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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}

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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）[1] 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.[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,[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 gene-specific primer set.[5－7,9－15]

S-allele designations are confusing; for instance, the *S*-genotype of ‘Bohnapfel’ was identified as $S_9S_xS_y$; x, y ＞11 and $S_9S_{16}S_{19}$ by cross pollination[3] and stylar protein analysis,[16] respectively. Recently, Broothaerts[2] 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_{6b} instead of S_{19} was present in ‘Bohnapfel’. From the results, S_{28} was left unchanged and S_{19} was re-numbered to S_{6b}.[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-

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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

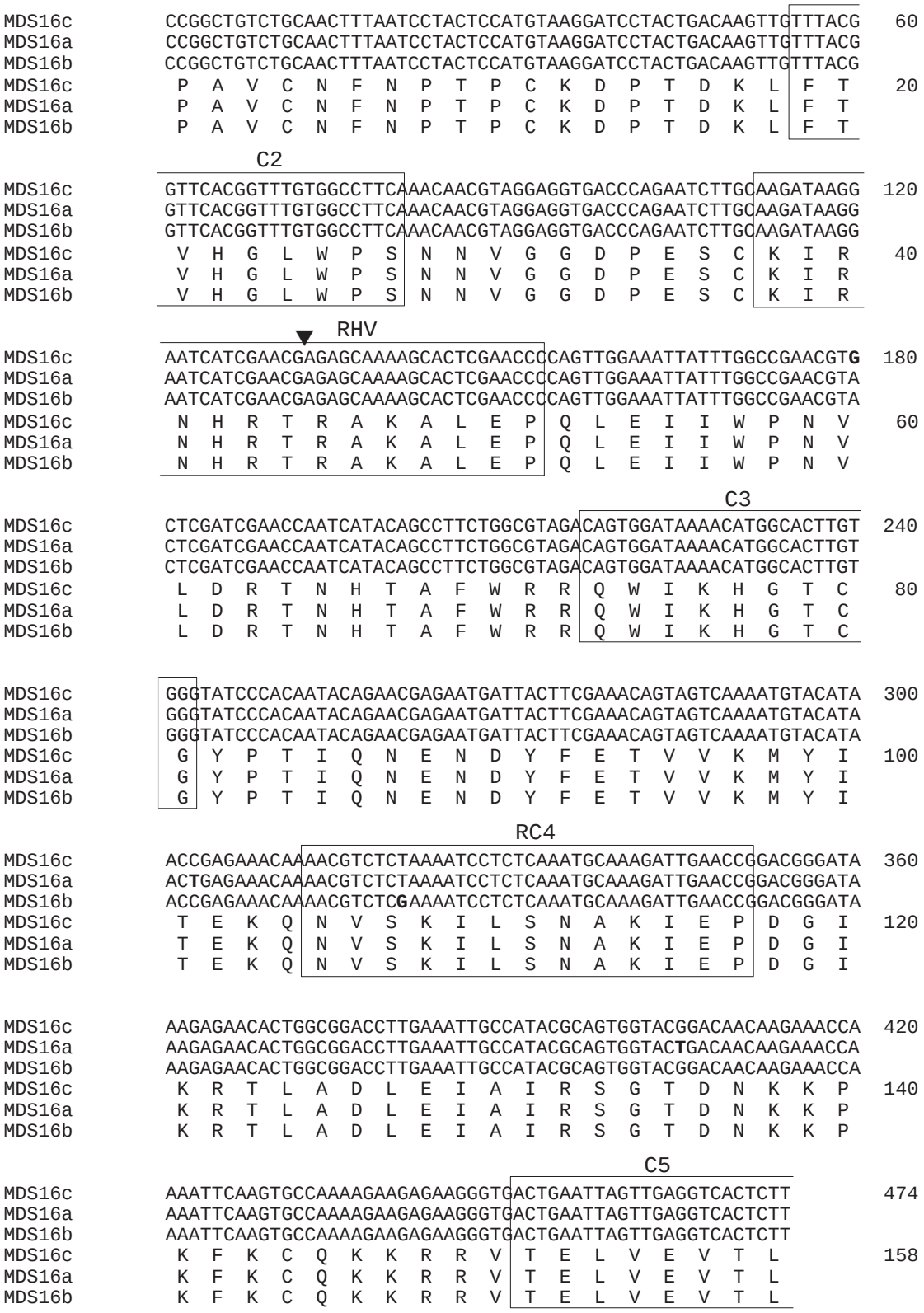

Fig.1 Alignment of the nucleotide and deduced partial amino acid sequences of $S_{16c(=16)}$-, $S_{16a(=16=27a)}$-, and $S_{16b(=22=27b)}$-RNase in 'Bohnapfel', 'Baskatong' and 'Alkmene' apple, respectively.

Genomic DNA encoding $S_{16c(=16)}$-RNase was amplified from S_{16}-allele of 'Bohnapfel' by PCR using the primers FTQQYQ[7] and OWB249.[13] PCR product was directly sequenced by dideoxy chain termination on an ABI PRISM™ 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 $S_{16b(=22=27b)}$(*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.

