Short Communication Plant Biotechnology, **20** (4), 323-329 (2003)

Partial Genomic Sequences of S_6-, S_{12}-, S_{13}-, S_{14}-, S_{17}-, S_{19}-, and S_{21}-RNases of Apple and Their Allele Designations

Shogo MATSUMOTO[1]*, Yui FURUSAWA[1], Kentaro KITAHARA[1] and Junichi SOEJIMA[2]

[1]*Department of Biology, Faculty of Education, Gifu University, Gifu 501-1193, Japan*
[2]*Apple Research Center, National Institute of Fruit Tree Science, Morioka 020-0123, Japan*
**Corresponding author* E-mail address: shmatsum@cc.gifu-u.ac.jp

Received 2 April 2003; accepted 4 August 2003

Abstract

We determined partial genomic sequences including a hypervariable HVa (RHV) region and introns of the S_6-, S_{12}-, S_{13}-, S_{14}-, S_{17}-, S_{19}-, and S_{21}-RNases of apple. Previously, it was suggested by Broothaerts (2003) that the S_6 corresponded to S_{25} in 'McIntosh'/'Wijcik' and 'Tydeman's Early Worcester', and S_{19} corresponded to S_{28}. However, S_6 and S_{19} are different from the S_{25} and S_{28}, respectively. The determined sequences of the S_6 and S_{12}, and S_{17} and S_{19} are identical, and these four alleles seem to act as the same allele. We newly assigned S_{6a} in place of S_6 and S_{12}, and S_{6b} in place of S_{17} and S_{19} from the results of sequence analyses, not pollination tests. The sequences of S_{13} and S_{14} were also identical. We also newly assigned S_{11} in place of S_{13} and S_{14}. We had speculated that S_{21} would correspond to S_t by PCR-digestion analysis, however, the determined sequence of S_{21} was different from S_t.

Key words: apple, *Malus* x *domestica*, *S*-allele, S-RNase, self-incompatibility.

Abbreviations

RHV, Rosaceae-specific hypervariable region; PCR, polymerase chain reaction.

Apple (*Malus* x *domestica* Borkh.) exhibits gametophitic self-incompatibility controlled by the multi-allelic *S*-locus (Kobel *et al.*, 1939; de Nettancourt, 1977). Self-incompatibility is a mechanism for preventing self-fertilization in flowering plants, and in gametophitic self-incompatibility, pollen-tube growth is inhibited when an *S*-haplotype of pollen matches one of the *S*-haplotypes of the pistil. Using cross pollination followed by analysis of pollen tube growth in the style or analysis of the fruit and seed, 11 (S_1 to S_{11}) and 10 (S_a to S_i, S_z) *S*-haplotypes have been identified (Kobel *et al.*, 1939; Komori *et al.*, 1999, 2000a). A pistil-specific glycoprotein with ribonuclease activity (**S**-RNase) encoded by the *S*-locus has been identified using molecular method (Broothaerts *et al.*, 1995), and shown to be related to the self-incompatibility response (Sassa *et al.*, 1992, 1993, 1997). As the *S*-RNase does not seem to act as a pollen factor other than a pistil factor, at least two kinds of polymorphic genes might be related to gametophytic self-incompatibility. Up to date, genes encoding S_c-, S_d-, S_e-, S_f-, S_g-, $S_{g'}$-, S_h-, $S_{10\,(\text{equivalent to } i)}$-, S_t-, and $S_{25\,(\text{equivalent to } z)}$-RNases have been cloned in Japan (Sassa *et al.*, 1996; Matsumoto *et al.*, 1999; Kitahara *et al.*, 2000; Matsumoto *et al.*, 2000; Matsumoto and Kitahara, 2000; Matsumoto *et al.*, 2001a; Kitahara and Matsumoto, 2002a, b), and genes encoding S_2-, S_3-, S_4-, S_5-, S_7-, S_9-, S_{24}-, S_{26}-, S_{27a}-, S_{27b}-, and $S_{30(28)}$-RNases in Europe (Broothaerts *et al.*, 1995; Janssens *et al.*, 1995; Verdoodt *et al.*, 1998; Schneider *et al.*, 2001; Van Nerum *et al.*, 2001). Recently, the nucleotide sequence of S_{29}-RNase was released on a database under the accession number AY039702. As described in previous reports (Kitahara and Matsumoto, 2002a, b), it was confirmed that the S_a-, S_b-, S_c-, S_d-, S_e-, S_f-, S_g-, S_h-, S_i-, and S_z-alleles established in Japan corresponded, respectively, to the S_2-, S_3-, S_9-, S_7-, $S_{30(=28)}$-, S_1-, S_{20}-, S_{24}-, S_{10}-, and S_{25}-alleles established in Europe. As the S-alleles taken up in this study are not included in these alleles, we investigated partial genomic sequences of unknown *S*-RNases as descrived below.

