Theor Appl Genet (2002) 105:222–228
DOI 10.1007/s00122-002-0980-7

R. Tao · T. Habu · A. Namba · H. Yamane
F. Fuyuhiro · K. Iwamoto · A. Sugiura

Inheritance of *S*^{f}-RNase in Japanese apricot (*Prunus mume*) and its relation to self-compatibility

Received: 27 September 2001 / Accepted: 7 March 2002 / Published online: 21 June 2002

Abstract Self-compatible cultivars of Japanese apricot (*Prunus mume* Shieb. et Zucc.), a tree species that normally shows *S*-RNase-based self-incompatiblity, have a horticultural advantage over self-incompatible cultivars. Inheritance of self-compatibility and a common *S*^{f}-RNase allele that is observed in self-compatible cultivars was investigated using progenies from controlled crosses. Total DNAs were isolated from the parents and progenies of seven crosses that included at least one self-compatible cultivar as a parent. These DNAs were PCR-amplified with the Pru-C2 and PCE-R primer pair to determine *S*-haplotypes of the parents and progenies. A novel *S*-haplotype, *S*^{8}, was found. In all crosses examined, the *S*^{f}-RNase gene was inherited from either the seed or pollen parent as a pistil *S*-allele in a non-functional *S*-haplotype. Self-compatibility of about 20 trees each from reciprocal crosses of 'Benisashi (*S*^{7}*S*^{f})' and 'Shinpeidayu (*S*^{3}*S*^{f})', and 26 selections from 16 different crosses was tested by pollination and pollen-tube growth studies. Cosegregation of the *S*^{f}-RNase allele and self-compatibility was confirmed with all but selection 1K0-26 (*S*^{3}*S*^{7}). Selection 1K0-26 (*S*^{3}*S*^{7}) that originated from 'Benisashi (*S*^{7}*S*^{f})' × 'Koshinoume (*S*^{3}*S*^{f})' appeared to be self-compatible even without the *S*^{f}-RNase allele. The possible role of pollen-*S*, a presumably existing pollen component of gametophytic self-incompatibility, is discussed.

Communicated by H.F. Linskens

R. Tao (✉) · T. Habu · A. Namba · H. Yamane · A. Sugiura
Graduate School of Agriculture, Kyoto University,
Kyoto 606-8502, Japan
e-mail: rtao@kais.kyoto-u.ac.jp
Fax: +81-75-753-6497

F. Fuyuhiro
Fukui Prefectural Horticultural Experiment Station,
Fukui 919-1123, Japan

K. Iwamoto
Horticultural Experiment Center
of Wakayama Research Center of Agriculture,
Forestry, and Fisheries, Wakayama 644-0024, Japan

Keywords Gametophytic self-incompatibility · Molecular marker · Pollen-*S* · *S*-RNase

Introduction

Gametophytic self-incompatibility is a widespread mechanism in flowering plants which prevents self-fertilization and promotes out-crossing. Although there are several different mechanisms of gametophytic self-incompatibility, the Solanaceae, Scrophulariaceae and Rosaceae share the same mechanism of the *S*-RNase-based gametophytic self-incompatibility system (de Nettancourt 1997). Since information about *S*-haplotypes is very important to ensure fruit set, *S*-RNase gene-specific PCR analysis has been developed to determine the *S*-haplotypes of various rosaceous fruit tree species, such as almond (*Prunus dulcis*) (Tamura et al. 2000), apple (*Malus* × *domestica*) (Janssens et al. 1995), Japanese pear (*Pyrus serotina*) (Ishimizu et al. 1999) sour cherry (*Prunus cerasus*) (Yamane et al. 2001) and sweet cherry (*Prunus avium*) (Tao et al. 1999; Hauck et al. 2001; Sonneveld et al. 2001; Wiersma et al. 2001).

Japanese apricot (*Prunus mume* Shieb. et Zucc.) that belongs to the Rosaceae exhibits *S*-RNase-based gametophytic self-incompatibility (Tao et al. 2000; Yaegaki et al. 2001), as do most other *Prunus* fruit tree species (Tao et al. 1997, 1999; Ushijima et al. 1998; Yamane et al. 1999, 2001). Although both self-incompatible and self-compatible cultivars are grown commercially in Japan, self-compatible cultivars have a horticultural advantage over self-incompatible cultivars because no cross-pollinizer is required. Consequently, one of the major breeding goals for Japanese apricot is to produce self-compatible cultivars of good horticultural quality.

We recently found that self-compatible cultivars of Japanese apricot had a common *S*-RNase gene, designated as *S*^{f}-RNase, by S-RNase gene-specific PCR and genomic DNA blot analyses (Tao et al. 2000). Since the *S*^{f}-RNase gene was not found in self-incompatible cultivars (Tao et al. 2000), it has been thought to be associat-

ed with self-compatibility in Japanese apricot although there appeared to be no distinct difference between the *S*^{f}-RNase and other *Prunus S*-RNases (Tao et al. 2002). The *S*^{f}-RNase gene is transcribed and translated into *S*^{f}-RNase with molecular weight, isoelectric focusing point and immunological characteristics similar to other *S*-RNases (Tao et al. 2002). Furthermore, the amount of *S*^{f}-RNase produced in the style appeared to be the same as those of other *S*-RNases although we have yet to compare the RNase activity of *S*^{f}- and other *S*-RNases (Tao et al. 2002).

