RESEARCH PAPER

Interhaplotypic heterogeneity and heterochromatic features may contribute to recombination suppression at the S locus in apple (*Malus × domestica*)

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Abstract

Gametophytic self-incompatibility (GSI) is controlled by a complex S locus containing the pistil determinant S-RNase and pollen determinant SFB/SLF. Tight linkage of the pistil and pollen determinants is necessary to guarantee the self-incompatibility (SI) function. However, multiple probable pollen determinants of apple and Japanese pear, SFBBs (*S locus F-box brothers*), exist in each S haplotype, and how these multiple genes maintain the SI function remains unclear. It is shown here by high-resolution fluorescence *in situ* hybridization (FISH) that SFB genes of the apple *S*³ haplotype are physically linked to the *S*-RNase gene, and the S locus is located in the subtelomeric region. FISH analyses also determined the relative order of *SFB* genes and *S-RNase* in the *S*³ haplotype, and showed that gene order differs between the *S*⁹ and *S*³ haplotypes. Furthermore, it is shown that the apple S locus is located in a knob-like large heterochromatin block where DNA is highly methylated. It is proposed that interhaplotypic heterogeneity and the heterochromatic nature of the S locus help to suppress recombination at the S locus in apple.


Introduction

Self-incompatibility (SI) is a mechanism to prevent self-fertilization and to promote outcrossing in plants (Franklin-Tong and Franklin, 2003). S-RNase-based gametophytic self-incompatibility (GSI) is the most widespread SI system, and has been found in the families Solanaceae, Rosaceae, and Plantaginaceae. In GSI systems, the S haplotype contains at least two tightly linked S-determinant genes, pistil S and pollen S. The pistil S gene encodes an extracellular ribonuclease called S-RNase, which is thought to act as a cytoxin to inhibit growth of the self pollen tube via degradation of pollen RNAs (Anderson et al., 1986; McClure et al., 1989, 1990; Lee et al., 1994; Murfett et al., 1994; Broothaerts et al., 1995; Xue et al., 1996; Sassa et al., 1996, 1997). For pollen S, F-box genes, SLF (*S locus F-box*)/SFB (*S haplotype-specific F-box*), were identified as candidates (Lai et al., 2002; Entani et al., 2003; Ushijima et al., 2003, 2004; Yamane et al., 2003; Sijacic et al., 2004; Sonneveld et al., 2005; Hauck et al., 2006). Recently, in *Petunia* (Solanaceae), a ‘collaborative non-self recognition model’ was proposed where at least three related SLF genes in an S haplotype encode pollen S determinants, and these factors work together to suppress non-self S-RNases (Kubo et al., 2010). Multiple F-box genes have also been identified as pollen S candidates in Japanese pear and apple of the tribe Pyreae (Rosaceae) (Sassa et al., 2007; Minamikawa et al., 2010; Kakui et al., 2011). Minamikawa et al. (2010) reported that the S locus of apple contains more than ten SFB genes. Recent findings on the SFBBs of Japanese pear suggested...
that GSI in Pyreae, like in petunia, a ‘non-self’ recognition by multiple factors’ system in which numerous SFBB genes in an S haplotype are components of the pollen determinant, and each SFBB targets a subset of non-self S-RNases (Kakui et al., 2011).

Tight linkage of the pollen S and pistil S genes is necessary for GSI to function. Recombination at the S locus would cause the breakdown of SI by generating differences in pollen and pistil specificity within a single S haplotype (Sijacic et al., 2004). As a result of recombination at the S locus, otherwise incompatible ‘self’ pollen would exhibit specificity different from the pistil and be accepted by the pistil. The centromeric localization of the S locus of the Solanaceae is considered important to suppress recombination at the locus and to maintain the SI system (Brewbaker and Natarajan, 1960; Pandy, 1965; Bernatzky, 1993; Bernacchi and Tanksley, 1997; ten Hoopen et al., 1998; Entani et al., 1999; Golz et al., 2001). Although the S locus is located at the distal end of the chromosome in Antirrhinum and Ipomoea, heterochromatinization at the locus is assumed to have epigenetic roles in the suppression of recombination (Suzuki et al., 2004; Yang et al., 2007). The S locus in apple was found to be located in the subtelomeric region by fluorescence in situ hybridization (FISH) (Minamikawa et al., 2010). However, the order and precise chromosomal location of the multiple SFBB genes remain unclear. In this study, a BAC-FISH analysis was conducted on the apple pachytene chromosome to determine the precise location and relative order of SFBB and S-RNase genes. The relative order of these genes differed between the S9 and S9 haplotypes, indicating structural heterogeneity at the S locus among S haplotypes. In addition, the BAC-FISH and immunostaining of 5-methylcytosine (5mC) revealed that the apple S locus shows heterochromatinization and DNA methylation compared with surrounding regions. The interhaploptic heterogeneity and heterochromatic and methylated nature of the apple S locus may contribute to the recombination suppression and maintenance of the SI function.

