

S-RNase allele nomenclature and designation in apple

Shogo MATSUMOTO

Summary

We propose a unified *S*-RNase allele designation in apple cultivars to avoid confusion. All alphabetical *S*-RNase allele designations for cultivars in Japan could be made to correspond to numerical designations in Europe. Using the numerical designation, one could avoid the confusion with alphabetical designations in Japan. We then classified the numerical designations in Europe using cross-pollination testing and sequence analysis, and cleared up the confusion of numerical designations in Europe. Finally, each of the 31 *S*-RNase alleles was identified with its own unique designation.

Key words: Apple; *S*-RNase allele; *Malus x domestica* Borkh.

Apple (*Malus x domestica* Borkh.) has a gametophytic self-incompatibility controlled by the multi-allelic *S*-locus (de Nettancourt, 1977; Kobel et al., 1939). In this type of self-incompatibility in the Rosaceae family, when one of the *S*-haplotypes (variants of the *S*-locus) of pollen matches either of the *S*-haplotypes of pistil, pollen tube growth is arrested within the pistil. This means that different cultivars possessing the same *S*-genotype show cross-incompatibility. For instance, the *S*-genotype of the main cultivar 'Fuji' ($S_i S_j$) is the same as that of the following cultivars: 'Akifu 1', 'Akifu 4', 'Alps Otome', 'Aofu 1', 'Blackjon', 'Kaori', 'Korin', 'Misora No Red', 'Nagara', Ryoka No Kise-tsu', 'Shinko', 'Spencer seedless', 'Spieton', 'Spitone', 'Takano Wase' and 'Yataka'. Crossing among the members in that group show them to be cross-incompatible because of having identical *S*-genotypes. Since combinations of the same *S*-genotypes are able to produce any fruit, it is very important for breeders and growers to determine accurately the *S*-genotype of apple cultivars.

S-RNase allele identification.

S-genotypes of apple cultivars in Europe and in Japan have already been identified by cross-pollination followed by an analysis of pollen tube growth in the style or by analyzing the fruit and seed set, and have been designated both numerically (11; S_i to S_{11}) and alphabetically (10; S_a to S_j , S_k), respectively (Kobel et al., 1939; Komori et al., 1998a, b, 1999, 2000). In addition, instead of the cross-pollination method, protein and genome analysis methods have been developed. The protein method is based on a pistil specific glycoprotein with ribonuclease activity (*S*-RNase), which has been shown to be related to the self-incompatible response in Rosaceae (Sassa et al., 1992, 1993, 1997). Since the *S*-RNase seems to act as a pistil factor rather than a pollen factor, at least two kinds of factors might be related to the gametophytic self-incompatibility system in Rosaceae. The genome method, called the PCR-digestion method, is based on different nucleotide sequences among *S*-RNase alleles (Table 1). Among the three methods, PCR-digestion is the most accurate and rapid, allowing us to determine the *S*-RNase alleles of more than 350 cultivars (Kitahara et al., 1999, 2000; Kitahara and Matsumoto, 2002a, b; Matsumoto et al., 1999a, b, 2000, 2001a, 2003a, b, c, 2006, 2007; Matsumoto and Kitahara, 2000; Matsumoto and Furusawa, 2005).

Table 1. Oligonucleotides and conditions for S-allele-specific PCR-digestion method

