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}

Shogo Matsumoto and Yui Furusawa

Summary

We determined a genomic DNA sequence including a hypervariable HVa(RHV) region and an intron of the S_{16} -RNase in 'Bohnapfel' apple. The deduced amino acid sequence of the S_{16} -RNase in 'Bohnapfel' ($S_{6b}S_9S_{16}$ genotype)' completely matched that of $S_{22(=27b)}$ -RNase in 'Alkmene' ($S_5S_{22=27b}$ genotype)² and $S_{16(=27a)}$ -RNase in 'Baskatong' ($S_{16=27a}S_{26}$ genotype)² however, the nucleotide sequences among them were different. We re-numbered $S_{16(=27a)}$ in 'Baskatong', $S_{22(=27b)}$ in 'Alkmene' and S_{16} in 'Bohnapfel' as S_{16a} , S_{16b} and S_{16c} , respectively.

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

Apple (*Malus x domestica* Borkh .) has a *S*-RNase-based gametophytic self-incompatibility controlled by the multi-allelic *S*-locus $\stackrel{3)}{,}$ *S*-RNases were detected in the style of apple, and cDNAs of 10 and 11 *S*-RNases from corresponding *S*-alleles have been cloned in Japan ^{4 · 10} and in Europe $\stackrel{11 - 14}{,}$ respectively. Knowledge of the *S*-genotypes of apple is very important for selection of compatible pollinators and facilitation of breeding programs. A PCR-digestion method for *S*-allele identification has been developed using each *S*-RNase genespecific primer set $\stackrel{5 - 7.9 - 15}{,}$

S-allele designations are confusing; for instance, the *S*-genotype of 'Bohnapfel' was identified as $S_9S_8S_9$; x, y > 11 and $S_9S_{16}S_{19}$ by cross pollination³ and stylar protein analysis ¹⁶, respectively. Recently, Broothaerts² found that the S_{27a} and S_{28} were present in 'Bohnapfel' and corresponded to S_{16} and S_{19} , respectively. From the results, S_{27a} and S_{28} were re-numbered to S_{16} and S_{19} respectively ². However, Matsumoto *et al* ¹. found that the S_{19} and S_{28} were different alleles, and the S_{66} instead of S_{19} was present in 'Bohnapfel'. From the results, S_{28} was left unchanged and S_{19} was re-numbered to S_{66} ¹. This confusion was caused by inadequate analysis; i.e., the correspondence between S_{28} and S_{19} was from *S*-allele-specific PCR-digestion analysis, not sequence analysis ². As the correspondence between S_{28} and S_{19} by PCR analysis was contradicted, we determined the partial genomic DNA sequences of S_{16} -RNase in 'Bohnapfel' to confirm the correspondence between S_{27a} and S_{16} .

Alignment of the nucleotide and deduced amino acid sequences for the exons of S_{16} -RNase in 'Bohnapfel' (AB126322) with those of $S_{16(=27a)}$ -and $S_{22(=27a)}$ -RNase determined by Van Nerum *et al*.¹⁴⁾ showed extremely high similarity (Fig .1). Deduced amino acid sequences of S_{16} , $S_{16=27a}$ and $S_{22=27b}$ are identical, whereas sequence comparison revealed that the S_{16} only deviates at three and two point mutations from $S_{16=27a}$ (nucleotide positions 180 from A to G , 303 from T to C and 405 from T to G) and $S_{22=27b}$ (nucleotide positions 180 from A to G and 321 from G to T) respectively (Fig .1). All S-RNases in apple contains one intron at the same location within the RHV region (Fig .1; Matsumoto *et al*.¹⁷⁾). The S_{16} -RNase also contained an intron deduced from the pres-

ence of plant 5 and 3 splice site consensus sequences at corresponding sites (Fig .1). The S_{16} intron size was 2218 bp (Fig 2), which was longest within apple S-RNases. Previously, apple S-alleles were