Materials and methods

Plant materials

Immature flower buds of Malus × domestica ‘Florina’ (S9) were collected from the Nagano Fruit Tree Experiment Station (Nagano prefecture, Japan) and fixed in Farmer’s solution [3:1 (v/v) ethanol:acetic acid] for more than 24 h. The fixed materials were transferred to 70% ethanol and stored at 4 °C.

FISH

Pachytene chromosomes were prepared using squashes of anthers from the fixed flower buds. Chromosome slides were prepared following established protocols (Minamikawa et al., 2010). Interphase nuclei were also prepared from fixed anthers to check the specificity of BAC probes.

BAC DNA was extracted by a standard alkaline lysis method and purified using PEG 8000 (Minamikawa et al., 2010). DNA probes were labelled by nick translation using the DIG Nick Translation Mix (Roche) or Biotin Nick Translation Mix (Roche). For triple-colour FISH, the third probe was labelled with a combination of 50% DIG-11-dUTP and 50% Biotin-16-dUTP, which would result in the detection of the intermediate.

Pretreatment, post-fixation, and FISH were conducted according to the procedures of Minamikawa et al. (2010) with some modifications. Briefly, the slides were incubated in 100 ng µl−1 of RNase at 37 °C for 60 min followed by 5 ng µl−1 of pepsin at 37 °C for 20 min, and post-fixed in 3:1 (v/v) ethanol:acetic acid for 10 min. Ten microlitres of hybridization solution (50% formamide, 10% dextran sulphate, 2× SSC, 20 ng of each probe, and 3 µg of apple genomic blocking DNA) was applied to each slide. Chromosomal DNA and probe DNA were denatured for 5 min at 80 °C. The slide was incubated at 37 °C for 2 d. After washing in distilled water, 125 µl of the 1st antibody cocktail [1% BSA, 4× SSC, 1 µg of FITC streptavidin conjugates (Molecular Probes), and 0.1 µg of mouse anti-digoxigenin (Roche)] was applied for 30 min at 37 °C. After washing, 125 µl of the 2nd antibody cocktail [1% BSA, 4× SSC, and 0.5 µg of biotinylated anti-streptavidin (Vector Laboratories)] was then applied and incubated. Finally, after washing, 125 µl of the 3rd antibody cocktail [1% BSA, 4× SSC, 1 µg of FITC streptavidin conjugates (Molecular Probes), and 1 µg of Alexa fluo 568-conjugated rabbit anti-mouse IgG (H+L) (Molecular Probes)] was applied. Chromosomes were counterstained with VECTASHIELD (Vector Laboratories) containing 5 µg ml−1 of 4, 6 diamidino-2-phenylindole (DAPI).

FISH signals were captured with a Leica DM RXA2 fluorescence microscope equipped with a CCD camera (Leica DC500F) and processed by Leica CW4000 and Adobe Photoshop v5.1. At least ten FISH images were analysed to determine the location on the chromosomes.

Immunodetection of 5mC

The chromosome slides for the immunofluorescence assay of 5mC were prepared according to the above FISH protocol. To detect 5mC, the slides were denatured in 70% formamide in 2× SSC, at 80 °C for 2 min, and then dehydrated in a series of cold (–20 °C) ethanol solutions (70%, 95%, and 100%) for 5 min each. 100 µl of blocking solution (1% BSA, 1× PBS) was dropped onto each slide and the slides were then incubated at 37 °C for 30 min in a wet chamber. After washes in 1× PBS, the slides were incubated with 125 µl of blocking solution containing mouse monoclonal antibody against 5-methylcytosine (1:50; Eurogentec) overnight at 4 °C. The mouse antibodies were detected using Alexa Fluor 568 rabbit anti-mouse IgG (1:1000; Invitrogen). Chromosomes were counterstained with 5 µg ml−1 of DAPI in VECTASHIELD.