S-RNase allele	Primers	Sense/ antisense	Nucleotide sequence (5' → 3')	Annealing temp (°C)	Band sizes (bp)	Restriction enzyme	Band sizes (bp) after digestion
$S_{1+}S_{20+}S_{24}^{\#}$	Sf-sense 2 Sf-antisense 2	Sense Antisense	ATTAAATCTGCCTCGCACATTG TTGGTGGGGCAGAAATTC	56 or 62	1047 (S_{1}) 1021 (S_{20}) 1040 (S_{24})	<i>RsaI</i>	337 ¹ (S_{1}) 122 ¹ , 78 ¹ (S_{20}) 540 ¹ (S_{24})
$S_{2}^{\#}$	OWB122 OWB123	Sense Antisense	GTTCAAACGCTGACTTATGCG GGTTTGGTTCCTTACCAATGG	64	449	<i>EcoRV</i>	349+100
$S_{3}^{\#}$	OWB134 OWB145	Sense Antisense	ATGGATCCGAATTCCTGAAACAAAYATTAATCAATG TAGAATTCATTTGCTAGGGACATCGATC	56	375	<i>PstI</i>	226+149
$S_{4+}S_{16a+}S_{16b}$ $+S_{16c}^{\#}$	FTC5 OWB249	Sense Antisense	ATGAATCCCAATACAGAACGAGA ATGAATTCATCTATGAAATGTGCTCTG	58	278	<i>TagI</i>	195+83 (S_{4}) 241+37 (S_{16a} , S_{16c}) 197+44+37 (S_{16b})
$S_{5}^{\#}$	S5-sense S5-antisense	Sense Antisense	TATCCTATCCAAAGCCAAATATCAAT CATCCTATATATGGATAAATGGTCAACCG	56	363	<i>EcoRV</i>	235+128
$S_{6a+}S_{6b+}S_{21}$ $+S_{30}$ (S_{28}) [†]	FTQ ^Q Q ^Q ^m anti-1/ ^m MIWPNV ^m	Sense Antisense	TTTACGCAGCAATATCAG ACGATCGCCAAAT [‡] /CATT	48	367 ($S_{6a,b}$) 369 (S_{28}) 375 (S_{21}) 378 (S_{30})	<i>MluI</i>	367 (S_{6a}) 251+116 (S_{6b}) 369 (S_{28}) 257+118 (S_{21}) 378 (S_{30})
$S_{7}^{\#}$	OWB126 OWB127	Sense Antisense	GCCTTCAGACTCGAATGGACA TGGCATTTACAATATCTTACC	52	440	<i>AccI</i>	228+212
$S_{8}^{\#}$	OWB154 OWB155	Sense Antisense	CAGCCGGCTCTCTGCCACTT CGGTTCGATCGAGTACGTTG	62	343	<i>EcoRI</i>	212+131
$S_{10+}S_{3}^{\#}$	Si-sense Si-antisense	Sense Antisense	AACAATCTTAAAGCCAGC GGTTTCTTATAGTCGATACATTG	60	282	<i>EheI</i>	185+97 (S_{10})
$S_{1+}S_{20+}S_{24}^{\#}$	Sf-sense 3 Sf-antisense 2	Sense Antisense	ACGATCATGAAGGCTTCTGGCG TTGGTGGGGCAGAAATTC	56	370	<i>SnaBI</i>	204+164 (S_{24})
$S_{25}^{\#}$	Sz-sense Sz-antisense	Sense Antisense	TTGTCCTGCTCCACTGTGGG GTAACATCCAAGGTGTGTGTT	60	198	<i>BamHI</i>	96+92
$S_{26}^{\#}$	FTC14 FTC9	Sense Antisense	GAAGATGCCATACGCAATGG ATGAATTCCTTAATACCGAATATTTGGCG	55	193	<i>KpnI</i>	170+23
$S_{28}^{\#}$	Se-sense Se-antisense	Sense Antisense	AAACGTCCTGCAATCTCG ATCGTGATCCTTGTGTGGT	60	227	<i>KpnI</i>	136+91
$S_{30+}S_{21}^{\#}$	St-sense St-antisense	Sense Antisense	CAATAGATAACGAGAACCAC CAATCTATGAAATGTCTCC	48	259	<i>RsaI</i>	214+45 (S_{30} , S_{21})

[‡]Data from Matsumoto et al. (1999b) and Kitahara et al. (2000); [†]Data from Broothaerts et al. (1995); [‡]Data from Verdoodt et al. (1998), Van Nerum et al. (2001) and Matsumoto and Furusawa (2005); [†]Data from Matsumoto et al. (1999a); [‡]Unpublished results; [†]Data from Janssens et al. (1995) and Matsumoto et al. (1999a); [‡]Data from Janssens et al. (1995); [†]Data from Kitahara and Matsumoto (2002a); [‡]Data from Kitahara et al. (2000); [†]Data from Kitahara and Matsumoto (2002b); [‡]Data from Matsumoto and Kitahara (2000); [†]Data from Matsumoto et al. (2003c); [‡]Other S-RNase alleles are amplified in different sizes; ¹Specific band(s) are shown.

Matching of alphabetical with numerical S-RNase allele designations.