MDS16C MDS16a MDS16b	CCG CCG CCG	GCT GCT GCT	GTC GTC GTC	TGC TGC TGC	AAC AAC AAC	TTT TTTT TTTT	TAA' 'AA'T 'AA'T	CCT CCT	ACT ACT ACT	CCA CCA CCA	TGT. TGT. TGT.	AAG AAG AAG	GAT GAT GAT	CCT CCT CCT	'ACT 'ACT ACT	GAC GAC GAC	AAG AAG AAG	TTG TTG	TTT TTT TTT	'ACG 'ACG 'ACG	60
MDS16c	P	А	V	С	Ν	F	Ν	Ρ	Т	Ρ	С	Κ	D	Ρ	Т	D	Κ	L	F	Т	20
MDS16a	P	A	V	С	Ν	F	Ν	Ρ	Т	Ρ	С	K	D	Ρ	Т	D	K	L	F	Т	
MDS16b	P	A	V	С	Ν	F	Ν	Ρ	Т	Ρ	С	K	D	Ρ	Т	D	K	L	F		
			С	2																	
MDS16c	GTT	CAC	GGT	TTG	тGG	CCT	TCA	AAC	AAC	GTA	GGA	GGT	GAC	CCA	GAA	тст	тGC	AAG	¦ата	AGG	120
MDS16a	GTT	CAC	GGT	TTG	TGG	CCT	TCA	AAC	AAC	GTA	GGA	GGT	GAC	CCA	GAA	TCT	TGC	AAG	ATA	AGG	
MDS16b	GTT	CAC	GGT	TTG	TGG	CCT	TCA	AAC	AAC	GTA	GGA	GGT	GAC	CCA	GAA	TCT	TGC	AAG	ATA	AGG	
MDS16c	V	Н	G	L	W	Ρ	S	Ν	Ν	V	G	G	D	Ρ	Е	S	С	K	I	R	40
MDS16a	V	Η	G	L	W	Ρ	S	Ν	Ν	V	G	G	D	Ρ	Е	S	С	K	I	R	
MDS16b	V	Η	G	L	W	Ρ	S	Ν	Ν	V	G	G	D	Ρ	Е	S	С	K	I	R	
						RHV	7														
MDS16c	AAT	CAT	CGA	ACG	AGA	GCA	AAA	GCA	CTC	GAA	ccd	CAG	TTG	GAA	ATT	ATT	TGG	CCG	JAAC	GT G	180
MDS16a	AAT	CAT	CGA	ACG	AGA	GCA	AAA	GCA	CTC	GAA	ccd	CAG	TTG	GAA	ATT	ATT	TGG	CCG	AAC	GTA	
MDS16b	AAT	CAT	CGA	ACG	AGA	GCA	AAA	.GCA	CTC	GAA	ccd	CAG	TTG	GAA	ATT	ATT	TGG	CCC	AAC	GTA	
MDS16c	Ν	Η	R	Т	R	А	Κ	А	L	Е	Ρ	Q	L	Е	I	I	W	Ρ	Ν	V	60
MDS16a	Ν	Η	R	Т	R	А	Κ	А	L	Е	Р	Q	L	Е	I	I	W	Ρ	Ν	V	
MDS16b	N	Η	R	Т	R	Α	K	Α	L	Е	Р	Q	L	Е	I	I	W	Ρ	Ν	V	
																	C3				
MDS16c	CTC	GAT	CGA	ACC	AAT	CAT	ACA	GCC	TTC	TGG	CGT.	AGA	CAG	TGG	ATA	AAA	CAT	'GGC	'ACT	TGT	240
MDS16a	CTC	GAT	CGA	ACC	AAT	'CAT	ACA	GCC	TTC	TGG	CGT	AGA	CAG	TGG	ATA	AAA	CAT	'GGC	ACT	TGT	210
MDS16b	CTC	GAT	CGA	ACC	AAT	CAT	ACA	GCC	TTC	TGG	CGT	AGA	CAG	TGG	ATA	AAA	CAT	GGC	ACT	TGT	
MDS16c	L	D	R	Т	Ν	Н	Т	А	F	W	R	R	Q	W	I	K	Н	G	Т	С	80
MDS16a	L	D	R	т	Ν	Н	т	А	F	W	R	R	õ	W	I	K	Н	G	Т	С	
MDS16b	L	D	R	Т	N	Н	Т	А	F	W	R	R	õ	W	I	K	Η	G	Т	С	
10010																					
MDS16C	GGG	TAT	CCC	ACA	ATA	CAG	AAC	'GAG	AAT	GAT	TAC	TTC	GAA	ACA	.GTA	GTC	AAA	ATG	TAC	ATA	300
MDS16C MDS16a	GGG GGG	TAT TAT	CCC	ACA ACA	ATA ATA	CAG	AAC AAC	'GAG 'GAG	AAT AAT	GAT GAT	TAC' TAC'	TTC TTC	GAA GAA	ACA ACA	.GTA .GTA	GTC GTC	AAA AAA	ATC ATC	STAC STAC	ATA ATA	300
MDS16c MDS16a MDS16b	GGG GGG GGG	TAT TAT TAT	CCC CCC	ACA ACA	ATA ATA ATA	CAG CAG CAG	AAC AAC AAC	'GAG 'GAG 'GAG	AAT AAT AAT	GAT GAT GAT	TAC' TAC' TAC'	TTC TTC TTC	GAA GAA GAA	ACA ACA ACA	.GTA .GTA .GTA	GTC GTC GTC	AAA AAA AAA	ATC ATC	STAC STAC STAC	ATA ATA ATA	300
MDS16c MDS16a MDS16b MDS16c	GGG GGG GGG G	TAT TAT TAT Y	CCC CCC CCC P	ACA ACA ACA T	ATA ATA ATA I	ICAG ICAG ICAG Q	AAC AAC AAC N	GAG GAG GAG E	AAT AAT AAT N	GAT GAT GAT D	TAC' TAC' TAC' Y	TTC TTC TTC F	GAA GAA GAA E	ACA ACA ACA T	.GTA .GTA .GTA .V	GTC GTC GTC V	AAA AAA AAA K	ATO ATO ATO M	TAC TAC TAC TAC	ATA ATA ATA I	300
MDS16c MDS16a MDS16b MDS16c MDS16a	GGG GGG GGG G G	TAT TAT TAT Y Y Y	CCC CCC CCC P P P	ACA ACA ACA T T	АТА АТА АТА І І	ICAG ICAG ICAG Q Q	AAC AAC AAC N N	GAG GAG GAG E E	AAT AAT AAT N N	GAT GAT GAT D D	TAC' TAC' TAC' Y Y Y	TTC TTC TTC F F	GAA GAA GAA E E	ACA ACA ACA T T	.GTA .GTA .GTA .V V V	GTC GTC GTC V V V	AAA AAA AAA K K	ATC ATC ATC M M	TAC TAC TAC TAC Y Y Y	ATA ATA ATA I I I	300 100
MDS16c MDS16a MDS16b MDS16c MDS16a MDS16b	GGG GGG GGG G G G G	TAT TAT TAT Y Y Y Y	CCC CCC P P P	ACA ACA ACA T T T	АТА АТА АТА І І І	ICAG ICAG ICAG Q Q Q	AAC AAC AAC N N N	GAG GAG GAG E E E	AAT AAT AAT N N N	GAT GAT GAT D D D	TAC TAC TAC Y Y Y Y	TTC TTC TTC F F F	GAA GAA GAA E E E	ACA ACA ACA T T T	.GTA .GTA .GTA V V V V	GTC GTC GTC V V V V	AAA AAA AAA K K K	ATC ATC ATC M M M M	TAC TAC TAC Y Y Y Y Y	ATA ATA ATA I I I I	300 100
MDS16c MDS16a MDS16b MDS16c MDS16a MDS16b	GGG GGG GGG G G G G	TAT TAT TAT Y Y Y Y	CCC CCC P P P	ACA ACA ACA T T T	ATA ATA ATA I I I	ICAG ICAG ICAG Q Q Q Q	AAC AAC AAC N N N	GAG GAG GAG E E E	AAT AAT AAT N N N	GAT GAT D D D	TAC TAC TAC Y Y Y Y RC	TTC TTC TTC F F F	GAA GAA GAA E E E	ACA ACA ACA T T T	.GTA .GTA .GTA V V V V	GTC GTC GTC V V V V	AAA AAA K K K K	ATC ATC ATC M M M	STAC STAC STAC Y Y Y Y	ATA ATA ATA I I I I	300 100