For the dual detection of 5mC and FISH signals, the slides were first processed for the detection of 5mC, dehydrated in an ethanol series, and air-dried. FISH was then conducted as mentioned above.

Results

SFBB-S9 genes, which show genetic linkage to S9-RNase, cluster in the small subtelomeric and haplotype-specific region of an apple chromosome

Previous genetic analysis showed that SFBB-S9 genes are closely linked to S9-RNase (Minamikawa et al., 2010). To verify whether they are physically linked to S9-RNase, BAC-FISH was conducted on pachytene chromosomes of ‘Florina’ (S9) with S9-haplotype BAC clones as probes. Out of 27 S9-haplotye BAC clones which constructed seven contigs (Minamikawa et al., 2010), 12 BAC clones covering all the contigs which contain S9-RNase and SFBB-S9 genes were used in this study (Table 1). All 12 BACs produced distinct signals on pachytene chromosomes and the signals completely colocalized to the subtelomeric region with S9-RNase (Fig. 1). Although BAC 9P16, BAC 69A4, and BAC90A15 also gave signals in subtelomeric regions of other chromosomes probably because of repetitive sequences, simultaneous detection of the three BACs provided distinct locations and the order of S9-RNase and SFBB-S9 genes on pachytene chromosome as mentioned below. The subtelomeric locations of FISH signals are consistent with genetic linkage analyses mapping the apple S locus to the bottom of linkage group 17 (Igarashi et al., 2008).
Table 1. The S\(^3\)-haplotype BAC clones used

<table>
<thead>
<tr>
<th>Contig</th>
<th>BAC</th>
<th>Gene(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>45M19(^b)</td>
<td>SFBB5-S(^3) (SFBB(^a3)-)</td>
</tr>
<tr>
<td></td>
<td>79B14</td>
<td>SFBB5-S(^3) (SFBB(^a3)-), S(^3)-RNase</td>
</tr>
<tr>
<td>3</td>
<td>34G16</td>
<td>S(^3)-RNase</td>
</tr>
<tr>
<td></td>
<td>96N6</td>
<td>S(^3)-RNase, SFBB7-S(^9) (SFBB(^a9)-)</td>
</tr>
<tr>
<td></td>
<td>14P21</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>90A15</td>
<td>SFBB7-S(^9) (SFBB(^a9)-)</td>
</tr>
<tr>
<td></td>
<td>72L2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>52B7</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>25J12</td>
<td>SFBB3-S(^9) (FBX17), FBX4</td>
</tr>
<tr>
<td></td>
<td>24C22</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>21O2</td>
<td>SFBB6-S(^9) (FBX15)</td>
</tr>
<tr>
<td></td>
<td>9P16</td>
<td>FBX12</td>
</tr>
<tr>
<td></td>
<td>10D16</td>
<td>FBX6, SFBB1-S(^9) (FBX16)</td>
</tr>
<tr>
<td></td>
<td>27P17</td>
<td>FBX6, SFBB1-S(^9) (FBX16)</td>
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<td></td>
<td>6N9</td>
<td>FBX6</td>
</tr>
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<tr>
<td></td>
<td>70J19</td>
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<tr>
<td></td>
<td>69A4</td>
<td>FBX19</td>
</tr>
<tr>
<td>5</td>
<td>9J18</td>
<td>SFBB8-S(^9) (FBX10)</td>
</tr>
<tr>
<td></td>
<td>22C6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>68N5</td>
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</tr>
<tr>
<td>6</td>
<td>33E2</td>
<td>FBX13</td>
</tr>
<tr>
<td></td>
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<tr>
<td></td>
<td>92P4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20J13</td>
<td></td>
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<tr>
<td>7(^a)</td>
<td>9L6</td>
<td>FBX11</td>
</tr>
</tbody>
</table>

\(^a\) FBX11 is a monomorphic gene and exists in both the S\(^3\)- and S\(^9\)-haplotypes (Minamikawa et al., 2010).