The alphabetical *S*-allele (variants of a polymorphic *S*-gene within the *S*-locus) designation in Japan is confusing. To resolve this confusion, we proposed to use the European numerical designations (Kitahara and Matsumoto, 2002a, b). How-

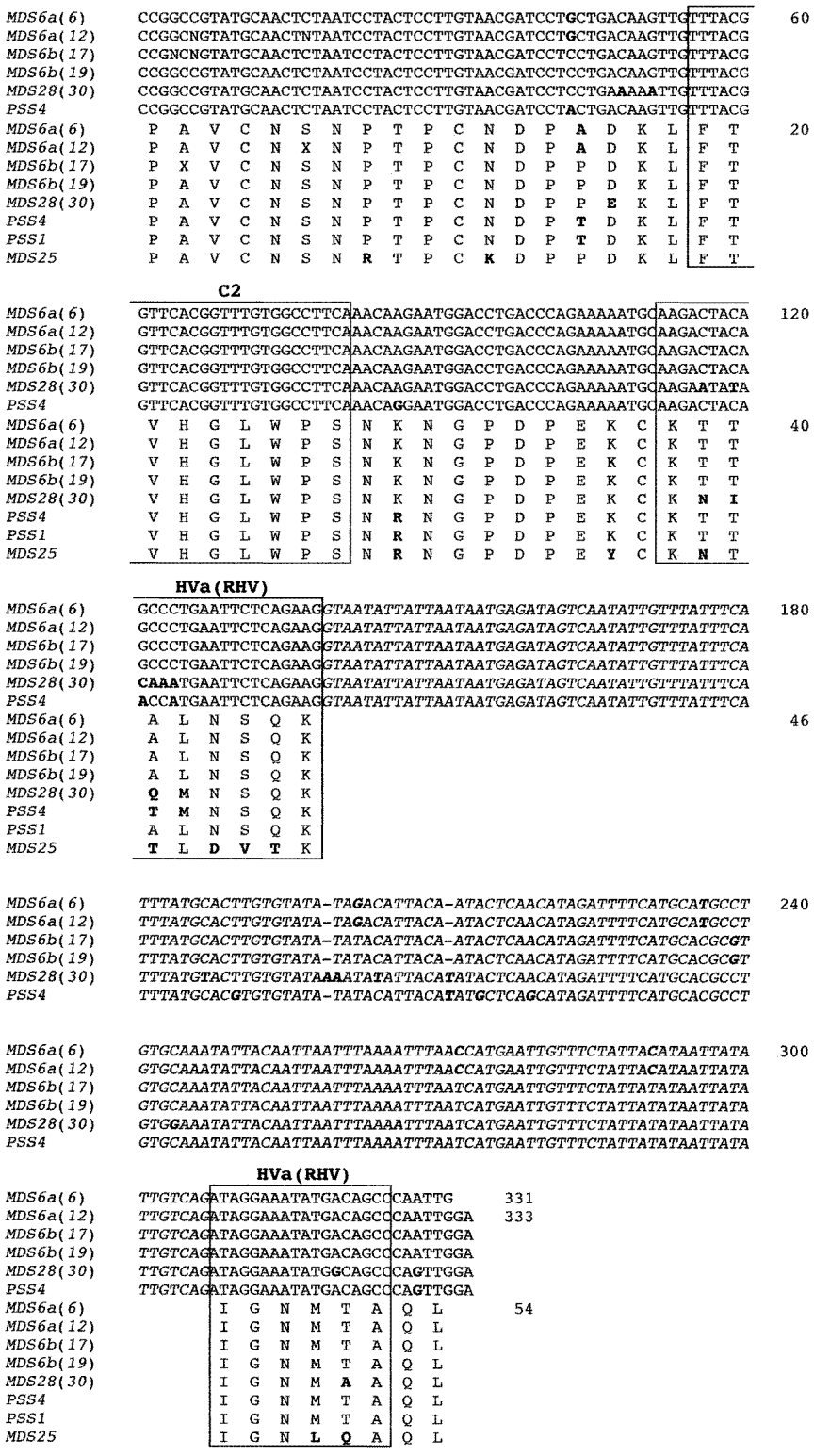

Fig. 1 Alignment of the partial nucleotide and deduced partial amino acid sequences of $S_{6a(=6)}$-, $S_{6a(=12)}$-, $S_{6b(=17)}$-, $S_{6b(=19)}$-, $S_{28(30)}$-, and S_{25}- (only amino acid sequence) RNase in apple and S_1- (only amino acid sequence) and S_4-RNase in Asian pear.

The conserved region C2 and hypervariable region HVa (RHV) are boxed. The sequences of $S_{6a(=6)}$ *(MDS6a (6))*, $S_{6a(=12)}$ *(MDS6a (12))*, $S_{6b(=17)}$ *(MDS6b (17))* and $S_{6b(=19)}$ *(MDS6a (19))* were deposited under the DDBJ accession numbers AB094495, AB105061, AB105062, and AB094493, respectively. Apple $S_{28(30)}$ *(MDS28 (30)* and apple S_{25} *(MDS25)*, Asian pear S_1 *(PSS1)* and Asian pear S_4 *(PSS4)* were from studies by Matsumoto and Kitahara (2000) together with Matsumoto *et al.* (2001b), Kitahara and Matsumoto (2002b), Ishimizu *et al.* (1999) and Norioka *et al.* (1995), respectively. The different sites are shown in bold. Intron sequences are italicized.

ever, the European numerical designation is also confusing, mostly due to the 11 S-alleles (S_{12} to S_{19}, S_{21} to S_{23}) that identified only in one cultivar by stylar protein analyses (Bošković and Tobutt, 1999). For instance, S_{22} and S_{23} which identified only in 'Alkmene' and 'Delbard Jubile', respectively (Bošković and Tobutt, 1999), were classified as S_{27b} by S-allele specific PCR and sequence analysis, not stylar protein analysis (Van Nerum *et al.*, 2001). Afterward, the S_{22}-, S_{23}-, and S_{27b}-alleles were renumbered as S_{22} (Broothaerts, 2003). Van Nerum *et al.* (2001) pointed out that the electrophoretic differences between several S-RNases identified by Bošković and Tobutt (1999) appeared to be small, and it is conceivable that some S-alleles do not exist. Mainly to verify this, we amplified the genomic DNA including a hypervariable HVa (RHV) and introns corresponding to S_6-, S_{12}- to S_{14}-, S_{17}-, S_{19}-, and S_{21}-alleles using primers derived from the amino acid sequences 'FTQQYQ' and 'anti-$^{I}/_{M}$