MDS16c MDS16a MDS16b MDS16c MDS16a MDS16b MDS16c	GGG GGG G G G G G ACC	TAT TAT TAT Y Y Y GAG	CCC CCC P P P P	ACA ACA T T T CAA	ATA ATA I I I AAC	CAG CAG Q Q Q Q	AAC AAC N N N	GAG GAG E E E ZAAA	AAT AAT N N N ATC	GAT GAT D D D CTC	TAC TAC TAC Y Y Y Y RC4 TCA	TTC TTC F F F 4 AAT	GAA GAA E E E GCA	ACA ACA T T T AAG	GTA GTA GTA V V V V	GTC GTC GTC V V V V	AAA AAA K K K CCC	ATO ATO M M M GAC	TAC TAC TAC Y Y Y Y	ATA ATA I I I I ATA	300 100 360
MDS16c MDS16b MDS16c MDS16c MDS16b MDS16c MDS16c MDS16a	GGG GGG G G G G G A C C A C T	TAT TAT TAT Y Y Y GAG GAG	CCC CCC P P P AAA	ACA ACA T T T CAA	ATA ATA I I AAC AAC	CAG CAG Q Q Q Q Q C C C C C C C C C C C C C C	AAC AAC N N N	GAG GAG E E E ZAAA	AAT AAT N N N ATC	GAT GAT D D D CTC CTC	TAC TAC Y Y Y RC TCA	TTC TTC F F AAT	GAA GAA E E E GCA GCA	ACA ACA T T T AAG AAG	GTA GTA GTA V V V ZATT	GTC GTC V V V GAA	AAA AAA K K K CCC CCC	ATC ATC ATC M M M GAC	TAC TAC TAC Y Y Y ZGGG	ATA ATA I I I ATA ATA	300 100 360
MDS16c MDS16b MDS16c MDS16c MDS16b MDS16c MDS16c MDS16a MDS16b	GGG GGG G G G G G A C C A C C A C C	TAT TAT Y Y Y GAG GAG GAG	CCC CCC P P P AAA AAA	ACA ACA T T CAA CAA	ATA ATA I I AAC AAC	CAG CAG Q Q Q Q C C C C C C C C C C C C C C C	AAC AAC N N N TCT	GAG GAG E E ZAAA AAA	AAT AAT N N N ATC ATC	GAT GAT D D CTC CTC CTC	TAC TAC Y Y Y RC TCA TCA	TTC TTC F F A AAT AAT	GAA GAA E E GCA GCA GCA	ACA ACA T T T AAG AAG	GTA GTA CTA V V V ZATT	GTC GTC V V V GAA GAA	AAA AAA K K K CCC CCC	ATC ATC M M GAC GAC	TAC TAC TAC Y Y Y SGGG	ATA ATA I I I ATA ATA ATA	300 100 360
MDS16c MDS16b MDS16c MDS16c MDS16b MDS16c MDS16a MDS16b MDS16b MDS16b	GGG GGG G G G G G G A C T	TAT TAT Y Y Y GAG GAG GAG	CCC CCC P P P AAAA AAAA AAAA K	ACA ACA T T T CAA CAA Q	ATA ATA I I AAC AAC AAC	CAG CAG Q Q Q Q Q C GTC GTC SGTC	AAC AAC N N N TCT	GAG GAG E E P AAA AAA K	AAT AAT N N N ATC ATC ATC	GAT GAT D D CTC CTC CTC L	TAC TAC Y Y Y Y TCA TCA TCA S	TTC TTC F F A AAT AAT AAT N	GAA GAA E E GCA GCA GCA A	ACA ACA T T AAG AAG AAG	GTA GTA V V V Z ATT ATT I	GTC GTC V V V GAA GAA GAA E	AAA AAA K K K CCC CCC CCC	ATC ATC M M GAC GAC GAC	TAC TAC TAC Y Y Y Y SGGG CGGG G GGG G	ATA ATA I I I ATA ATA ATA I	300 100 360 120
MDS16c MDS16b MDS16c MDS16c MDS16b MDS16c MDS16a MDS16b MDS16b MDS16c MDS16c MDS16c	GGG GGG G G G G G G A C T A C C T T	TAT TAT Y Y GAG GAG GAG E E	CCC CCC P P P AAAA AAA AAA K K	ACA ACA T T T CAA CAA Q Q	ATA ATA I I AAC AAC AAC N N	CAG CAG Q Q Q Q Q C C C C C C C C C C C C C C	AAC AAC N N N TCT TCT TCT S S	GAG GAG E E E PAAA AAA K K K	AAT AAT N N N ATC ATC ATC I I	GAT GAT D D D CTC CTC CTC L L	TAC TAC Y Y Y Y TCA TCA TCA S S	TTC TTC F F AAT AAT AAT N N	GAA GAA E E E GCA GCA GCA A A A	ACA ACA T T AAG AAG AAG K K	GTA GTA V V V V ZATT ATT I I	GTC GTC V V V GAA GAA GAA E E	AAA AAA K K K CCC CCC CCC P P P	ATC ATC M M M GAC GAC GAC GAC D D	TAC TAC TAC Y Y Y Y S GGG C GGG C G G G G G	ATA ATA I I I ATA ATA ATA I I I	300 100 360 120
MDS16c MDS16b MDS16c MDS16c MDS16b MDS16c MDS16a MDS16b MDS16c MDS16c MDS16c MDS16c MDS16c MDS16a MDS16a	GGG GGG G G G G G G A C C A C C T T T T	TAT TAT Y Y GAG GAG GAG E E E	CCC CCC P P P AAAA AAA K K K K	ACA ACA T T CAA CAA CAA Q Q Q	ATA ATA I I AAC AAC AAC N N N N	CAG CAG Q Q Q Q Q C GTC SGTC SGTC V V V V	AAC AAC N N N TCT TCT S S S	GAG GAG E E ZAAA AAA K K K K	AAT AAT N N N ATC ATC ATC I I I	GAT GAT D D CTC CTC CTC L L L	TAC TAC Y Y Y TCA TCA TCA S S S	TTC TTC F F AAT AAT AAT N N N	GAA GAA E E GCA GCA GCA A A A A	ACA ACA T T AAG AAG AAG K K K	GTA GTA V V V V ATT ATT I I I I	GTC GTC V V V GAA GAA GAA E E E	AAA AAA K K K CCCG CCG P P P	ATC ATC M M M GAC GAC GAC D D D	TAC TAC TAC TAC TAC T Y Y Y ZGGG ZGGG ZGGG ZGGG G G G G G G G	ATA ATA I I I ZATA ATA ATA I I I I	300 100 360 120