\(^b\) BAC clones and genes used in this study are indicated in bold.

\(^c\) New naming system for SFBB genes was adopted, i.e. SFBBX-S\(^x\) for the type-x SFBB from the S\(^x\) haplotype (Kakui et al., 2011). Previous gene names (Minamikawa et al., 2010) are in parentheses. Genes that were not classified into the eight SFBB types (Kakui et al., 2011) are shown with their old names (Minamikawa et al., 2010).

Although interhaplotypic homology of the same types of SFBB genes is generally very high (Minamikawa et al., 2010; Kakui et al., 2011), each of the 12 BAC probes detected single signal on a chromosome except for probable repetitive sequence-derived signals for some BACs (Fig. 1, see above). For some BACs, S\(^x\)-specificities were further confirmed by FISH on nuclei and chromosomes of somatic cells (see Supplementary Figs S1 and S2 at JXB online). Lack of cross-hybridization of S\(^x\)-BAC probes on the S\(^x\) chromosome may be because the flanking regions of the SFBB genes are rich in S-haplotype-specific sequences.

High-resolution mapping and the relative order of S\(^9\)-RNase and SFBB-S\(^9\) genes on pachytene chromosome

FISH mapping on pachytene chromosomes is a powerful approach for high-resolution mapping because pachytene chromosomes are, on average, 10–50 times longer than somatic chromosomes (de Jong et al., 1999; Cheng et al., 2002). Previous BAC-FISH analysis showed that S\(^3\)-RNase and SFBB5-S\(^9\) (SFBB\(^a9\)-) genes, which are separated by 42 kb from each other (Sassa et al., 2007), are located in a subtelomeric region of a somatic chromosome (Minamikawa et al., 2010). However, their relative orientation could not be determined by FISH using somatic chromosomes. Therefore, high-resolution FISH mapping was conducted on pachytene chromosomes to determine the location and order of all SFBB-S\(^9\) genes.

First, rough mapping of the seven contigs were conducted by using eight BAC clones as probes. FISH analysis using the eight BAC probes showed that five contigs, contigs 1 (45M19), 2 (25J12), 3 (21O2), 4 (69A4), and 6 (33E2), were located between contig 7 (BAC 9L6 including FBX11) and contig 5 (BAC 9J18/BAC 22C6 including SFBB8-S\(^9\)) (Fig. 1A–G). The signals of BAC 9L6 (contig 7) and BAC 22C6 (contig 5) were localized to the side of the centromere and telomere, respectively (Fig. 1D). Next, the relative order of the five contigs located between contigs 5 and 7 was analysed. BAC 9P16 (contig 4) was located between BAC 9L6 (contig 7) and BAC 8913 (contig 6) (Fig. 1H), i.e. contig 4 was between contig 7 and contig 6. Similarly, BAC 21O2 (contig 3) was located between BAC 9P16 (contig 4) and BAC 45M19 (contig 1) (Fig. 1J), and BAC 9P16 (contig 4) between BAC 25J12 (contig 2) and BAC 45M19 (contig 1) (Fig. 1J). The signals of BAC 33E2/BAC 8913 (contig 6) and BAC 21O2 (contig 3) overlapped even on pachytene chromosome (data not shown), thus the relative order of contigs 3 and 6 among the seven contigs could not be determined. Together, the relative order of the seven contigs were determined to be as follows; contigs 5, 1, 3/6, 4, 2, and 7 (Fig. 2).

Because contig 1 and contig 4 contain more than one SFBB gene, FISH mapping was conducted to determine the orientation of SFBB1-S\(^9\), and FBX6, 12 and 19 in contig 4 and S\(^9\)-RNase, SFBB5-S\(^9\) and SFBB7-S\(^9\) in contig 1. Two BAC clones of each contig and BAC 9L6 (contig 7) or 45M19 (contig 1) were used as FISH probes. For contig 1, BAC 90A15 (SFBB7-S\(^9\)) was closer to BAC 9L6 (contig 7) than BAC 45M19 (SFBB5-S\(^9\)) (Fig. 1K). For contig 4, BAC 69A4 (FBX19) was closer to BAC 45M19 (contig 1) than BAC 9P16 (FBX12) (Fig. 1L). Based on the results of FISH mapping, therefore, an ideogram was constructed of the order of the S\(^9\)-RNase and SFBB-S\(^9\) genes from the telomere to centromere, including SFBB8-S\(^9\), SFBB5-S\(^9\), S\(^9\)-RNase, SFBB7-S\(^9\), FBX13/SFBB6-S\(^9\), FBX19, FBX6, SFBB1-S\(^9\), FBX12, SFBB3-S\(^9\), and FBX11 (Fig. 2). Because the BAC 33E2/BAC 8913 (FBX13) and BAC 21O2 (SFBB6-S\(^9\)) signals overlapped (see above), the two genes were placed at the same position in the ideogram.