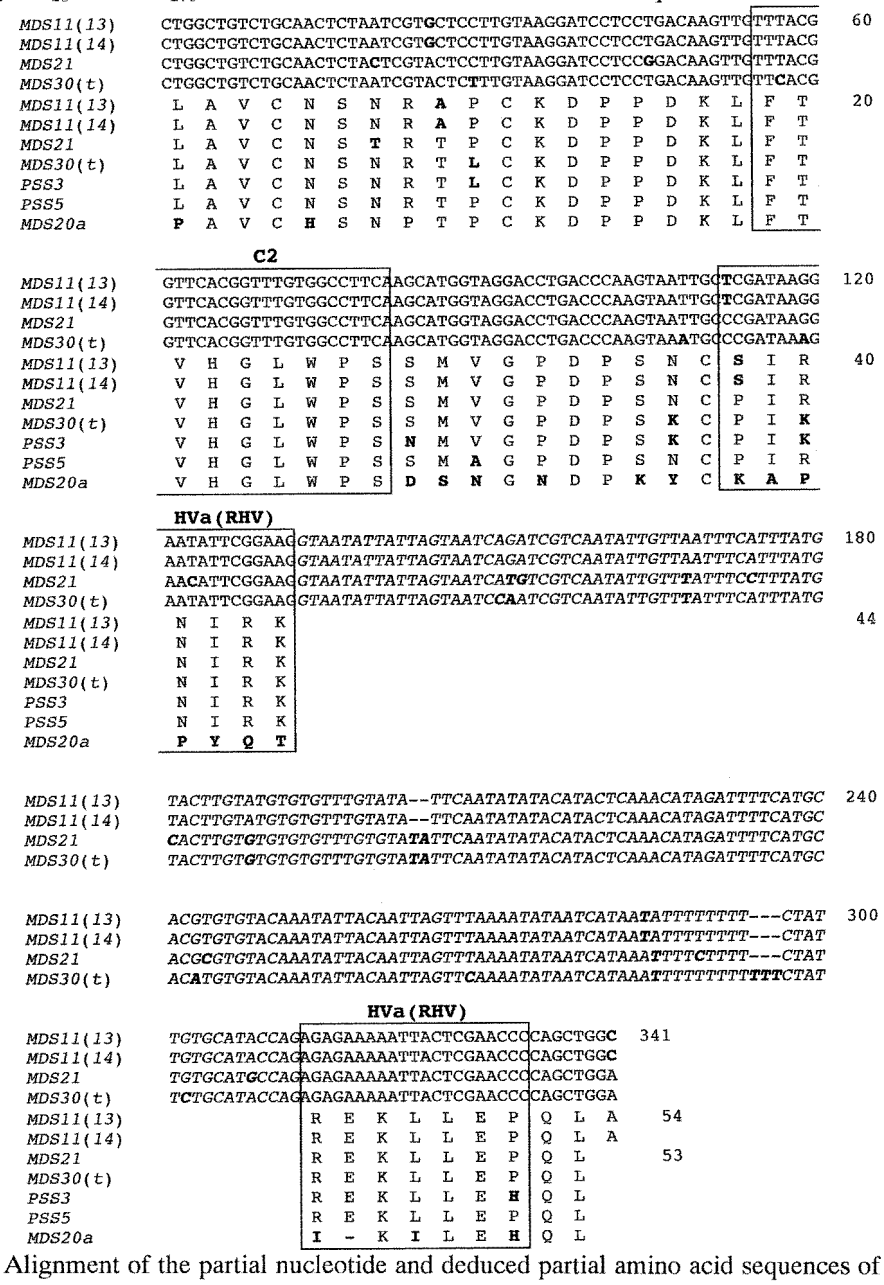

Fig. 2 Alignment of the partial nucleotide and deduced partial amino acid sequences of $S_{11(=13)}$-, $S_{11(=14)}$-, S_{21}-, $S_{30(=t)}$-, and S_{20a}- (only amino acid sequence) RNase in apple and S_3- and S_5- RNase in Asian pear (only amino acid sequence).

The conserved region C2 and hypervariable region HVa (RHV) are boxed. The sequences of $S_{11(=13)}$ *(MDS11 (13))*, $S_{11(=14)}$ *(MDS11 (14))* and S_{21} *(MDS21)* were deposited under the DDBJ accession numbers AB 105060, AB094492, and AB094494, respectively. Apple $S_{30(=t)}$ *(MDS30(t))*, apple S_{20a} *(MDS20a)*, and Asian pear S_3 *(PSS3)* together with S_5 *(PSS5)* were from studies by Matsumoto *et al.* (2000, 2001b), Matsumoto *et al.* (1999) and Ishimizu *et al.* (1998), respectively. The different sites are shown in bold. Intron sequences are italicized.

Table 1 *S*-alleles of apple cultivars and a species.

Proposed *S*-allele	Former *S*-allele	Cultivar/Species	*S*-alleles			Database Accession #
6a	6	**Oetwiler Reinette**	3	6a		AB094495
	12	**Citron d'Hiver**	3	5	6a	AB105061
6b	17	**Blenheim Orange**	1	3	6b	AB105062
	19	**Bohnapfel**	6b	9	16	AB094493
11	10/11,13	**Gravenstein**	4	11	20a	AB105060
	14	**Jacques Lebel**	1	3	11	AB094492
21	21	**Ribston Pippin**	1	9	21	AB094494
28	19,30,e,d,de,g	Delicious	9	28		AB035273
	28,30	Red Delicious	9	28		AF201748
30	t	*Malus transitoria*	20b	30	?	AB035928

Malus plants used in this study are in bold face.

