SFBs of Japanese Plum (Prunus salicina): Cloning Seven Alleles and Determining Their Linkage to the S-RNase Gene

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Abstract. Japanese plum (Prunus salicina Lindl.), a species of the Rosaceae family, carries the S-RNase-mediated gametophytic self-incompatibility system. Self-incompatibility is manifested if the S-haplotype of the pollen is carried also by the pollinated flower. Thus, for fertilization to occur, the cultivars have to be genetically compatible. The haplotype is conferred by a S-locus, which contains the style-specific expressed S-RNase and the pollen-specific expressed F-box genes (SFB). Since both the S-RNase and the SFB genes are multiallelic and are characteristic of each of the S-haplotypes, they are ideal markers for molecular S-typing. In this work, seven SFBs, from eight Japanese plum cultivars, were cloned and sequenced. Five of the alleles were published recently and two SFBc and SFBb are new. The physical linkage of SFBb and SFBb to their adjacent S-RNase was determined; it is 544 base pairs (bp) and 404 bp for the Sb and Sc loci, respectively.

Self-incompatibility (SI) is the ability of a fertile hermaphrodite flowering plant to prevent self-fertilization by discriminating between self and nonself pollen. Japanese plum (Prunus salicina Lindl.), a species of the Rosaceae family, carries the S-RNase-mediated gametophytic self-incompatibility system (GSI system). This system was first identified in Solanaceae (Anderson et al., 1986) and later in Rosaceae (Sassa et al., 1992) and in Scrophulariaceae (Xue et al., 1996). Research in the three botanical families is brought together in an attempt to explore the S-RNase-mediated GSI system.

In GSI, the inhibition of a pollen grain is based on its haploid genotype (termed S-haplotype). SI is manifested if the S-haplotype of the pollen is carried also by the pollinated flower (McCubbin and Kao, 2000). The haplotype is conferred by a S-locus, which contains, among others, the style-specific expressed S-RNase and the pollen-specific expressed F-box genes (McClure et al., 1989; Zhu et al., 2004). Both genes are heteroallelic and are suspected of being involved in determining the specific self-pollen rejection; however, the mechanism of the system is still not fully understood. Several reviews describing the current perception of the S-RNase-mediated GSI system have been published recently (Goldway et al., 2007; McClure and Franklin-Tong, 2006; Takayama and Isogai, 2005).

As a result of the SI, for obtaining satisfactory yield, it is essential that Japanese plum orchards contain at least two cultivars that serve as pollinators of each other. Before the molecular genetic era, compatibility was determined in field experiments by using natural and hand-pollination of cultivar couples. However, because agronomic and environmental factors affect fruit-set levels, the method is inaccurate. Although S-RNase alleles are well known and have been studied for more than a decade, the pollen F-Box gene was identified only recently, first in Antirrhinum (Lai et al., 2002) and then in almond (Prunus dulcis) and Japanese apricot (Prunus mume) (Entani et al., 2003; Ushijima et al., 2003), both of the Prunus genus included in Prunoideae, a subfamily of Rosaceae, in Petunia inflata of the Solanaceae (Sijacic et al., 2004), and recently in apple (Malus domestica) and Japanese pear (Prunus pyrifolia) (Cheng et al., 2006; Sassa et al., 2007), which are in Maloideae, another subfamily of Rosaceae. The gene was termed SLF (S-Locus F-box) by Entani et al. (2003) and also SFB (S-haplotype-specific F-Box protein) by Ushijima et al. (2003).

In this article, we followed the latter, which has been already applied to other Prunus species. Because both the S-RNase and the SFB genes are multiallelic and are characteristic of each of the S-haplotypes, they are ideal markers for molecular S-typing. To date, 14 S-RNases were cloned from Japanese plum (Beppu et al., 2002, 2003; Sapir et al., 2004). In the present work, seven Japanese plum SFBs were cloned from nine cultivars. Five SFBs were also described in a recent work of Zhang et al. (2007).
sequenced from two different clones in both directions with automated sequencing using dye terminator cycle sequencing with fluorescent-labeled dye terminators on an ABI PRISM 377 DNA sequencer (PE Bio System, Foster City, CA).