During the last 12 years, S-RNase alleles (variants of a given polymorphic gene at the S-locus) encoding S_{2-} , S_{3-} , S_{4-} , S_{5-} , S_{7-} , S_{9-} , S_{10-} , S_{24-} , S_{26-} , S_{27a-} , S_{27b-} , S_{30} (S_{28})⁻ and S_{29} -RNase allele have been isolated in Europe (Broothaerts et al., 1995; Janssens et al., 1995; Matityahu et al., 2005; Schneider et al., 2001; Van Nerum et al., 2001; Verdoodt et al., 1998); the S_{1-} , S_{4-} , S_{6-} , S_{7-} , S_{8-} , S_{9-} , S_{10} (equivalent to i)⁻, S_{1-} and S_{25} (equivalent to z)⁻ RNase allele has also been isolated in Japan (Kitahara et al., 1999, 2000; Kitahara and Matsumoto, 2002a, b; Matsumoto et al., 1999b, 2000, 2001a, b; Matsumoto and Kitahara, 2000; Sassa et al., 1996). Comparing the sequence information and S-genotypes of the same cultivars between Europe and Japan, we have succeeded in matching the S_{a-} , S_{b-} , S_{c-} , S_{d-} , S_{e-} , S_{f-} , S_{g-} , S_{h-} , S_{i-} and S_{z-} RNase allele in Japan with the S_{2-} , S_{3-} , S_{9-} , S_{7-} , S_{28-} , S_{1-} , S_{20-} , S_{24-} , S_{10-} and S_{25-} RNase allele in Europe (Table 2). To reconcile the S-RNase allele designations with the numerical ones, we used an alphabetical sign after the numerical number in some S-RNase alleles. As a result, some S-RNase alleles, e.g., S_{7a} and S_{7b} , and S_{9a} and S_{9b} , now correspond to S_7 and S_d , and S_9 and S_c , respectively (Table 2). In these cases, though nucleotide polymorphisms were observed between the two alleles, both were functionally identical. Using the numerical designations, variable alphabetical designations within one cultivar in Japan such as S_{d_1} , S_{d_2} , S_e and S_g for 'Delicious' and 'Starking Delicious' (Sport of 'Delicious'), S_d and S_e for 'Jonathan', and S_d and S_g for 'Mutsu', which were caused by designations at the convenience of the individual researchers, were reconciled so that now correspond to S_{28} for 'Delicious' and 'Starking Delicious', S_7 for 'Jonathan', and S_{20} for 'Mutsu' (Table 2).