IWPNV' (Ishimizu *et al.*, 1999; Matsumoto and Kitahara, 2000). The conditions for PCR were as described by Ishimizu *et al.* (1999). The amplified fragments were directly sequenced by dideoxy chain termination on an ABI PRISM™ 310 DNA sequencer (Perkin-Elmer) using dRhodamine Terminator Cycle Sequencing Kits (Perkin-Elmer).

The seven *S*-RNases were divided into three groups of four, two and one, respectively: S_6, S_{12}, S_{17}, and S_{19} (**Fig. 1**); S_{13} and S_{14} (**Fig. 2**); and S_{21} (**Fig. 2**), based on sequence similarity. Genes encoding all *S*-RNases in apple contain one intron at the same location within the hypervariable HVa (RHV) region (Matsumoto *et al.*, 2001b), and the seven *S*-RNases also contain an intron deduced from the presence of plant 5 and 3 splice site consensus sequences at corresponding sites (**Fig. 1, 2**). As shown in **Fig. 1**, the determined sequence of S_6 and S_{12}, and S_{17} and S_{19} were completely identical, and the deduced amino acid sequence of S_6 (S_{12}) was identical to S_{17} (S_{19}) except for one amino acid located outside of a hypervariable region (position 15) as described below, where alanine was replaced by proline (**Fig. 1**). In closely related *S*-RNases in Solanaceae, the specificity of cell-cell recognition phenomenon has been shown to depend on the amino acid sequences at the two hypervariable regions (HVa and HVb) (Kao *et al.*, 1996; Matton *et al.*, 1997). HVa and HVb regions alone are sufficient for *S*-allele discrimination (Matton *et al.*, 1997). The rosaceous *S*-RNases have only one hypervariable region (RHV) located at a position corresponding to that of the solanaceous region HVa (Ushijima *et al.*, 1998). Since the HVa and HVb regions merely control allelic specificity (Matton *et al.*, 1997, 1999; Luu *et al.*, 2001), the RHV region must be sufficient for allele discrimination of closely related apple *S*-alleles. Up to date, functionally different alleles having identical RHV regions are unknown not only in apple, but also in other plants with gametophytic self-incompatibility. Since the RHV regions of S_6, S_{12}, S_{17}, and S_{19} were identical (**Fig. 1**), the four alleles were thought to be functionally identical. We designated S_6 and S_{12} as S_{6a}, and S_{17} and S_{19} as S_{6b}, respectively (**Table 1**).

Recently, Broothaerts (2003) re-numbered $S_{30(28)}$ to S_{19}. This re-numbering was based on the finding of the $S_{30(28)}$-allele in 'Bohnapfel' ($S_9S_{16}S_{19}$) by PCR-digestion analysis, not sequence analysis. Broothaerts (2003) detected both $S_{30(28)}$ and S_{19} using his $S_{30(28)}$-allele specific primers; however, we could not detect the $S_{30(28)}$-allele in 'Bohnapfel' with our PCR-digestion method (Matsumoto and Kitahara, 2000; data not shown). Moreover, from our sequence analysis, the five amino acids in the HVa (RHA) region of the S_{19}-allele differed from those of the $S_{30(28)}$-allele in 'Delicious' and 'Red Delicious' (**Fig. 1**), suggesting that S_{19} and $S_{30(28)}$ were different alleles.

Broothaerts (2003) also speculated from a PCR analysis that 'McIntosh'/'Wijcik' ($S_{10}S_{25}$ from stylar protein analysis by Bošković and Tobutt, 1999) and 'Tydeman's Early Worcester' ($S_{24}S_{25}$ from stylar protein analysis by Bošković and Tobutt, 1999) contained the S_6-allele, instead of the S_{25}-allele. Using S_{20}-primers, he observed a weak PCR product of a different size from that of the true S_{20}-fragment in 'Oetwiler' (S_3S_6), 'McIntosh'/'Wijcik' and 'Tydeman's Early Worcester'. Within the cultivars of 'Merlijn', 'McIntosh'/'Wijcik', 'Telamon', 'Trajan', and 'Tydeman's Early Worcester', which were shown to possess the S_{25}-allele from stylar protein analysis (Bošković and Tobutt, 1999), S_{25} in 'Merlijn' and 'Telamon' were replaced by S_{27b}- and S_{10}-alleles, respectively (Van Nerum *et al.*, 2001). As a result, the presence of the S_{25}-allele was contradicted (Van Nerum *et al.*, 2001). However,