MDS16c MDS16b MDS16c MDS16a MDS16b MDS16c MDS16a MDS16b MDS16c MDS16c MDS16c MDS16a MDS16b	GGG GGG G G G G G ACC ACC T T T T	TAT TAT Y Y GAG GAG GAG GAG E E E	CCC CCC P P P AAAA AAAA K K K K	ACA ACA T T CAA CAA Q Q Q	ATA ATA I I AAC AAC AAC N N N	CAG CAG Q Q Q Q C GTC C GTC C C GTC V V V V	AACC AAC N N N TCT TCT TCT TCT S S S S	GAG GAG E E CAAA CAAA K K K K K	AAT AAT N N ATC ATC ATC I I I I	GAT GAT D D CTC CTC CTC CTC L L L	TAC TAC TAC Y Y Y Y RC4 TCA TCA S S S S	TTC TTC F F AAT AAT N N N	GAA GAA E E GCA GCA GCA A A A A	ACA ACA T T AAG AAG AAG K K K K	GTA GTA GTA V V V V ATT I ATT I I I I I I	GTC GTC V V V GAA GAA E E E E	AAA AAA K K CCCG CCCG P P P	ATG ATG M M GAC GAC GAC D D D	GTAC GTAC Y Y Y CGGG CGGG G G G G G G G G G G G	ATA ATA I I I ATA ATA ATA I I I I	300 100 360 120
MDS16c MDS16b MDS16c MDS16a MDS16b MDS16c MDS16c MDS16c MDS16c MDS16c MDS16c MDS16a MDS16c	GGG GGG G G G G G G G C ACT T T T	TAT TAT TAT Y Y GAG GAG GAG GAG E E E	CCC CCC P P P AAAA AAAA AAAA K K K	ACA ACA T T CAA CAA CAA Q Q Q Q	ATA ATA I I AAC AAC AAC AAC N N N	CAG CAG Q Q Q Q C GTC C GTC C C GTC V V V V	AAC AAC AAC N N N TCT TCT TCT TCT S S S	GAG GAG E E E AAAA AAA K K K K	AAT AAT N N ATC ATC I I I I	GAT GAT D D CTC CTC CTC CTC CTC CTC	TAC TAC TAC Y Y Y TCA TCA TCA S S S	TTC TTC F F AAT AAT AAT N N N	GAA GAA E E GCA GCA GCA A A A	ACA ACA T T AAG AAG AAG K K K K	GTA GTA GTA V V V V ATT ATT I I I I	GTC GTC V V V GAA GAA GAA E E E	AAA AAA K K K CCCG CCCG P P P P	ATG ATG ATG M M M GAC GAC D D D D	TAC TAC TAC Y Y Y Y CGGG CGGG CGGG G G G G G G	ATA ATA I I I ATA ATA I I I I	300 100 360 120
MDS16c MDS16a MDS16c MDS16a MDS16b MDS16c MDS16a MDS16b MDS16c MDS16c MDS16a MDS16b MDS16c MDS16a MDS16b	GGG GGG G G G G G G G G C ACT T T T T	TAT TAT TAT Y Y GAG GAG GAG E E E AGA	CCC CCC P P P P AAAA AAAA K K K K K	ACA ACA T T CAA CAA Q Q Q Q CTG CTG	ATA ATA I I AAC AAC N N N GCG	CAG CAG CAG Q Q Q Q C GTC C GTC V V V V V C GTC C GTC C C GTC C C GTC C C GTC C C GTC C C GTC C C GTC C C G C C C C	AACCAACCAACCAACCAACCAACCAACCAACCAACCAA	GAG GAG E E E CAAA CAAA K K K CGAA	AAT AAT N N ATC ATC ATC I I I I ATT	GAT GAT D D CTC CTC CTC CTC CTC CTC CTC CTC CT	TAC TAC TAC Y Y Y TCA TCA TCA TCA S S S ATA	TTC TTC F F AAT AAT N N N CGC	GAA GAA E E E GCA GCA A A A A A GT	ACA ACA T T AAGG AAGG K K K K GGT	GTA GTA GTA V V V V ATT I I I I ATT ATT	GTC GTC V V V GAA GAA E E E GAC GAC	AAA AAA K K K CCCC CCCC P P P P AAC	ATG ATG ATG M M M GAC GAC GAC D D D D CAAG	TAC TAC TAC TAC Y Y Y Y Y CGGG CGGG G G G G G G G G G	ATA ATA I I I ATA ATA ATA I I I I CCA	300 100 360 120 420
MDS16c MDS16a MDS16b MDS16c MDS16a MDS16c MDS16c MDS16c MDS16a MDS16b MDS16c MDS16a MDS16b	GGG GGG GGG G G G G G G G G C C T T T T	TAT TAT Y Y GAG GAG GAG GAG E E E AGA AGA	CCC CCC P P P AAAA AAAA K K K K K ACA ACA	ACA ACA T T CAA CAA Q Q Q CTG CTG CTG	ATA ATA I I AAC AAC N N N GCCG GCCG	CAG CAG Q Q Q Q CGTC CGTC CGTC V V V V V C CGTC CGTC CG	AACCAACCAACCAACCAACCAACCAACCAACCAACCAA	GAG GAG E E ZAAA CAAA K K K CGAA	AAT AAT N N ATC ATC I I I ATT ATT	GAT GAT D D D CTC CTC CTC L L L GCC GCC	TAC' TAC' Y Y RC4 TCA TCA TCA S S S ATA ATA	TTC TTC F F AAT AAT N N CGC CGC	GAA GAA E E GCA GCA A A A A A A GT	ACA ACA T T AAGG AAGG K K K GGT GGT	GTA GTA GTA V V V V ATT I I I I ZACG ACT	GTC GTC V V GAA GAA E E E GAC GAC GAC	AAA AAA K K K CCCC CCCC P P P P AAC AAC	ATG ATG ATG M M M GAC GAC D D D D C AAG	TAC TAC TAC TAC Y Y Y Y CGGG CGGG G G G G G G G G G G	ATA ATA I I I ATA ATA ATA I I I I CCA CCA	300 100 360 120 420
MDS16c MDS16a MDS16b MDS16c MDS16a MDS16b MDS16c MDS16c MDS16c MDS16c MDS16c MDS16c MDS16c MDS16c MDS16c MDS16c	GGG GGG GG G G G G G G G G C T T T T T AAGC AAGG AAGG	TAT TAT Y Y GAGGGAG GAG GAG E E E AGA AGA AGA	CCCC CCCC P P P AAAA AAAA K K K K K K ACA ACA ACA	ACA ACA T T CAA CAA CAA Q Q Q Q CTG CTG CTG	ATA ATA I I AACC AACC AACC AACC C A C G C G C G G C G G C G G C G G C G G C G G C G G C G G C G G C G G C G	CAG CAG Q Q Q Q C GTC C GTC C C GTC C C GTC C C GTC C C GTC C C GTC C C GTC C C GTC C C GTC C C G C C A G Q Q Q Q Q Q Q C A G G G C A G Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q	AACCAAACAAACAAACAAACAAACAAACAAACAAACAA	GAG GAG E E P AAA AAA K K K C GAA GAA GAA E	AAT AAT N N ATC ATC ATC I I I ATT ATT	GAT GAT D D CTC CTC CTC CTC CTC CTC CTC CTC CT	TAC' TAC' Y Y RC4 TCA TCA TCA S S S ATA ATA	TTC TTC F F AAT AAT N N CGC CGC CGC CGC	GAA GAA E E GCA GCA GCA A A A A GT AGT AGT S	ACA ACA T T AAG AAG AAG K K K K GGT GGT GGT	GTA GTA GTA V V V V V ATT I I I I Z ACG ACT T	GTC GTC V V V GAA GAA GAA E E E GAC GAC GAC D	AAAA AAAA K K CCCG CCCG P P P P AACC AACC AACC	ATG ATG ATG M M M GGAC GGAC D D D D CAAG CAAG CAAG CAAG C	TAC TAC TAC TAC Y Y Y SGGG CGGG C G G G G G G G G G G K AAA K	ATA ATA I I I ATA ATA ATA I I I I CCA CCA CCA P	300 100 360 120 420