Recently, molecular analysis revealed that the *S*-locus of the Rosaceae is bipartite, with different genes encoding the style component (*S*-RNase) and the pollen component (pollen-*S*) (Sassa et al. 1992). These genes are very tightly linked to each other so that they behave as if they were a single gene. On the basis of this finding, the term "haplotype" has been adopted to denote variants of the locus and the term "allele" to denote variants of a given polymorphic gene within the *S*-locus (McCubbin and Kao 2000). Thus, if the *S*^{f}-RNase gene is located at the *S*-locus region as a pistil *S*-allele, it is likely to be linked to the pollen-*S* gene. Dysfunction of either the *S*^{f}-RNase gene or the pollen-*S* gene product could lead to self-compatibility. Thus, even if only the pollen-*S* gene that is linked to the *S*^{f}-RNase gene is dysfunctional, we could expect cosegregation of the *S*^{f}-RNase gene and self-compatibility and the possibility of using the *S*^{f}-RNase gene as a molecular marker for self-compatibility in Japanese apricot.

With a molecular marker for self-compatibility, the time required for breeding of self-compatible Japanese apricots could be considerably shortened. Assessment of self-compatibility, as determined by conventional methods of pollination and pollen-tube growth tests, usually requires several years after the tree reaches the flowering age. Molecular markers for self-compatibility make it possible to select self-compatible progenies soon after germination because they can be used on vegetative material, independently of age or season.

The *S*^{f}-RNase gene may also help elucidate the mode of inheritance of self-compatibility that is presently uncertain because of the complicated segregation for self-compatibility in Japanese apricot (Miyake et al. 1995). A complicated segregation would be expected if the *S*^{f}-RNase gene is located at the *S*-locus region because both individual self-incompatible alleles, as well as associated *S*-haplotypes, also affect the ratio of the self-compatible progenies obtained. The objective of this study is to investigate the inheritance of the *S*^{f}-RNase gene and self-compatibility. Progenies from controlled crosses were analyzed to determine if the *S*^{f}-RNase gene was located at the *S*- locus region and cosegregated with self-compatibility.

Materials and methods

Plant material

Fourteen cultivars and a selection TK (Tamaume × Koshinoume) (Fig. 1), involving seven progenies consisting of 13 to 98 trees and seedlings (Table 1), and 26 selections from various controlled crosses among Japanese apricot were used in this study (Table 2).

DNA isolation and PCR

Total DNA was isolated from young leaves by the miniprep method described by Edwards et al. (1991) or using the Nucleon Phytopure for the Plant DNA extraction kit (Amersham Pharmacia Biotech UK, Buckinghamshire, England). PCR was performed using an *S*-RNase gene-specific primer pair, Pru-C2 (5′-CTATG GCCAA GTAAT TATTC AAACC-3′) (Tao et al. 1999) and PCE-R (5′-TGTTT GTTCC ATTCG CYTTC CC-3′) (Yamane et al. 2001), to determine the *S*-haplotypes of the cultivars and progenies. These primers were designed from the previously identified C2 and C3 conserved regions of *Prunus S*-RNase, respectively (Ushijima et al. 1998). The PCR reaction mixture contained 10 mM of Tris–HCl (pH 8.3), 50 mM of KCl, 1.5 mM of $MgCl_2$, 200 μM each of dNTPs, 400 nM each of primers, 50 ng of template DNA, and 1 U of TaKaRa Ex *Taq* polymerase (Takara Shuzo Co., Shiga, Japan) in a 50-μl reaction volume. PCR reactions were run with a program of 35 cycles at 94 °C for 1 min, 56 °C for 1 min and 72 °C for 1 min 30 s, with an initial denaturing at 94 °C

Table 1 Segregation of *S*-RNase alleles in seven progenies of Japanese apricot as determined by PCR

Seed parent (*S*-haplotype)	Pollen parent (*S*-haplotype)	Observed numbers (*S*-haplotype)				*P*[a]
Nanko (S^1S^7)	Jizoume (S^3S^f)	10 (S^1S^f)	11 (S^1S^3)	9 (S^3S^7)	3 (S^7S^f)	0.195
Hachiro (S^8S^f)	Nanko (S^1S^7)	5 (S^1S^8)	5 (S^1S^f)	6 (S^7S^8)	9 (S^7S^f)	0.632
Benisashi (S^7S^f)	Shinpeidayu (S^3S^f)	10 (4[b]) (S^3S^7)	15 (4[b]) (S^3S^f)	17 (5[b]) (S^7S^f)	12 (4[b]) (S^fS^f)	0.542
Shinpeidayu (S^3S^f)	Benisashi (S^7S^f)	7 (4[b]) (S^3S^7)	11 (7[b]) (S^3S^f)	16 (6[b]) (S^7S^f)	12 (4[b]) (S^fS^f)	0.312
Hachiro (S^8S^f)	Hachiro (S^8S^f)	57 (S^8S^f)	41 (S^fS^f)			0.106
Nanko (S^1S^7)	Kensaki (S^fS^f)	23 (S^1S^f)	19 (S^7S^f)			0.537
Kensaki (S^fS^f)	Kensaki (S^fS^f)	13 (S^fS^f)				