we recently sequenced the S_{25}-allele in 'McIntosh' (Kitahara and Matsumoto, 2002b). We determined the *S*-genotype of 'McIntosh' and 'Tydeman's Early Worcester' to be $S_{10}S_{25}$ and $S_{24}S_{25}$, respectively, by PCR-digestion, which were the same results as those from stylar protein analysis (Kitahara and Matsumoto, 2002b). The *S*-genotype of 'Trajan' (S_2S_{25}) was also confirmed by PCR-digestion (data not shown). Finally, from our sequence analysis, the deduced amino acid sequence of S_6 was different from that of the S_{25}-allele in 'McIntosh' (**Fig. 1**), suggesting that S_6 and S_{25} were different alleles. S_{6a} and S_{6b} showed a marked similarity to the Asian pear (*Pyrus pyrifolia* (Burm) Nak.) S_1 and S_4 (**Fig. 1**) (Norioka *et al.*, 1995; Ishimizu *et al.*, 1998).

As the sequences of the S_{13} and S_{14} were completely identical to those observed between S_6 and S_{12} or S_{17} and S_{19} (**Fig. 2**), we designated them as neither S_{13} nor S_{14}, but S_{11} (**Table 1**). We confirmed S_{11} corresponded to S_{13} (=S_{14}) from the following. The S_{11}-allele was identified in 'Adam's Pearmain' (Kobel *et al.*, 1939), but we identified the S_1-allele instead of the S_{11}-allele (Matsumoto *et al.*, 2003). As the S_{11}-allele within 'Adam's Pearmain' was refuted, S_{11} was possibly left only in 'Gravenstein' (S_4S_{10} (or S_{11}) S_x by Kobel *et al.*, 1939). As we did not identify the S_{10}-alleles in 'Gravenstein' by PCR-digestion (data not shown), its *S*-genotype was thought to be $S_4S_{11}S_x$, not $S_4S_{10}S_x$. On the other hand, 'Gravenstein' is the only cultivar in which the S_{13}-allele has been identified, and its *S*-genotype was identified as $S_4S_{13}S_{20}$ by stylar protein analysis (Bošković and Tobutt, 1999). As the RHV region of S_{13} was different from that of S_{20} (**Fig. 2**), we unified S_{11} and S_{13} (= S_{14}) to S_{11} (**Table 1**).

The S_{21}-allele seemed to correspond to the S_t-allele in *Malus transitoria* (Matsumoto *et al.*, 2000) since a PCR product very close to 259 bp was obtained from *M. transitoria* ($S_{20b=g'}S_{30=t}$) and 'Ribston Pippin' ($S_1S_9S_{21}$) using the S_t-allele-specific primers 'St-sense' (5'-CAATAGATAACGAGAACCAC-3') and '*St*-antisense' (5'-CAATCTATGAAATGTTCTCC-3') (data not shown). Digestion of the 259 bp fragment by *Rsa*I cleaved it into fragments of the expected size, 214 bp and 45 bp (data not shown). To confirm that S_{21} corresponded to S_t, we determined the partial genomic sequence of the S_{21}-allele. As shown in **Fig. 2**, the deduced amino acid sequence of S_{21} was slightly different from that of the S_t. In particular, one amino acid difference was observed within the HVa (RHV) region (**Fig. 2**). The $S_{11(13,14)}$ also seemed to be close to S_{21} and S_t since only one or two amino acid differences were detected within the HVa (RHV) region (**Fig. 2**). $S_{11(13,14)}$, S_{21} and S_t showed a high similarity to the Asian pear [*Pyrus pyrifolia* (Burm) Nak.] S_3 and S_5 (**Fig. 2**) (Ishimizu *et al.*, 1998). The *P. pyrifolia* S_3- and S_5-alleles are functionally different, and differed by two amino acids in the HVa (RHV) region (**Fig. 2**) (Ishimizu *et al.*, 1998). In this case, the lysine and histidine residues of S_3-RNase differed from the arginine and proline residues of S_5-RNase, respectively (**Fig. 2**). Within the two amino acid differences, the site of the one amino acid difference between histidine and proline corresponded to that between S_g and $S_{g'}$-allele in apple. Previously, we established that one amino acid difference of S_g- and $S_{g'}$-RNase within the HVa (RHV) region was not enough to distinguish them as different alleles (Matsumoto *et al.*, 2001a). If the difference between histidine and proline is not enough to distinguish them as functionally different alleles, another difference between lysine and arginine must be essential to enable the S_3- and S_5-allele discrimination in the Asian pear. Such a difference was observed between $S_{11(13,14)}$ and S_t, and S_{21} and S_t. The difference was not observed between $S_{11(13,14)}$ and S_{21}, but the serine residue at the start of the HVa (RHV) region of $S_{11(13,14)}$ differed from the proline residue of S_{21}. In this case, it is unknown whether the two alleles are the same or not. We could not carry out cross-pollination tests among $S_{11(13,14)}$ S_{21} and S_t, because the alleles of all the identified cultivars are triploid. Consequently, we did not change the allele designation except for S_t, but did change the S_t to S_{30} when converting from the alphabetical to the numerical designation (**Table 1**). Since S_{30} has been designated as the $S_{30(28)}$, we proposed the unification of S_{30} and S_{28} as S_{28} (**Table 1**).