S-RNase allele nomenclature and designation in apple

Table 2. S-haplotypes and S-RNase alleles of apple cultivars and a species

Proposed S-haplotype	Proposed S-RNase allele	Former S-RNase allele	Cultivar / Species	S-RNase alleles			Database Accession #	Reference
1	1	1, f	Fuji	1	9b		D50837	Sassa et al., 1996
2	2	2, a	Golden Delicious	2	3		U12199	Broothaerts et al., 1995
3	3	3, b	Golden Delicious	2	3		U12200	Komori et al., 1998a, b Broothaerts et al., 1995
4	4	4	Gravenstein	4	11	20	AF327223	Komori et al., 1998a, b Kobel et al., 1939 Boskovic and Tobutt, 1999 Van Nerum et al., 2001
5	5	5	Queen's Cox	5	9a		U19791	Janssens et al., 1995
6	6a	6	Oetwiler Reinette	3	6a		AB094495	Kobel et al., 1939
		12	Citron d'Hiver	3	5	6a	AB105061	Matsumoto et al., 2003c Boskovic and Tobutt, 1999
	6b	17	Blenheim Orange	1	3	6b	AB105062	Boskovic and Tobutt, 1999 Matsumoto et al., 2003c
		19	Bohnapfel	6b	9	16	AB094493	Boskovic and Tobutt, 1999 Matsumoto et al., 2003c
7	7a	7	Idared	3	7a		U19792	Janssens et al., 1995
	7b	d, e ^z	Akane	7b	24		AB032246	Kitahara et al., 2000
8	8	8	Ontario Reinette	1	8			Kobel et al., 1939
9	9a	9	Queen's Cox	5	9a		U19793	Janssens et al., 1995
	9b	c	Fuji	1	9b		D50836	Sassa et al., 1996
10	10a	10, 25 ^y	Prima	2	10a		AF327221	Van Nerum et al., 2001
	10b	i	McIntosh	10b	25		AB052683	Kitahara and Matsumoto, 2002a
11	11	10/11, 13	Gravenstein	4	11	20	AB105060	Kobel et al., 1939 Boskovic and Tobutt, 1999 Matsumoto et al., 2003c
		14	Jacques Lebel	1	3	11	AB094492	Kobel et al., 1939 Boskovic and Tobutt, 1999 Matsumoto et al., 2003c
15	15	15	Kaiserapfel	1	10	15		Boskovic and Tobutt, 1999
16	16a	27, 27a	Baskatong	16a	26		AF016919	Verdoordt et al., 1998 Van Nerum et al., 2001
	16b	22, 27b	Alkmene	5	16b		AF327222	Boskovic and Tobutt, 1999 Van Nerum et al., 2001
		23, 27b	Delbard Jubile	2	16b		AF327222	Boskovic and Tobutt, 1999 Van Nerum et al., 2001
		25 ^x , 27b	Merlijin	3	16b		AF327222	Boskovic and Tobutt, 1999 Van Nerum et al., 2001
	16c	16, 27a ^w	Bohnapfel	6b	9	16c	AB126322	Boskovic and Tobutt, 1999 Broothaerts, 2003
18	18	18	Menzauer Jagerapfel	3	5	18		Boskovic and Tobutt, 1999
20	20a	g, d ^v	Indo	7	20a		AB019184	Matsumoto et al., 1999b
	20b	g'	<i>Malus transitoria</i>	20b	30	?	AB096138	Matsumoto et al., 2001a
21	21	21	Ribston Pippin	1	9	21	AB094494	Boskovic and Tobutt, 1999 Matsumoto et al., 2003c
23	23	10 ^u	Granny Smith	3	23		AF239809	Schneider et al., 2001
24	24a	24	Braeburn	9	24a		AF016920	Verdoordt et al., 1998
	24b	h	Akane	7	24b		AB032247	Kitahara et al., 2000
25	25	25, z	McIntosh	10b	25		AB062100	Kitahara and Matsumoto, 2002a, b
26	26	26	Baskatong	16	26		AF016918	Verdoordt et al., 1998
28	28	19, 30, e, d ^t , d ^e , e ^t , g ^t	Delicious	9	28		AB035273	Matsumoto and Kitahara, 2000 Broothaerts, 2003
		28, 30	Red Delicious	9	28		AF201748	Schneider et al., 2001
29	29	29	Anna	3	29		AY039702	Matityahu et al., 2005
30	30	t	<i>Malus transitoria</i>	20b	30	?	AB035928	Matsumoto et al., 2000 Matsumoto et al., 2001b

^zS_e-allele in 'Hokuto', 'Toko' and 'Jonathan', ^yS₂₅-allele in 'Telamon', ^xS₂₅-allele in 'Merlijin', ^wS_{27a}-allele in 'Bohnapfel',
^vS_d-allele in 'Mutsu', ^uS₁₀-allele in 'Granny Smith', ^tS_d, S_{de}- and S_g-allele in 'Delicious' etc.

Re-numbering of numerical S-RNase allele designations.

Within the 21 numerical S-RNase allele designations in Europe, 11 S-RNase alleles (S_{12} to S_{19} , S_{21} to S_{23}) were identified in only one cultivar by stylar protein analyses (Bošković and Tobutt, 1999). We indicated that S_{12} might be identical to S_6 (Matsumoto et al., 2003c), and S_{17} and S_{19} are functionally identical (Matsumoto et al., 2006a). Although the partial genomic sequences of S_{12} and S_6 were slightly different from those of S_{17} and S_{19} , all of them have an identical rosaceous hypervariable (RHV) region at the nucleotide level. Since that region is related with controlling allelic specificity, we re-numbered S_6 and S_{12} as S_{6a} , and S_{17} and S_{19} as S_{6b} (Table 2; Matsumoto et al., 2003c).

Although Broothaerts (2003) has re-numbered $S_{30(28)}$ as S_{19} , $S_{30(28)}$ and S_{19} are different alleles both structurally and functionally (Matsumoto et al., 2003c, 2006a). We proposed the simplification of $S_{30(28)}$ to S_{28} (Table 2; Matsumoto et al., 2003c).