[a] Probability to fit with a 1:1:1:1 or 1:1 ratio using the chi-square test
[b] Number of trees tested for self-(in)compatibility in parenthesis

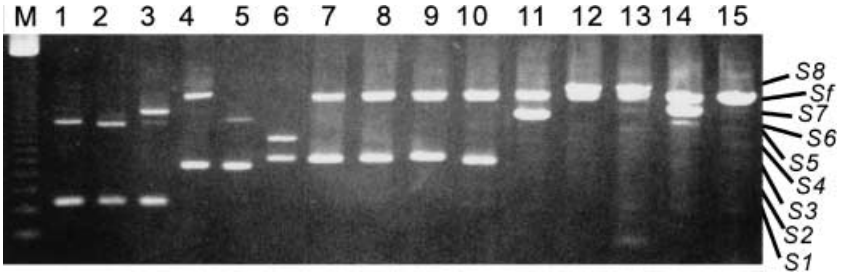

Fig. 1 PCR analysis for 14 Japanese apricot cultivars and a selection line (Tamaume × Koshusaisyo) with a *S*-RNase gene-specific primer pair, Pru-C2 and PCE-R. *Lane M*: 123-bp DNA ladder, *lane 1*: Gessekai (S^1S^6), *lane 2*: Oushuku (S^1S^5), *lane 3*: Nanko (S^1S^7), *lane 4*: Tamaume × Koshusaisyo (S^2S^8), *lane 5*: Gyokuei (S^2S^6), *lane 6*: Kairyouchidaume (S^3S^4), *lane 7*: Koshinoume (S^3S^f), *lane 8*: Shinpeidayu (S^3S^f), *lane 9*: Rinshu (S^3S^f), *lane 10*: Jizoume (S^3S^f), *lane 11*: Orihime (S^6S^f), *lane 12*: Hachiro (S^8S^f), *lane 13*: Ryukyokoume (S^8S^f), *lane 14*: Benisashi (S^7S^f), and *lane 15*: Kensaki (S^fS^f)

for 3 min and a final extension of 72 °C for 7 min. The PCR products were analyzed in 1% agarose gel in TAE buffer and visualized by staining with ethidium bromide. Three different PCR machines, TaKaRa PCR Thermal Cycler MP and TaKaRa PCR Thermal Cycler Personal (Takara Shuzo Co., Shiga, Japan), and PERKIN ELMER DNA Thermal Cycler PJ2000 (Perkin-Elmer, Connecticut, USA), were employed.

Pollination and pollen-tube growth test

Fruit set after self-pollination was investigated for 26 selections resulting from controlled crosses (Table 2), and 17 and 21 trees, respectively, from reciprocal crosses of 'Benisashi (S^7S^f)' and 'Shinpeidayu (S^3S^f)' (Table 1). Fifty to 100 flower buds were bagged just before anthesis. When they opened, previously collected self-pollen was applied to stigmas with a glass rod and the flowers were bagged again to exclude outside pollen. Fruit set was recorded about 3 months after pollination when the fruit matured. Pollen-tube growth tests were also performed for 'Koshinoume', 'Benisashi' and selection 1K0-26, as well as progenies from reciprocal crosses of 'Benisashi' and 'Shinpeidayu' that showed a fruit set of less than 10% after self-pollination based on the methods of Lansari and Lezzoni (1990) but with substantial modifications as described by Yamane et al. (2001). Pollen was individually collected from trees being tested (self-pollen) as well as bulk pollen collected from several cultivars (outcross pollen). Ten of 20 emasculated flowers per branch were hand-pollinated when receptive (24 h after emasculation) with self-pollen in the laboratory (20 °C). The other ten emasculated flowers that were chosen at random were pollinated with outcross pollen. The outcross and self-pollinated pistils were collected 72 h after pollination and immersed in a fixing solution [(1 chloroform:3 (95%) ethanol:1 glacial acetic acid) (v/v)] for 24 h, transferred to 100% ethanol, and stored at 4 °C until stained. The pistils were washed thoroughly under running tap water and incubated in 10 N NaOH for 5 to 6 h to soften the tissues. The pistils were then soaked in 0.1% aniline blue solution with 33 mM of K_3PO_4 for 1 h. Pollen tubes were observed by ultraviolet ffluorescent microscopy (BX60; Olympus, Tokyo, Japan) equipped with a digital camera (DP50, Olympus, Tokyo, Japan).