**Analysis of the physical distance between S-RNase and SFB in the cultivar ‘Golfrose’**.

The physical distance between S-RNase and SFB in the cultivar ‘Golfrose’ was analyzed by PCR amplification using 20 ng of DNA, 5 mM of 10x PCR buffer containing 27.5 mM MgCl2, (Roche Diagnostics, Mannheim, Germany), 0.5 mM dNTP mix, 1 μM of PRL-C2 primer, designed from S-RNase C2 conserved region (Tao et al., 1999), 1 μM of SFBc-F primer, designed from the F-box motif of SFB (Romero et al., 2004), and 1 μL of Expand Long Template PCR System (5 unit/μL; Roche Diagnostics) in a 50-μL reaction mixture. The PCR conditions were as follows: initial cycle of 2 min at 94°C followed by 10 cycles of 10 s at 94°C, 30 s at 54°C, and 10 min at 68°C followed by 25 cycles of 15 s at 94°C, 30 s at 54°C, and 10 min + 20 s for each successive cycle at 68°C. The final cycle was 7 min at 68°C.

**DNA sequence and its putative protein analysis.** Analysis of the consensus contigs of each SFB genomic DNA and deduced amino acid sequence data were performed by LaserGene 6 (Madison, WI) software.

**Results and Discussion**

PCR products of SFBs were identified from cultivars with a previously determined S-RNase genotype (Sapir et al., 2004; unpublished data). For preventing misidentification, each SFB allele was cloned and sequenced at least twice from two or three different cultivars (Fig. 1). A total of seven alleles were cloned from the following nine cultivars as follows: SFB f from ‘Wickson’, SFB e from ‘Black Diamond’, SFB b from ‘Royal Zee’, SFB c from ‘440’, SFB g from ‘Songold’, SFB h from ‘Shiro’, SFB b from ‘Golfrose’, and SFB e from ‘Newyorker’.

DNA alignment revealed that all seven Japanese plum SFB alleles carry the previously described features of the SFB gene: a single F-box domain, four (hyper) variable regions (V1, V2, HVa, and HVb), and no introns (Fig. 1). The comparison of their putative amino acid showed they were highly polymorphic, ranging from 73.3% to 82.5% (Table 1). These levels of polymorphism resemble those of other Prunus SFBs. For example, in almond (Prunus dulcis), it ranges from 68.4% to 76.4% (Ushijima et al., 2003), in Japanese apricot (Prunus mume) from 74.9% to 80.2% (Entani et al., 2003), in sweet cherry (Prunus avium) from 75.3% to 81.1% (Ikeda et al., 2004), and in European apricot (Prunus armeniaca) from 67% to 83% (Romero et al., 2004). Furthermore, this SFB polymorphism is similar to the Japanese plum S-RNases polymorphism (63.8% to 84.1%) (Table 1).
Table 1. Identities of the putative amino acid sequences of Japanese plum S-locus genes. The upper half represents amino acid sequence identities (%) between SFBs and the lower half between the S-RNases. SFBs: GenBank accession numbers: SFBb (DQ464688), SFBa (DQ464689), SFBc (DQ464690), SFBd (DQ989578), SFBf (DQ989579), and SFBe (DQ992485). S-RNase genes: GenBank accession numbers: S-RNaseb (AB084143), S-RNasec (AB084146), S-RNased (AB084147), S-RNasee (AB099311), and S-RNasef (AB092313).

| Sb | Sc | Sd | Se | Sf | Sg |
|----|----|----|----|----|----|
| 92.0 | 81.4 | 76.7 | 77.0 | 82.5 | 77.0 |
| 76.7 | 73.3 | 76.9 | 74.7 | 76.1 | 74.1 |
| 80.6 | 82.5 | 76.7 | 78.6 | 77.1 | 77.7 |
| 78.1 | 76.9 | 78.3 | 75.8 | 71.0 | 73.1 |
| 79.4 | 74.7 | 63.8 | 78.8 | 70.0 | 78.2 |
| 80.3 | 73.8 | 65.2 | 78.0 | 69.1 | 71.9 |
| 74.4 | 83.8 | 72.0 | 84.1 | 74.7 | 76.6 |

PCR was carried out with primers complementary to the F-Box motif of the SFB and the C2 conserved region of S-RNase (see “Materials and Methods”). PCR amplification of 'Golfrose' DNA with primer pair SFBc-F and Pru-C2. PCR products (left) were run on a 0.7% agarose gel, stained with ethidium bromide. (B) A scheme illustrating the distances between the S-RNase and SFB genes for Sb and Sb japanese plum haplotypes. Full Arrows represent the transcriptional direction of the genes, solid line represent intergenic region.

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