We also confirmed that S_{11} corresponded to S_{13} . As S_{13} and S_{14} were identical at the determined nucleotide sequence level, we re-numbered S_{13} and S_{14} as S_{11} (Table 2; Matsumoto et al., 2003c).

S_{27a} was re-numbered as S_{16} since S_{27a} and S_{28} were present in ‘Bohnappel’ and corresponded to S_{16} and S_{19} (Broothaerts, 2003). However, as I mentioned, S_{28} and S_{19} are different S-RNase alleles. We examined whether or not S_{27a} and S_{16} are the same. As the partial deduced amino acid sequences, including the RHV region of S_{16} , S_{16-27a} and S_{22-27b} , are identical, these three alleles are thought to be functionally identical. We re-numbered S_{16} in ‘Bohnappel’, S_{16-27a} , and S_{22-27b} as S_{16c} , S_{16a} and S_{16b} , respectively, (Table 2; Matsumoto and Furusawa, 2005).

Conclusions

Accurate knowledge of the S-RNase allele in each apple cultivar is one of the most important things for apple breeders and growers to understand to avoid cross-incompatibility. According to the nucleotide sequence information of S-RNase alleles and cross-pollination tests, we modified the alphabetical S-RNase allele designations in Japan to match their numerical counterparts in Europe. We also classified the numerical S-RNase alleles, and finally identified each of the 31 S-RNase alleles with their unique designation.

Acknowledgements

This research was supported by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (No. 17580024) and the Ministry of Education, Culture, Sports, Science and Technology of Japan (No. 15208004).

Literature Cited

- Bošković, R. and K.R. Tobutt. 1999. Correlation of stylar ribonuclease isoenzymes with incompatibility alleles in apple. *Euphytica* 107:29-43.
- Broothaerts, W., G.A. Janssens, P. Proost, and W.F. Broekaert. 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, p.28-57. In: R. Frankel, G.A.E. Gal, and H.F. Linskens (eds.). *Monographs on theoretical and applied genetics*. Springer-Verlag.
- Janssens, G.A., I.J. Goderis, W.F. Broekaert, and W. Broothaerts. 1995. A molecular method for S-