Table 2 *S*-haplotype, percentage of fruit set after self-pollination, and parentages of 26 selection lines of Japanese apricot

Line[a]	Fruit set %	Seed parent[a]	Pollen parent[a]
1A0-03 (S^7S^f)	52.5	Benisashi (S^7S^f)	Benisashi(S^7S^f)
1C1-10 (S^3S^7)	0	Benisashi (S^7S^f)	Kairyouchidaume (S^3S^4)
1C3-05 (S^4S^f)	8.5	Benisashi (S^7S^f)	Kairyouchidaume (S^3S^4)
1E3-10 (S^1S^7)	0	Benisashi (S^7S^f)	Gessekai (S^1S^6)
1E3-28 (S^1S^7)	0	Benisashi (S^7S^f)	Gessekai (S^1S^6)
1F0-02 (S^3S^f)	15.1	Benisashi (S^7S^f)	Rinshu (S^3S^f)
1F0-06 (S^3S^f)	19.4	Benisashi (S^7S^f)	Rinshu (S^3S^f)
1F3-12 (S^7S^f)	13.9	Benisashi (S^7S^f)	Rinshu (S^3S^f)
1F3-18 (S^fS^f)	15.3	Benisashi (S^7S^f)	Rinshu (S^3S^f)
1G3-01 (S^7S^f)	47.5	Benisashi (S^7S^f)	Kensaki (S^fS^f)
1K0-26 (S^3S^7)	54.3	Benisashi (S^7S^f)	Koshinoume (S^3S^f)
1L0-23 (S^7S^8)	0	Benisashi (S^7S^f)	Ryukyokoume (S^8S^f)
1L0-39 (S^7S^f)	28.8	Benisashi (S^7S^f)	Ryukyokoume (S^8S^f)
1O3-01 (S^5S^f)	43.1	Benisashi (S^7S^f)	Oushuku (S^1S^5)
1O3-14 (S^1S^7)	0	Benisashi (S^7S^f)	Oushuku (S^1S^5)
1P3-14 (S^2S^7)	0	Benisashi (S^7S^f)	Tamaume × Koshusaisho (S^2S^8)
2B3-09 (S^fS^f)	39.5	Shinpeidayu (S^3S^f)	Shinpeidayu (S^3S^f)
2L3-34 (S^fS^f)	42.1	Shinpeidayu (S^3S^f)	Ryukyokoume (S^8S^f)
2M3-10 (S^3S^f)	35.0	Shinpeidayu (S^3S^f)	Orihime (S^6S^f)
2M3-41 (S^3S^f)	24.3	Shinpeidayu (S^3S^f)	Orihime (S^6S^f)
2P3-09 (S^2S^f)	12.1	Shinpeidayu (S^3S^f)	Tamaume × Koshusaisho (S^2S^8)
2P3-24 (S^2S^f)	35.0	Shinpeidayu (S^3S^f)	Tamaume × Koshusaisho (S^2S^8)
3A3-09 (S^7S^f)	65.6	Kensaki (S^fS^f)	Benisashi (S^7S^f)
3H3-02 (S^7S^f)	53.0	Kensaki (S^fS^f)	Nanko (S^1S^7)
3H3-07 (S^1S^f)	51.4	Kensaki (S^fS^f)	Nanko (S^1S^7)
71KO-04 (S^1S^f)	62.2	Kensaki (S^fS^f)	Oushuku (S^1S^5)

[a] *S*-haplotypes of selection lines and parents are shown in parenthesis

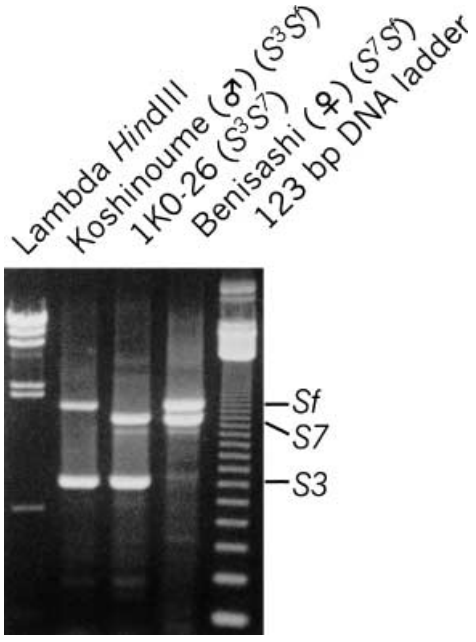

Fig. 2 PCR analysis for selection 1K0-26 (S^3S^7) and its parents, Koshinoume (S^3S^f) and Benisashi (S^7S^f), with a *S*-RNase gene-specific primer pair, Pru-C2 and PCE-R. Selection 1K0-26 (S^3S^7) showed self-compatibility despite lacking the S^f-RNase allele