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MDS16c MDS16a MDS16b MDS16c MDS16a MDS16b MDS16c MDS16c MDS16c MDS16c MDS16b MDS16c MDS16b MDS16c MDS16a MDS16b MDS16c MDS16a MDS16b MDS16c MDS16c MDS16a MDS16c	GGG GGG GGG G G G G G G G G G C T T T T	TAT TAT TAT Y Y GAGGGAG GAGGGAG E E AGAAGAA R R R TTCC F F	CCCC CCCC P P P AAAA AAAA K K K K ACAA ACA T T T T AAGG AAGG K	ACA ACA T T T CCAA CCAA CCAA CCAA Q Q Q Q CCTG CCTG C CTGC C TGCC C C C C C C C C	ATA ATA I I AACC AACC AACC AACC A CAA CAA CAA	CAG CAG CAG CAG Q Q Q Q C C C C C C C C C C C C C C C	AAAC AAAC N N N TCT TCT TCT S S S S CCTT L L L L CCTT L L L CCTT CCTT	GAGAG GAG E E E AAAA K K K C GAAA K K K C GAAA C C GAA C AAAA K K C GAAA C AAAA K K C GAAG A A C AAAA C C AAAA C C AAAA C C AAAA C C AAAA C C AAAA C C AAAA C C AAAA C C AAAA C C AAAA C C AAAA C C C AAAA C C C AAAA C C C AAAA C C C AAAA C C C AAAA C C C AAAA C C C AAAA C C C AAAA C C C AAAA C C C AAAAA C C C AAAA C C C AAAA C C C AAAA C C C AAAA C C C C AAAA C C C C AAAAA C C C C AAAAA C	AATT AAT N N ATC ATC ATC ATC I I I ATT ATT I I AGG AGG AGG R P	GAT GAT D D CTC CTC CTC CTC CTC CTC CTC CTC CT	TAC' TAC' Y Y TCA TCA TCA S S S ATAA ATAA ATAA I I I I ACT' T	TTCC F F AATT AATT N N CCGCC CCGC CCGC CCGC R R R GAAA GAAA GAAA E	GAA GAA E E GCA GCA GCA A A GCA A A GCA A S S S S S S TTA TTA TTA L T	ACA ACA T T T AAG AAG AAG AAG K K K GGT GGT GGT GGT GGT GTT V V	GTA GTA GTA V V V V V ATT I I I I I C ACG T T T C GAGG GAGG E F	GTC GTC V V GAAA GAA GAA E E E GAC GAC D D D GTC GTC GTC V V	AAAA AAAA K K K CCCG CCCG P P P P AACC AACC AACC AACC N N N ACT ACT T T	ATC ATC ATC ATC M M M GAC GAC GAC D D D D C T C T C C T I L T	TAC TAC TAC Y Y Y CGGC CGGC G G G G G G G G G G C G C	ATA ATA I I I ATA ATA ATA I I I I CCCA CCA CCA P P P	300 100 360 120 420 140 474 158
MDS16c MDS16a MDS16b MDS16c MDS16a MDS16b MDS16c MDS16c MDS16c MDS16c MDS16b MDS16c MDS16a MDS16b MDS16c MDS16a MDS16b MDS16c MDS16a MDS16b MDS16c MDS16a MDS16b	GGG GGG GGG G G G G G G G G G G C T T T T	TAT TAT TAT Y Y GAGGGAG GAGGGAG E E AGAAGAA R R R TTCC F F F	CCCC CCCC P P P AAAA AAAA K K K K ACAA AACA T T T AAGG K K K K	ACA ACA T T T CCAA CCAA CCAA CCAA Q Q Q Q CCTG CCTG C CTGC C TGCC C C C C C C C C	ATA ATA I I AACC AACC AACC AACC AACCAA CAAA CAAA CAAA CAAA Q Q O	CAG CAG CAG CAG Q Q Q Q C C C C C C C C C C C C C C C	AACCAACCAACCAACCAACCAACCAACCAACCAACCAA	GAGAG GAG E E CAAA CAAAA CAAAA K K CAAAA CAAAA CAAAA CAAAA CAAAA CAAAA CAAAA CAAAA CAAAA CAAAA CAAAA CAAAAA CAAAAA CAAAAA CAAAAA CAAAAA CAAAAA CAAAAA CAAAAA CAAAAA CAAAAA CAAAAA CAAAAA CAAAAA CAAAAA CAAAAA CAAAAAA	AATT AAT N N ATC ATC ATC I I I I ATT ATT I I I AGG AGG R R R R	GAT GAT D D CTC CTC CTC CTC CTC CTC CTC CTC CT	TAC' TAC' Y Y TCA TCA TCA TCA S S S ATA' ATA' ATA' I I I I ACT' T T T	TTCC F F AAAT N N CCGCCCGC R R R GAAA GAAA GAAA E E F	GAA GAA E E GCA GCA A GCA A GCA A GCA A GCA S S S S S S S S TTA TTA L L L I.	ACA ACA T T T T AAG AAG AAG K K K GGT GGT GGT GGT GGT GGT C GGT C C GTT V V V V	GTA GTA GTA V V V V V ATT I I I I I C C C C GAG GAG GAG C GAG C GAG C C C C	GTC GTC V V GAAA GAA GAA E E GAC GAC D D D GTC GTC GTC V V V	AAAA AAAA K K K CCCG CCCG P P P P P AACC AACC AACC N N N ACT ACT T T T	ATC ATC ATC ATC M M M GGAC GGAC GGAC D D D D C T C C T I L L I, I	TAC TAC TAC Y Y Y CGGG CGGG G G G G G G G G G G C G C	ATA ATA I I I ATA ATA ATA I I I I I CCCA CCA CCA P P P	 300 100 360 120 420 140 474 158