Finally, we concluded that the S_6 and S_{12}, and S_{17} and S_{19} should be re-numbered to S_{6a} and S_{6b}, respectively. These four alleles are thought to act as the same allele. Similarly, S_{13} and S_{14} are re-numbered as S_{11}. S_{21} was left unchanged, and S_t was re-numbered as S_{30}.

Acknowledgments

We wish to thank Dr. Wim Broothaerts for supplying the plant samples and DNAs. This research was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (No. 14360019 and 15208004).

References

Boškovic, R., Tobutt, K. R., 1999. Correlation of stylar ribonuclease isoenzymes with incompatibility alleles in apple. Euphytica, **107**: 29-43.

Broothaerts, W., Janssens, G. A., Proost, P., Broekaert, W. F., 1995. cDNA cloning and molecular analysis of two self-incompatibility alleles from apple. Plant Mol. Biol., **27**: 499-511.

Broothaerts, W., 2003. New findings in apple S-genotype analysis resolve previous confusion and request the re-numbering of some S-alleles. Theor. Appl. Genet., **106**: 703-714.

de Nettancourt, D., 1977. Incompatibility in Angiosperms. In: Frankel, R. *et al.* (Eds.): Monographs on theoretical and applied genetics, pp. 28-57. Springer-Verlag, New York.

Ishimizu, T., Shinkawa, T., Sakiyama, F., Norioka, S., 1998. Primary structural features of rosaceous S-RNases associated with gametophytic self-incompatibility. Plant Mol. Biol., **37**: 931-941.

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, G. A., Goderis, I. J., Broekaert, W. F., Broothaerts, W., 1995. A molecular method for *S*-allele identification in apple based on allele-specific PCR. Theor. Appl. Genet., **91**: 691-698.

Kao, T-H., Mccubbin, A. G., 1996. How flowering plants discriminate between self and non-self pollen to prevent inbreeding. Proc. Natl. Acad. Sci. U. S. A., **93**: 12059-12065.

Kitahara, K., Soejima, J., Komatsu, H., Fukui, H., Matsumoto, S., 2000. Complete sequences of the *S*-genes, Sd- and Sh-RNase cDNA in apple. HortSci., **35**: 712-715.

Kitahara, K., Matsumoto, S., 2002a. Sequence of the S_{10} cDNA from 'McIntosh' apple and a PCR-digestion identification method. HortSci., **37**: 187-190.

Kitahara, K., Matsumoto, S., 2002b. Cloning of the S_{25} cDNA from 'McIntosh' apple and an S_{25}-allele identification method. J. Hortic. Sci. Biotechnol., **77**: 724-728.

Kobel, F., Steinegger, P., Anliker, J., 1939. Weitere Untersuchungen über die Befruchtungsverhältnisse der Apfel- und Birnsorten. Landw. Jahrb. Schweiz, **53**: 160-191.

Komori, S., Soejima, J., Abe, K., Kato, H., Kotoda, N., Kudo, K., 1999. Analyses of *S*-allele genotypes of 'McIntosh', 'Kitakami', 'Worcester Pearmain', etc. J. Japan. Soc. Hort. Sci., **68** (Suppl. 2): 94.