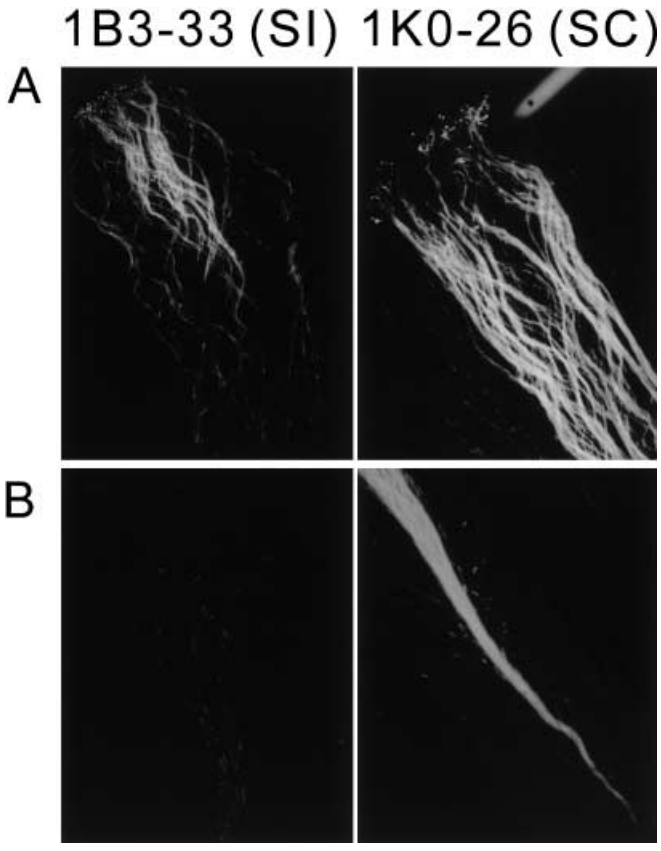

Fig. 3A, B Pollen-tube growth after self-pollination in two selections of Japanese apricot. *Left* two panels show the photographs of selection 1B3-33 (S^3S^7) that is from Benisashi (S^7S^f) × Shinpeidayu (S^3S^f) (Table 1), while *right* two photographs show those of selection 1K0-26 (S^3S^7) that is from Koshinoume (S^3S^f) × Benisashi (S^7S^f). Selection 1K0-26 is self-compatible even with no S^f-RNase allele (Table 2). The upper two photographs (**A**) show the stigma and upper style while the lower photographs (**B**) show the base of the style of pistils. Note that the pollen tubes of self-incompatible (SI) 1B3-33 stopped at the upper part of the pistil while those of the self-compatible (SC) selection 1K0-26 continued towards the ovule

Results

PCR determination of *S*-haplotypes

All cultivars and selection TK tested, except for 'Kensaki', yielded two different bands by PCR with an *S*-RNase gene-specific primer pair (Fig. 1 and Table 1). In terms of the size of the PCR products, at least one novel *S*-RNase allele or *S*-haplotype, designated as S^8, was found.

Segregation for S^f-RNase in progenies

PCR with an *S*-RNase gene-specific primer pair, Pru-C2 and PCE-R, effectively determined *S*-haplotypes of the progenies (Table 1). The S^f-RNase allele was present only in self-compatible cultivars and not in self-incompatible cultivars, supporting the hypothesis of Tao et al. (2000) that the S^f-RNase allele is associated with the *S*-haplotype that confers self-compatibility. Chi-square tests of the segregation data also support this hypothesis. In crosses in which parents did not share any *S*-RNase alleles in common, including 'Nanko (S^1S^7)' × 'Jizoume (S^3S^f)', 'Hachiro (S^8S^f)' × 'Nanko (S^1S^7)', 'Benisashi (S^7S^f)' × 'Shinpeidayu (S^3S^f)', and 'Shinpeidayu (S^3S^f)' × 'Benisashi (S^7S^f)', resultant progenies segregated into four distinct *S*-haplotypes with segregation ratios of approximately the expected 1:1:1:1. Self-pollination of 'Hachiro (S^8S^f)' segregated into two *S*-haplotypes, S^fS^f and S^8S^f, with 41 seedlings being of the former haplotype and 57 the latter. This could be approximated to the 1:1 ratio expected in semi-compatible crosses in which parents share only one common *S*-haplotype although the significance is marginal (Table 1). The *S*-haplotype of 'Kensaki', which gave only an S^f-band by PCR, appeared to be S^fS^f rather than S^fS^{novel} because all seedlings from self-pollination of 'Kensaki' yielded the S^f-band and 'Nanko (S^1S^7)' × 'Kensaki' segregated into the two expected *S*-haplotypes, S^1S^f and S^7S^f, with the expected ratio of 1:1.