Fig .1 Alignment of the nucleotide and deduced partial amino acid sequences of $S_{16c(=16)}$ - $S_{16a(=16=27a)}$ -, and $S_{16b(=22=27b)}$ -RNase in `Bohnapfel', `Baskatong' and `Alkmene' apple, respectively.

Genomic DNA encoding $S_{16c(=16)}$ -RNase was amplified from S_{16} -allele of `Bohnapfel' by PCR using the primers FTQQYQ⁷) and OWB249 .¹³⁾ PCR product was directly sequenced by dideoxy chain termination on an ABI PRISMTM 310 DNA sequencer (Perkin-Elmer) using dRhodamine Terminator Cycle Sequencing Kits (Perkin-Elmer). The conserved regions C2 ,C3 ,RC4 and C5 ,and hypervariable region RHV are boxed. The sequence of $S_{16c(=16)}$ (*MDS* 16 *c*) in `Bohnapfel' was deposited under the DDBJ accession number AB126322 . $S_{16a(=16=27a)}$ (*MDS* 16 *a*) and $S_{10b(=22=27b)}$ (*MDS* 16 *b*) were from the studies of Verdoodt *et al* .¹³) and Van Nerum *et al* .¹⁴ respectively. The different sites are shown in bold. The site of the intron is shown by a triangle.