Previously, it was reported that gene order is not conserved between S\(^3\) and S\(^9\) haplotypes of apple (Minamikawa et al., 2010). Comparison of the gene order of S\(^9\) haplotype determined by high-resolution FISH with previously reported S\(^3\) contigs showed additional structural difference between the two S haplotypes. Although the two proximal SFBBs of S\(^3\)-RNase are SFBB\(^a9\) and SFBB\(^a9\)- (Minamikawa et al., 2010), their probable alleles, FBX19 and FBX6, respectively, were shown to be the 4th and 5th SFBB genes from S\(^3\)-RNase, respectively (Fig. 2).

S-RNase and SFBB genes were located on heterochromatic and heavily methylated DNA sequence

Pachytene chromosomes provide high-resolution cytogenetic information on euchromatin and heterochromatin. To elucidate the cytogenetical basis of recombination suppression of the region of S\(^3\)-RNase and SFBB genes, the chromatin structure and DNA methylation were examined at the S locus in apple.
FISH and black-and-white images of DAPI-stained chromosomes showed that the chromosomal region containing the \(S\)-RNase and SFBB genes was embedded in a large knob-like heterochromatin block (Fig. 3). There were nine heterochromatin blocks on the chromosome, and the \(S\) locus was located in the third heterochromatic domain from the nearest telomere end.

Cytosine DNA methylation is a conserved epigenetic silencing mechanism in higher eukaryotes, and plays an important role in many biological and biochemical processes including...
heterochromatinization (Zhang et al., 2008) and DNA recombination suppression (Meyers et al., 2002). To investigate whether the methylation is associated with the apple S locus, an immunofluorescence assay was conducted on relaxed chromosomes of early pachytene of ‘Florina’ apple using the anti-5mC antibody. The S locus showed much brighter fluorescence than the surrounding regions (Fig. 4). This pattern is similar to the distribution of heterochromatin along the chromosomes, but there were several spots where fluorescence signals were missing (Fig. 4).

Discussion

SFBB and S-RNase genes are physically linked

In a previous study, more than ten SFBB genes were identified by screening a BAC library five times the size of the apple genome and it was characterized as having pollen-specific expression, S haplotype-specific polymorphisms, and genetic linkage to S-RNase (Minamikawa et al., 2010). On the other hand, the physical linkage of multiple genes and the size of the apple S locus are still unknown. It has been demonstrated here that the S9-haplotype SFBBs and S9-RNase genes clustered in the subtelomeric region on the apple chromosome. Among SFBBs, FBXII was considered to be a monomorphic gene and existing in the S3 and S9 haplotypes (Minamikawa et al., 2010). Although two chromosomes form FISH signals of BAC 9L6 (including FBXII), the signal on the chromosome with the signal from the S9 haplotype was much stronger than that on the other chromosome (S Wang, unpublished data). The difference in intensity suggests that the former signal was specific and the latter unspecific. Therefore, it is considered that, although FBXII is present in both the S9 and S9 haplotypes (Minamikawa et al., 2010), BAC 9L6 was derived from the S9 haplotype and is rich in S9 haplotype-specific sequences.