Cosegregation of S^f-RNase and self-compatibility

All trees and selections with no S^f-RNase allele, except for selection 1K0-26, did not set fruit. Selection line 1K0-26 (S^3S^7) from 'Koshinoume (S^3S^f)' × 'Benisashi (S^7S^f)' set fruit after self-pollination, although it does not have the S^f-RNase allele (Fig. 2 and Table 2). Trees from reciprocal crosses of 'Benisashi' and 'Shinpeidayu' (Table 1) showing a fruit set less than 10%, as well as 'Koshinoume', 'Benisashi' and selection 1K0-26, were

subjected to the pollen-tube growth test to confirm their self-(in)compatibility. Self-pollen-tube growth of all the trees showing no fruit set after self-pollination was inhibited in the upper middle part of stylar tissue and no self-pollen-tube reached the ovule (Fig. 3 left). Self-pollen-tubes of all the trees showing a fruit set of greater than 0% successfully reached the ovule. The pollen-tube growth test also confirmed that selection 1K0-26, 'Benisashi' and 'Koshinoume' were self-compatible (Fig. 3 right).

Discussion

PCR determination of *S*-haplotypes

PCR using an *S*-RNase gene-specific primer pair was conducted to determine the *S*-haplotypes of the parents for progenies used in this study. Yaegaki et al. (2001) successfully applied the PCR analysis to identify *S*-haplotypes of Japanese apricot by using the *S*-RNase gene-specific primer set, Pru-C2 and Pru-C5 (Tao et al. 1999). These primers were developed from the DNA sequences at the conserved domains C2 and C5, respectively, of sweet cherry *S*-RNase (Tao et al. 1999). In this study, we used a primer pair of Pru-C2 and PCE-R that is different from that used by Yaegaki et al. (2001). The PCE-R primer was designed from the conserved domain C3 of *Prunus S*-RNase (Yamane et al. 2001). An intron varying in size has been reported to be present universally in the DNA sequences encoding the hyper-variable region (RHV) located between the C2 and C3 domains of *S*-RNase of *Prunus* species, which makes it possible to distinguish *S*-haplotypes by PCR (Tamura et al. 2000; Yamane et al. 2000; Tao et al. 2002). Although one additional intron seemed to be present at the sequence encoding the N-terminal of *S*-RNase (Tao et al. 1999; Yamane et al. 2000), no other introns have been reported so far for *S*-RNase genes of *Prunus*. Furthermore, there has been reported to be little difference in the size of the exon sequences of *Prunus S*-RNase genes. Thus, we could compare our results with those of Yaegaki et al. (2001), because the relative sizes of the PCR products from different *S*-RNase genes with the two primer sets used in the present study and that of Yaegaki et al. (2001) should be substantially the same. Among the six cultivars that Yaegaki et al. (2001) used, three cultivars, 'Oushuku (S^1S^5)', 'Nanko (S^1S^7)' and 'Kairyouchidaume (S^3S^4)', were employed as parent cultivars for controlled crosses in this study and subjected to PCR analysis. Although they could represent the S^1-, S^3-, S^4-, S^5- and S^7-haplotypes, there were no references for the S^2- and S^6-haplotypes. Thus 'Gyokuei (S^2S^6)' that was also used by Yaegaki et al. (2001) was subjected to PCR analysis to represent the S^2- and S^6-haplotypes, although it was not used as a parent for controlled crosses in the present study. We tentatively assigned respective *S*-haplotypes to cultivars and selection TK based on PCR analysis (Fig. 1 and Table 1). A novel *S*-haplotype, S^8, has been identified in this study. However, further confirmation of *S*-haplotypes by RFLP and sequence analyses, as well as pollination tests, are necessary because different *S*-haplotypes sometimes yielded PCR products of the same size and vice versa (Tao et al. 1999; Hauck et al. 2001; Yamane et al. 2001).

S^f-RNase as a marker for self-compatibility

A major finding of this study was the demonstration that the S^f-RNase gene cosegregated with self-compatibility and was located at the presumed *S*-locus region of Japanese apricot like other *S*-RNase genes. Since the segregation data suggested that the S^f-haplotype was a non-functional *S*-haplotype that confers self-compatibility, we conducted the pollination and fruit-set studies for the trees obtained from the reciprocal crosses of 'Benisashi' and 'Shinpeidayu' (Table 1), and selection lines from various crosses (Table 2) to see if the S^f-RNase allele cosegregated with self-compatibility. Furthermore, we also conducted a pollen-tube growth test for the trees showing a fruit-set less than 10% because our investigation for fruit-set was conducted at a mature stage of fruit development and the data obtained may have been greatly affected by environmental and growing conditions. All data except that on selection 1K0-26 were consistent with the fact that the S^f-RNase allele cosegregated with self-compatibility, although the percentages of fruit-set after self-pollination varied depending on the tree and selection (Table 2). Furthermore, all segregation data were consistent with the S^f-RNase gene located at the presumed *S*-locus region. With the discovery of *S*-RNase alleles and associated *S*-haplotypes (Yaegaki et al. 2001), including data obtained from our current and previous studies on S^f-RNase (Tao et al. 2000, 2002), we can now predict the proportion of self-compatible offspring obtained from certain crosses. As the S^f-haplotype is inherited in a dominant fashion, self-compatible cultivars can be readily obtained from crosses with only one self-compatible parent. The expected ratio of self-compatible offspring obtained would be 50%, 75% or 100% depending on the *S*-haplotypes of the parents employed. 'Kensaki' would be an especially useful cultivar in the breeding program for self-compatible Japanese apricots since 'Kensaki' appeared to be S^fS^f and all progenies of 'Kensaki' would be expected to be self-compatible.