GTAATATTATTAGTAGTAAGACAGTCAATATTGTTTATTTCATTTATCTATATTTATCTG	60
TATATATCAAGGGAGAAGACAAAAATGATGAAACATTCATAATTTCAAAATTGCCCTCTT	20
CCATTAAATTTTCAAATAAAGTAAGAAATTCACAAAATTAAAAAATTAAAATATCTCCA	180
TGGAGTGGTTAGCAACCAACTTTATTATTTTTTAAATGAGATTTTTGAACTTTAATCCTT	240
GACTATATAAATAAAGGGAGAAGACAAAAGTGGTGAAACATTCATAATTCCAAAATTGCC	300
CTCTTCCATTAAATTTCAAATAAAGTAAAAAATTCACAAAATAAAAAAAA	360
GAGTAGTTAGCAACTAACTTTATTATTTTTTTTTTTTTT	420
AGAAGATTAAAAATTTAATAAAAACTAGGTGTTAGATTGGATATTGATTTTAGTCCTTTAA	480
TGAGTACTTAATTCAAATTCATATTATATTTTGTTTTAATATTTAATAGATATATTTTTT	540
AATTCCATTACACTAAATTTTAAATTTATTATTATACACATTTTAATTCATTAATTTCAT	600
TTAACTTCCTCTATTTAGTGTACATAAACGCCTGTATCATAATATATTTTTAGAAACCTT	660
CATTTTAATCCTTGGCAAAGATGACTTTTAGATTTAAACCATGMGTAAGACCAAAATCTA	720
ATTTTAGTCATTTGATACATTAATAACCTGATTYATTAATTATATTTTGATTTTTTTTMA	780
TTATTTATCTTTTGYTCCTAATTTTTATTTTCATTTCATT	840
GTAAACATTTAAATAAATTAATTTTTGTTATACAAYWTATTTCAATGCACTTTGTCTTCT	900
TCATTGAGGTACCATTAAATTCATTATACTACATTTCAGTACATACA	960
ATTTTCGTACATACCATTTTGATACAAACATTTAGGTACATTAATTTTATATAATACATT	1020
TTGGTACTAACATTTCGGTGCATACATTTCAATATACAAATTTAGATACATTAACTTTAA	1080
TATAATACATTTCGGTGCATATATTTCGGTACATACATTTCGGTACTAACATTTAAATAC	1140
ATTTGTTCAATATAATACATTTCGGTACACACATTTTGGTACATTAATTGAGTCTTTCAA	1200
GTTTGAAGAATGTTAAATAAAATTAAAAATTAAAAAAAA	1260
AATTAAAATTTGTGTAGTAATAAATTTAAATATTTTATGGGAGACATTAAATAAA	1320
CATTAATTATTTTGAATTAAGGACACATCAAGAGATTATAATCAATATGTAATCTAACAT	1380
CAGGTTTATTTTAAATTTCAATCTTCTTAAAGGATTAAAGTTAAATGCTAAATTAGTGTA	1440
CTGAAGTGTCTGTTCCTAAATATATTGTAGTACCTAACTATATTGTAGGGATTTAGTGGC	1500
ACATAAGAAAATATGCAAAAACGCAAGTGTACTGAAAATTTTGTACCTAAATATTTGATA	1560
CTAAACTAGTGTACCGAAATGTCGGTGTCTAAATGTATTATACTAAGCCAGTGCACCGAA	1620
ATGTCCTTATCTAAACATATTATATTAACTTAGTGTACCGAAATATTTGTACCTAAATGT	1680
ATTATAATGAATTAGTGGCACTTACGAAAAATACGCAAAAAACATAAGTGTACCGAAAAAATC	1740
TCTATGAAAATATTTTCTTATATAAGATACTTACCAGAATGACAATTAAAAATTTATAATA	1800
TTTTCATAAAATTTAATTTATGAACACCATAAATATAAAAAAAA	1860
ACATGTGCTGCCATTAAATAGAGTTTAGGGACTAGAATCAATTTTTAATCTGGTATAAGA	1920
CATTTTTCTAAACTTCATCTTTTTAAGGATTAAAATCTAGTTTTATATTATTATTTTTTT	1980
TTCTATTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT	2040

