of self-compatibility.

Acknowledgements

This work was supported in part by grant in aid 13460014 for scientific research (B) to R Tao and 14760017 for Young Scientists (B) to H Yamane from the Japanese Society for the Promotion of Science. We thank J Soejima at the National Institute of Fruit Tree Science (Morioka, Japan) for providing us with plant materials.

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