Self-compatibility in selection 1K0-26 with no S^f-RNase

An exception to the cosegregation of the S^f-RNase allele with self-compatibility was observed with selection 1K0-26, which is from 'Benisashi (S^7S^f)' × 'Koshinoume (S^3S^f)', and was self-compatible even with no S^f-RNase allele detected (Fig. 3, Table 2). 'Benisashi' and 'Koshinoume' are known to be self-compatible cultivars as confirmed by pollen-tube growth studies. It is possible that genes other than *S*-RNase including pollen-*S* genes

could account for the self-compatibility in selection 1K0-26. It has recently been shown that proteins other than *S*-proteins, such as the HT-protein, are necessary for the self-incompatible reaction in *Nicotiana* (McClure et al. 1999). Alternatively, it is plausible that pollen-*S* of the S^3- or S^7-haplotype of selection 1K0-26 may have recombined with the S^f-haplotype to become self-compatible. If selection 1K0-26 has recombined *S*-haplotype with a combination of the S^3- or S^7-RNase gene and the pollen-*S* of the S^f-haplotype, it should be self-compatible.

Possible mechanism of self-compatibility observed with S^f-RNase

Several mechanisms for self-compatibility exist. The S^f-RNase gene may code for a non-functional *S*-RNase although no differences between the S^f- and other *S*-RNases that are involved in the self-incompatible reaction in Japanese apricot and other *Prunus* species have been detected (Tao et al. 2002). Since RNase activity is shown to be essential for the self-incompatibility reaction (Huang et al. 1994: Royo et al. 1994), it is possible that the S^f-RNase gene may encode an *S*-RNase that has weak or no RNase activity so that the S^f-haplotype cannot reject self-pollen. Alternatively, it is possible that genes that are involved in self-incompatibility and are tightly linked to the S^f-RNase gene may account for the self-compatibility observed with the S^f-RNase allele. As mentioned, the *S*-locus of the Rosaceae is bipartite, with different genes encoding the style component (*S*-RNase) and the pollen component (pollen-*S*) (Sassa et al. 1997). Pollen-*S* mutations could be responsible for the self-compatibility observed with the S^f-RNase allele. If pollen-*S* of the S^f-haplotype is dysfunctional, the S^f-haplotype could be a self-compatible haplotype and also a universal pollen donor. Also, if recombination occurred only between the pollen-*S* genes of the S^f-haplotype and other self-incompatible *S*-haplotypes, but not within respective *S*-RNase genes, a self-compatible haplotype would result. Alternatively, if two or more different pollen-*S* genes are present at the *S*-locus region, self-compatibility could result from the competitive interaction phenomena as reported in solanaceous plants (Chawla et al. 1997; Entani et al. 1999; Golz et al. 1999). Recent progress towards identifying the pollen component in almond, *P. dulcis* (Ushijima et al. 2001), may help determine the mechanism involved. Further insight into S^f-haplotype function may lead to the identification of unknown pollen components conferring gametophytic self-incompatibility in the Rosaceae as well as in the Solanaceae and Scrophulariaceae.

Acknowledgements This work was supported in part by grant-in-aid no. 13460014 for scientific research (B) to R. Tao from the Ministry of Education, Culture, Sports, Science and Technology. We gratefully acknowledge the National Institute of Fruit Tree Science for providing us with plant materials and Hidenori Sassa and Koichiro Ushijima at Yokohama City University for a critical review of this manuscript.

References

Chawla B, Bernatzky R, Liang W, Marcotrigiano M (1997) Breakdown of self-incompatibility in tetraploid *Lycopersicon peruvianum*: inheritance and expression of *S*-related proteins. Theor Appl Genet 95:992–996

de Nettancourt (1997) Incompatibility in angiosperms. Sex Plant Reprod 10:185–199

Edwards K, Johnstone C, Thompson C (1991) A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. Nucleic Acids Res 19:1349

Entani T, Takayama S, Iwano M, Shiba H, Che F-S, Isogai A (1999) Relationship between polyploidy and pollen self-incompatibility phenotype in *Petunia hybrida* Vilm. Biosci Biotech Biochem 63:1882–1888

Golz JF, Su V, Clarke AE, Newbigin E (1999) A molecular description of mutations affecting the pollen component of the *Nicotiana alata S* locus. Genetics 152:1123–1135

Hauck NR, Iezzoni AF, Yamane H, Tao R (2001) Revisiting the *S*-allele nomenclature in sweet cherry (*Prunus avium*) using RFLP profiles. J Am Soc Hort Sci 126:654–660

Huang S, Lee HS, Karunanandaa B, Kao T-H (1994) Ribonuclease activity of *Petunia inflata S* proteins is essential for rejection of self-pollen. Plant Cell 6:1021–1028

Ishimizu T, Inoue K, Shimonaka M, Saito T, Terai O, Norioka S (1999) PCR-based method for identifying the *S*-genotypes of Japanese pear cultivars. Theor Appl Genet 98:961–967