- allele identification in apple based on allele-specific PCR. *Theor. Appl. Genet.* 91:691-698.
- Kitahara, K., H. Fukui, J. Soejima, and S. Matsumoto. 1999. Cloning and sequencing of a new S-gene 'Sg-RNase' (Accession No. AB019184) from *Malus x domestica* Borkh. 'Indo'. (PGR99-046) *Plant Physiol.* 119:1567.
- Kitahara, K., J. Soejima, H. Komatsu, H. Fukui, and S. Matsumoto. 2000. Complete sequences of the S-genes, Sd- and Sh-RNase cDNA in apple. *HortScience* 35:712-715.
- Kitahara, K., and S. Matsumoto. 2002a. Sequence of the S_{10} cDNA from 'McIntosh' apple and a PCR-digestion identification method. *HortScience* 37:187-190.
- Kitahara, K., and S. Matsumoto. 2002b. Cloning of the S_{25} cDNA from 'McIntosh' apple and an S_{25} -allele identification method. *J. Hortic. Sci. Biotechnol.* 76:163-166.
- Kobel, F., P. Steinegger, and J. Anliker. 1939. Weitere Untersuchungen über die Befruchtungsverhältnisse der Apfel- und Birnsorten. *Landw. Jahrb. Schweiz* 53:160-191.
- Komori, S., J. Soejima, Y. Ito, H. Bessho, K. Abe, and N. Kotoda. 1998a. Analyses of the self-incompatibility genotypes in some apple cultivars. *J. Jpn. Soc. Hort. Sci.* 67:917-926.
- Komori, S., J. Soejima, K. Kudo, H. Kyotani, K. Abe, N. Kotoda, H. Komatsu, Y. Ito, and H. Bessho. 1998b. Determination of S-allele genotypes of 21 apple cultivars and strains and confirmation of 45 others by controlled crosses. *J. Jpn. Soc. Hort. Sci.* 67:880-889.
- Komori, S., J. Soejima, K. Abe, H. Kato, N. Kotoda, and K. Kudo. 1999. Analyses of S-allele genotypes of 'McIntosh', 'Kitakami', 'Worcester Pearmain', etc. *J. Japan. Soc. Hort. Sci.* 68 (Suppl. 2):94.
- Komori, S., J. Soejima, K. Abe, N. Kotoda, and H. Kato. 2000. Analyses of S-allele genotypes and genetic diversity in the apple. *Acta Hort.* 538:83-86.
- Matityahu, A., R.A. Stern, D. Schneider, and M. Goldway. 2005. Molecular identification of a new apple S-RNase-S29-cloned from 'Anna', a low-chilling-requirement cultivar. *HortScience* 40:850-851.
- Matsumoto, S., S. Komori, K. Kitahara, S. Imazu, and J. Soejima. 1999a. S-genotypes of 15 apple cultivars and self-compatibility of 'Megumi'. *J. Jpn. Soc. Hort. Sci.* 68:236-241.
- Matsumoto, S., K. Kitahara, S. Komori, and J. Soejima. 1999b. A new S-allele in apple 'Sg', and its similarity to the 'Sf' allele from Fuji. *HortScience* 34:708-710.
- Matsumoto, S., and K. Kitahara. 2000. Discovery of a new self-incompatibility allele in apple. *HortScience* 35:1329-1332.
- Matsumoto, S., M. Suzuki, K. Kitahara, and J. Soejima. 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., K. Kitahara, H. Komatsu, and J. Soejima. 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., S. Hayashi, K. Kitahara, and J. Soejima. 2001b. Genomic DNA sequences encoding *Malus x domestica* Borkh. "Akane", "Delicious" and *Malus transitoria* S-RNases. *DNA Seq.* 12:381-383.
- Matsumoto, S., K. Kitahara, Y. Furusawa, J. Soejima, H. Komatsu, and H. Fukui. 2003a. S-allele genotype of apple cultivars and selections. *Acta Hort.* 622:389-396.
- Matsumoto, S., Y. Furusawa, H. Komatsu, and J. Soejima. 2003b. S-allele genotypes of apple pollenizers, cultivars and lineages including those resistant to scab. *J. Hortic. Sci. Biotechnol.* 78:634-637.
- Matsumoto, S., Y. Furusawa, K. Kitahara, and J. Soejima. 2003c. 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. *Plant Biotech.*

20:323-329.

- Matsumoto, S., and Y. Furusawa. 2005. Genomic DNA sequence of $S_{16c(-16)}$ -RNase in apple: re-numbering of $S_{16(-27a)}$ - and $S_{22(-27b)}$ -allele to S_{16a} and S_{16b} . *Sci. Rep. Fac. Educ. Gifu Univ. (Nat. Sci.)* 29:7-12.
- Matsumoto, S., K. Kitahara, H. Komatsu, and K. Abe. 2006. Cross-compatibility of apple cultivars possessing S-RNase alleles of similar sequence. *J. Hortic. Sci. Biotechnol.* 81: 934-936.
- Matsumoto, S., T. Eguchi, H. Bessho, and K. Abe. 2007. Determination and confirmation of S-RNase genotypes of apple pollinators and cultivars. *J. Hortic. Sci. Biotechnol.* 82: 323-329.
- Sassa, H., H. Hirano, and H. Ikehashi. 1992. Self-incompatibility-related RNases in style of Japanese pear (*Pyrus serotina* Rehd.). *Plant Cell Physiol.* 33:811-814.
- Sassa, H., H. Hirano, and H. Ikehashi. 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., T. Nishio, Y. Kowayama, H. Hirano, T. Koba, and H. Ikehashi. 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., H. Hirano, T. Nishio, and T. Koba. 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., R.A. Stern, D. Eisikowitch, and M. Goldway. 2001. Analysis of S-alleles by PCR for determination of compatibility in the 'Red Delicious' apple orchard. *J. Hort. Science Biotechnol.* 76:596-600.
- Van Nerum, I., M. Geerts, A. Van Haute, J. Keulemans, and W. Broothaerts. 2001. Re-examination of the self-incompatibility genotype of apple cultivars containing putative 'new' S-alleles. *Theor. Appl. Genet.* 103:584-591.
- Verdoodt, L., A. Van Haute, I.J. Goderis, K. De Witte, J. Keulemans, and W. Broothaerts. 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.