Komori, S., Soejima, J., Abe, K., Kotoda, N., Kato, H., 2000a. Analyses of S-allele genotypes and genetic diversity in the apple. Acta Hort., **538**: 83-86.

Luu, D. -T., Qin, X., Laublin, G., Yang, Q., Morse, D., Cappadocia, M., 2001. Rejection of S-heteroallelic pollen by a dual-specific S-RNase in *Solanum chacoense* predicts a multimeric SI pollen component. Genetics, **159**: 329-335.

Matsumoto, S., Kitahara, K., Komori, S., Soejima, J., 1999. A new *S*-allele in apple '*Sg*', and its similarity to the '*Sf*' allele from Fuji. HortSci., **34**: 708-710.

Matsumoto, S., Suzuki, M., Kitahara, K., Soejima, J., 2000. Possible involvement of a new S-gene 'St-RNase' (Accession No. AB035928) in the wild apple possessing high similarity to the 'S3-' and 'S5-RNase' in the Japanese pear. Plant Physiol., **122**: 620.

Matsumoto, S., Kitahara, K., 2000. Discovery of a new self-incompatibility allele in apple. HortSci., **35**: 1329-1332.

Matsumoto, S., Kitahara, K., Komatsu, H., Soejima, J., 2001a. A functional *S*-allele, '*Sg*', in the wild apple possessing a single amino acid, S-RNase 'Sg'-RNase', different from 'Sg-RNase' in *Malus* x *domestica* cultivars. J. Hortic. Sci. Biotechnol., **76**: 163-166.

Matsumoto, S., Hayashi, S., Kitahara, K., Soejima, J., 2001b. Genomic DNA sequences encoding *Malus* x *domestica* Borkh. "Akane", "Delicious" and *Malus transitoria* S-RNases. DNA Seq., **12**: 381-383.

Matsumoto, S., Kitahara, K., Furusawa, Y., Soejima, J., Fukui, H., Komatsu, H., 2003. *S*-allele genotype of apple cultivars and selections. Acta Hort., **622**: 389-396.

Matton, D. P., Maes, O., Laublin, G., Xike, Q., Bertrand, C., Morse, D., Cappadocia, M., 1997. Hypervariable domains of self-incompatibility RNases mediate allele-specific pollen recognition. Plant Cell, **9**: 1757-1766.

Matton, D. P., Luu, D. T., Xike, Q., Laublin, G., O'Brien, M., Maes, O., Morse, D., Cappadocia, M., 1999. Production of an S RNase with dual specificity suggests a novel hypothesis for generation of new *S* alleles. Plant Cell, **11**: 2087-2097.

Norioka, N., Ohnishi, Y., Norioka, S., Ishimizu, T., Nakanishi, T., Sakiyama, F., 1995. Nucleotide sequences of cDNAs encoding S2- and S4-RNases (D49527 and D49528 for EMBL) from Japanese Pear (*Pyrus Pyrifolia* Nakai 1). Plant Physiol., **108**: 1343.

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., Ikehashi, H., 1993. Identification and characterization of stylar glycoproteins associated with self-incompatibility genes of Japanese pear, *Pyrus serotina* Rehd. Mol. Gen. Genet., **241**: 17-25.

Sassa, H., Nishio, T., Kowyama, Y., Hirano, H., Koba, T., Ikehashi, H., 1996. Self-incompatibility (*S*) alleles of the Rosaceae encode members of a distinct class of the T_2/S ribonuclease superfamily. Mol. Gen. Genet., **250**: 547-557.

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

Schneider, D., Stern, R. A., Eisikowitch, D., Goldway, M., 2001. Analysis of *S*-alleles by PCR for determination of compatibility in the 'Red Delicious' apple orchard. J. Hortic. Sci. Biotechnol., **76**: 596-600.

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

Van Nerum, I., Geerts, M., Van Haute, A., Keulemans, J., Broothaerts, W., 2001. Re-examination of the self-incompatibility genotype of apple cultivars containing putative 'new' S-alleles. Theor. Appl. Genet., **103**: 584-591.

Verdoodt, L., Van Haute, A., Goderis, I. J., De Witte, K., Keulemans, J., Broothaerts, W., 1998. Use of the multi-allelic self-incompatibility gene in apple to assess homozygosity in shoots obtained through haploid induction. Theor. Appl. Genet., **96**: 294-300.