Janssens GA, Goderis IJ, Broekaert WF (1995) A molecular method for *S*-allele identification in apple based on allele-specific PCR. Theor Appl Genet 91:691–698

Lansari A, Lezzoni A (1990) A preliminary analysis of self-incompatibility in sour cherry. HortScience 25:1636–1638

McClure B, Mou B, Canevascini S, Bernatzky R (1999) A small asparagine-rich protein required for *S*-allele-specific pollen rejection in *Nicotiana*. Proc Natl Acad Sci USA 96:13,548–13,553

McCubbin AG, Kao T-H (2000) Molecular recognition and response in pollen and pistil interactions. Annu Rev Cell Dev Biol 16:333–364

Miyake M, Yamaguchi M, Haji T (1995) The self-compatibility in Mume cultivars (in Japanese). J Japan Soc Hort Sci 64 (Suppl 2):116–117

Royo J, Kunz C, Kowyama Y, Anderson M, Clarke AE, Newbigin E (1994) Loss of a histidine residue at the active site of *S*-locus ribonuclease is associated with self-compatibility in *Lycopersicon peruvianum*. Proc Natl Acad Sci USA 91:6511–6514

Sassa H, Hirano H, Ikehashi H (1992) Self-incompatibility-related RNases in styles of Japanese pear (*Pyrus serotina* Rehd.). Plant Cell Physiol 33:811–814

Sassa H, Hirano H, Nishio T, Koba T (1997) Pistil-specific self-compatibility mutation caused by deletion of the *S*-RNase gene in Japanese pear (*Pyrus serotina*). Plant J 12:223–227

Sonneveld T, Robbins TP, Boskovic R, Tobutt KR (2001) Cloning of six cherry self-incompatibility alleles and development of allele-specific PCR detection. Theor Appl Genet 102:1046–1055

Tamura M, Ushijima H, Sassa H, Hirano H, Tao R, Gradziel TM, Dandekar AM (2000) Identification of self-incompatibility genotypes of almond by allele-specific PCR analysis. Theor Appl Genet 101:344–349

Tao R, Yamane H, Sassa H, Mori H, Gradziel TM, Dandekar AM, Sugiura A (1997) Identification of stylar RNases associated with gametophytic self-incompatibility in almond (*Prunus dulcis*). Plant Cell Physiol 38:304–311

Tao R, Yamane H, Sugiura A, Murayama H, Sassa H, Mori H (1999) Molecular typing of *S*-alleles through identification, characterization and cDNA cloning for *S*-RNases in sweet cherry. J Am Soc Hort Sci 124:224–233

Tao R, Habu T, Yamane H, Sugiura A, Iwamoto K (2000) Molecular markers for self-compatibility in Japanese apricot (*Prunus meme*). HortScience 35:1121–1123

Tao R, Habu T, Yamane H, Sugiura A (2002) Characterization and cDNA cloning for *Sf*-RNase, a molecular marker for self-compatibility, in Japanese apricot (*Prunus mume*). J Japan Soc Hort Sci (in press)

Ushijima K, Sassa H, Tao R, Yamane H, Dandekar AM, Gradziel TM, Hirano H (1998) Cloning and characterization of cDNAs encoding *S*-RNases in almond (*Prunus dulcis*): primary strucutural features and sequence diversity of the *S*-RNases in Rosaceae. Mol Gen Genet 260:261–268

Ushijima K, Sassa H, Tamura M, Kusaba M, Tao R, Gradziel TM, Dandekar AM, Hirano H (2001) Characterization of the *S*-locus region of almond (*Prunus dulcis*): analysis of a somaclonal mutant and a cosmid contig for an *S* haplotype. Genetics 158:379–386

Wiersma PA, Wu Z, Zhou L, Hampson C, Kappel F (2001) Identification of new self-incompatibility alleles in sweet cherry (*Prunus avium* L.) and clarification of incompatibility groups by PCR and sequencing analysis. Theor Appl Genet 102:700–708

Yaegaki H, Shimada T, Moriguchi T, Hayama H, Haji T, Yamaguchi M (2001) Molecular characterization of *S*-RNase genes and *S*-genotypes in the Japanese apricot (*Prunus mume* Sieb. et Zucc.). Sex Plant Reprod 13:251–257

Yamane H, Tao R, Sugiura A (1999) Identification and cDNA cloning for *S*-RNases in self-incompatible Japanese plum (*Prunus salicina* Lindl. cv Sordum). Plant Biotech 16:389–396

Yamane H, Tao R, Murayama H, Sugiura A (2000) Determining the *S*-genotypes of several sweet cherry cultivars based on PCR-RFLP analysis. J Hort Sci Biotech 75:562–567

Yamane H, Tao R, Sugiura A, Hauck NR, Iezzoni AF (2001) Identification and characterization of *S*-RNases in tetraploid sour cherry (*Prunus cerasus* L.). J Am Soc Hort Sci 126:661–667