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GTAATATTATTAGTAGTAAGACAGTCAATATTGTTTATTTCATTTATCTATATTTATCTG    60
TATATATCAAGGGAGAAGACAAAAATGATGAAACATTCATAATTTCAAAATTGCCCTCTT    20
CCATTAAATTTTCAAATAAAGTAAGAAATTCACAAAATTAAAAAAATTAAAATATCTCCA   180
TGGAGTGGTTAGCAACCAACTTTATTATTTTTTAAATGAGATTTTTGAACTTTAATCCTT   240
GACTATATAAATAAAGGGAGAAGACAAAAGTGGTGAAACATTCATAATTCCAAAATTGCC   300
CTCTTCCATTAAATTTTCAAATAAAGTAAAAAATTCACAAAATAAAAAAAAATCTCCACG   360
GAGTAGTTAGCAACTAACTTTATTATTTTTTAAATGAGATTTTTGAACTTTAATCCTTGA   420
AGAAGATTAAAATTTAATAAAAACTAGGTGTTAGATTGGATATTGATTTTAGTCCTTTAA   480
TGAGTACTTAATTCAAATTCATATTATATTTTGTTTTAATATTTAATAGATATATTTTTT   540
AATTCCATTACACTAAATTTTAAATTTATTATTATACACATTTTAATTCATTAATTTCAT   600
TTAACTTCCTCTATTTAGTGTACATAAACGCCTGTATCATAATATATTTTTAGAAACCTT   660
CATTTTAATCCTTGGCAAAGATGACTTTTAGATTTAAACCATGMGTAAGACCAAAATCTA   720
ATTTTAGTCATTTGATACATTAATAACCTGATTYATTAATTATATTTTGATTTTTTTTMA   780
TTATTTATCTTTTTGYTCCTAATTTTTATTTTCATTTCATTTATTGTAATATATTTCGTT   840
GTAAACATTTAAATAAATTAATTTTTGTTATACAAYWTATTTCAATGCACTTTGTCTTCT   900
TCATTGAGGTACCATTAAATTCATTATACTACATTTCAGTACATACATTTAGGTMMACAT   960
ATTTTCGTACATACCATTTTGATACAAACATTTAGGTACATTAATTTTATATAATACATT  1020
TTGGTACTAACATTTCGGTGCATACATTTCAATATACAAATTTAGATACATTAACTTTAA  1080
TATAATACATTTCGGTGCATATATTTCGGTACATACATTTCGGTACTAACATTTAAATAC  1140
ATTTGTTCAATATAATACATTTCGGTACACACATTTTGGTACATTAATTGAGTCTTTCAA  1200
GTTTGAAGAATGTTAAATAAAATTAATAAATTAAAAAAAATCTTTTTTGGTAAAAAAATA  1260
AATTAAAATTTGTGTAGTAATAAATTTAAATATTTTATGGGAGACATTAAATAAAATGCA  1320
CATTAATTATTTTGAATTAAGGACACATCAAGAGATTATAATCAATATGTAATCTAACAT  1380
CAGGTTTATTTTAAATTTCAATCTTCTTAAAGGATTAAAGTTAAATGCTAAATTAGTGTA  1440
CTGAAGTGTCTGTTCCTAAATATATTGTAGTACCTAACTATATTGTAGGGATTTAGTGGC  1500
ACATAAGAAAATATGCAAAAACGCAAGTGTACTGAAAATTTTGTACCTAAATATTTGATA  1560
CTAAACTAGTGTACCGAAATGTCGGTGTCTAAATGTATTATACTAAGCCAGTGCACCGAA  1620
ATGTCCTTATCTAAACATATTATATTAACTTAGTGTACCGAAATATTTGTACCTAAATGT  1680
ATTATAATGAATTAGTGGCACTTACGAAAATACGCAAAAACATAAGTGTACCGAAAAATC  1740
TCTATGAAAATATTTTCTTATATAAGATACTTACCAGAATGACAATTAAAATTTATAATA  1800
TTTTCATAAAATTTAATTTATGAACACCATAAATATAAATAAAAAAGAGAGACATTGAAT  1860
ACATGTGCTGCCATTAAATAGAGTTTAGGGACTAGAATCAATTTTTAATCTGGTATAAGA  1920
CATTTTTCTAAACTTCATCTTTTTAAGGATTAAAATCTAGTTTTATATTATTATTTTTTT  1980
TTCTATTTTTTTTTCTATTTTTTTTTCAAATAAAAAATCCAAAGAGCCAATTGCTAGTAC  2040
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ATTTTTTGTTTTTTTTTTCTTTCAAAAAAATAAAATCAAAAGACCCAATTGCTACACACT  2100
TACGCATGTAGCAAGGGGCTAGTATACTTTTGTGTGTGCGCACGGGTGTATACTTTTTAA  2160
TACAATTAATTTAAAATCTAATCATAAATGTTTTATATTGTACATATTACCTTGTCAG    2218
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Fig. 2 DNA sequence of the intron in $S_{16c(=16)}$-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.[5,8,17] The third group consisting of S_2-, $S_{7=d}$-, $S_{9=c}$-, $S_{28=e}$- 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,[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, Broothaerts[2] 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 re-numbered to S_{16c}, S_{16a} and S_{16b}, respectively (Table1).

Table 1. *S*-alleles of apple cultivars.

Proposed *S*-allele	Former *S*-allele	Cultivar	*S*-alleles			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[z], 27b	Merlijin	3	16b		AF327222
16c	16[y], 27a[x]	Bohnapfel	6b	9	16c	AB126322

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

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