ATTTTTTGTTTTTTTTTTTTTTTCTTTCAAAAAAATAAAATCAAAAGACCCAATTGCTACACACT	2100
TACGCATGTAGCAAGGGGCTAGTATACTTTTGTGTGTGCGCACGGGTGTATACTTTTTAA	2160
TACAATTAATTTAAAATCTAATCATAAATGTTTTATATTGTACATATTACCTTGTCAG	2218

Fig 2 DNA sequence of the intron in $S_{I6c(=16)}$ -allele.

The sequence was determined as described in Fig .1 .

divided into three groups based on the length of the intron within *S*-RNases .¹⁷⁾ The first group consisting of S_{3-} and S_{5} -RNase had ca .1100bp of intron .^{11,12)} The second group consisting of S_{1-f} - $S_{g=20a}$ - $S_{g=20b}$ - and $S_{24=h}$ -RNase had 318-344 bp of intron .^{5, β,17)} The third group consisting of S_{2-} - $S_{7=a-}$ - $S_{9=c-}$ - $S_{28=e-}$ and $S_{30=t}$ -RNase had118-180 bp of intron .^{15,17)} Since the introns of closely related *S*-alleles, such as those belonging to the second group showed an extremely high similarity (83 9 - 94 4%) $S_{16=27a}$ and $S_{22=27b}$ may have similar size with S_{16} in 'Bohnapfel'. We grouped the alleles into a fourth group. We could not find any retrotransposon-like sequence in the intron, which is linked to the *S*-locus of *Nicotiana alata* .¹⁸⁾

Two hypervariable regions (HVa and HVb) alone are sufficient for *S*-allele discrimination in closely related Solanaceae *S*-RNases .¹⁹ Since rosaceous *S*-RNases have only one hypervariable region RHV located at a position corresponding to that of the solanaceous region HVa $_{r}^{20}$ it is reasonable that the RHV region is sufficient for allele discrimination of closely related apple *S*-alleles. As the deduced amino acid sequence of S_{16} in 'Bohnapfel' is identical to $S_{16=27a}$ and $S_{22=27b}$, these three alleles are thought to be functionally identical. Van Nerum *et al* .¹⁴ described the two identical alleles in S_{27} to be S_{27a} and S_{27b} . Afterward, Broothaerts² re-numbered S_{27a} and S_{27b} to S_{16} and S_{22} respectively.

We determined the S_{16} -RNase sequence, and concluded that the S_{16} in 'Bohnapfel', $S_{16=27a}$ and $S_{22=27b}$ should be renumbered to S_{16c} , S_{16a} and S_{16b} , respectively (Table1).

Proposed S-allele	Former S-allele	Cultivar	S-al	leles	Database Accession #		
16a	16, 27, 27a	Baskatong	16a	26		AF016919	
16b	22, 27b	Alkmene	5	16b		AF327222	
	23, 27b	Delbard Jubile	2	16b		AF327222	
	25, 27b	Merlijin	3	16b		AF327222	
16c	$16^{y}, 27a^{x}$	Bohnapfel	6b	9	16c	AB126322	

Table 1. S-alleles of apple cultivars.

^z S_{25} -allele in 'Merlijin', ^y S_{16} -allele in 'Bohnapfel', ^x S_{27a} -allele in 'Bohnapfel'

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