The apple S locus has been mapped to the bottom of linkage group 17 (Igarashi et al., 2008) and physically located in a subtelomeric region of a somatic chromosome using FISH (Minamikawa et al., 2010). The subtelomeric location of the apple S locus was further confirmed by high-resolution FISH on pachytene chromosomes in this study. To maintain SI, pollen S and pistil S must be tightly linked. Otherwise, intergenic recombination would cause the breakdown of SI by generating differences in pollen and pistil specificity within a single S-haplotype (Sijacic et al., 2004). In addition, the entire apple S locus containing SFBB-S9 and S9-RNase genes is estimated to be ~1 Mb or more, because contig 1 contains the sequenced 312 kb region (Sassa et al., 2007) and extends the region, and contigs 2–7 are located outside contig 1. Consistent with this, in Japanese pear (Pyrus pyrifolia), Okada et al. (2011) found six and ten SFBB genes in 649 kb around S4-RNase and 378 kb around S4-RNase, respectively, and PpSFBB4α and PpSFBB4γ (Sassa et al., 2007) were located outside the sequenced regions. Thus, to protect the broad apple S locus from meiotic recombination, mechanisms of suppression are necessary.

Structural and genetic features of the apple S locus might help to suppress recombination in this region

The suppression of recombination around the S locus has been widely observed in different plants, such as Brassica, Petunia...
inflata, Ipomoea trifida, Antirrhinum, and Hordeum bulbosum (Casselman et al., 2000; Wang et al., 2004; Rahman et al., 2007; Yang et al., 2007; Kakeda et al., 2008). It was also observed in apple, for example, no recombination occurred between SFBB and S-RNase genes in 239 segregants (Minamikawa et al., 2010). Sequence heterogeneity among S haplotypes has been suggested to be one of the genetic factors responsible for the suppression of recombination in Brassica and Ipomoea (Iwano et al., 1998; Casselman et al., 2000; Suzuki et al., 2004; Rahman et al., 2007). In apple, the FISH results showed that the relative order of probable alleles of SFBB genes was not conserved between the S^6 and S^9 haplotypes (Fig. 2; Minamikawa et al., 2010). The order of SFBB genes in the S^3 haplotype determined in this study provides further evidence of interhaplotypic heterogeneity. Although SFBB^{3-α} and SFBB^{3-β} are located next to S^3-RNase (Minamikawa et al., 2010), their probable alleles FBX19 and FBX6 were shown to be the 4th and 5th SFBB genes from S^3-RNase, respectively. In Japanese pear S^4 and S^2 haplotypes (Okada et al., 2011), sequence heterogeneity was also found. The FISH results of this study provide further evidence of sequence heterogeneity of the apple S locus region. The 12 BAC probes containing SFBB-S^9 genes detected S^9-specific signals, although the haplotypic homology of SFBB genes are very high (Sassa et al., 2007; Minamikawa et al., 2010; Kakui et al., 2011). Previous study has also shown that two BAC probes containing SFBB-S^9 detected S^9-specific signals on a somatic chromosome of a heterozygous (S^9S^9) apple seedling (Minamikawa et al., 2010). This is probably because SFBB genes are embedded in S haplotype-specific sequences. Hence, sequence heterogeneity might help to inhibit homologous recombination at the apple S locus and guarantee inheritance of SFBBs and S-RNase as a unit.

Heterochromatin is concentrated in pericentromeric and telomeric regions in most eukaryotes, is abundant in repetitive sequences and transposable elements, and has a relatively low gene density (Peng and Karpen, 2008). The DNA sequences in heterochromatic chromosomal domains are often hypermethylated (Zhang et al., 2008). Heterochromatinization is considered to be an important factor inhibiting genetic recombination at involved loci in different genetic systems (Haber et al., 1998; Meyers et al., 1998; Wei et al., 2002; Walsh et al., 2003; Zhang et al., 2008). In this research, it has been shown that the apple S locus is located in the third heterochromatin knob of the chromosome and the DNA elements in the heterochromatin were highly methylated, which might also contribute to the suppression of
recombination at the apple S locus. Recent findings on Japanese pear, a close relative of apple, suggested that multiple SFBB genes are involved in determining pollen S specificity (Kakui et al., 2011), suggesting that many, if not all, apple SFBB genes located in the large S locus region would also be the components of pollen determinants. Structural heterogeneity and the heterochromatic nature of the apple S locus would be necessary to guarantee stable inheritance of GSI by suppressing recombination in the large region.

Supplementary data
Supplementary data can be found at JXB online.

Supplementary Fig. S1. FISH to interphase nuclei in somatic cells probed with BACs in respective contigs.

Supplementary Fig. S2. FISH mapping of BAC 34G16 and BAC 45M19 on somatic chromosomes of Florina (S'S').

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