Molecular Identification of a New Apple *S*-RNase—S29—Cloned from ‘Anna’, a Low-chilling-requirement Cultivar

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‘Anna’, a low-chilling-requirement (LCR) apple cultivar is the progeny of an hybridization between ‘Golden Delicious’ and ‘Hadassiya Red’, which was carried out by Abba Stein in 1956 (Brooks and Olmo, 1972). It has become an economically important cultivar especially in South America, since it can be grown in moderate climate regions, which are not suitable for the common apple cultivars (Hauagge and Cummins, 2000). ‘Golden Delicious’ is one of the classic, most popular, apple cultivars, while ‘Hadassiya Red’ is a Middle Eastern, low-quality, LCR variety which has not been cultivated for >40 years.

Apples (Rosaceae) carry the gametophytic self-incompatibility (GSI) (Kao and Tsukamoto, 2004) system, and therefore apple orchards contain at least two cultivars that are compatible with each other. GSI, which was first identified in Solanaceae and is also found in Scrophulariaceae and other families, is determined by a single genetic locus (*S*-locus). The *S*-locus encodes, among other genes, for a multi-allelic RNase (*S*-RNase) that inhibits pollen growth in the style in a haplotype specific manner (Kao and Tsukamoto, 2004). Thus, compatibility may be full, when the cultivars carry different *S*-RNase alleles and semi when they share one of their two *S*-RNase alleles. Semi-compatibility may lead to reduction in fruit set and yield (Goldway et al., 1999). Many *S*-RNase alleles have been identified, including 15 from apples (Broothaerts, 2003). Molecular analysis of the *S*-RNase allele content of cultivars enables the determination of their genetic compatibility. Here we present the *S*-RNase content of ‘Anna’, which includes the identification of a new *S*-RNase, S29.

Since ‘Golden Delicious’ is *S*2 *S*3 (Broothaerts et al., 1995), it would be expected that

one of ‘Anna’s’ *S*-RNases would be either *S*2 or *S*3, while its second *S*-RNase, inherited from ‘Hadassiya Red’, remained to be identified. As shown in Fig. 1, PCR analysis, of DNA extracted from ‘Anna’, with specific primers for *S*2 and *S*3 (primers for *S*2 were OWB122 and OWB123, and for *S*3, OWB145 [Broothaerts et al., 1995] and AM12, 5'-TAA TCT GCC TCG CTG -3', generated according to the nucleotides 21-35 of the sequence with the accession number U12200), revealed that it carries *S*3. For identification of ‘Anna’s’ second *S*-RNase, universal primers NSS2 and NSA1 (NSS2, 5'-CAC GGT/ATTG TGG CCTTC-3' and NSA1, 5'-GACCTCAACC/TAATTCAG -3' corresponding respectively, to nucleotides 244-260 and 635-651, from sequence accession number U12199), were used and a single PCR product was obtained. The segment was cloned and sequenced. It contains 811 bp, which makes up most of the gene, spanning between the conserved regions, C1 to C5. Like other *S*-RNases it contains an intron (232 bp). DNA alignment by the Blast program of NCBI revealed that it is a new *S*-RNase. To avoid confusion with identification-numbers appearing in the literature, we have named the new allele *S*29 (accession number AY039702). Alignment of the exon regions (579bp) (Fig.

2) revealed that the highest degree of identity (89%) was to the *S*16 allele of rowan (*Sorbus aucuparia*). Interestingly, *Sorbus aucuparia* grows in regions with extreme conditions in the Middle East, such as in the Sinai Desert or close to the summit of Mount Hermon (2200m). In addition, a high degree of similarity, 87%, was also found between *S*29 and *S*24/Sh of apple and *S*b of the European pear (*Pyrus communis*). When *S*29 was compared with *S*-RNases from the Prunoideae subfamily of Rosaceae, the similarity was much lower (45% to 50%). This relationship between alleles is explained by the hypothesis that the divergence of *S*-alleles predated speciation of the Rosaceae, but occurred shortly after the divergence into subfamilies (Ushijima et al., 1998).

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Fig. 1. Identification of the ‘Anna’ *S*-RNase alleles. PCR for *S*2, *S*3 and *S*29 RNase alleles was carried out on DNA from ‘Anna’. DNA of ‘Golden Delicious’ served as the control. Lanes 1 and 2 = primers for *S*2; Lanes 3 and 4 = primers for *S*3; Lanes 5 and 6 = primers for *S*29. Lane 1,3 and 5 = ‘Anna’, lanes 2,4, and 6 = ‘Golden Delicious’ and lane M = size marker.

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MdS 24/Sh 360 CTCCTGTGCGTCTTC OCC AA TACAG AAC CA GAAGC ATT AC TTTGA TAC AG TAATC AAA AT
 MdS g 357 CTCCTGTGCGTCTTC OCC AA TACAG AAC CA GAAGC ATT ACTTTGA TAC AG TAATC AAA AT
 SaS 16 230 CTCCTGTGCGTCTTC OCC AA TACAG AAC CA GACGC ATT AC TTTGA TAC AG TAATC AAA AT
 PcS b 485 CTCCTGTGCGTCTTC OCC AA TACAG AAC CA GACGC ATT AC TTTGA TAC AG TAATC AAA AT
 MdS 29 577 CTCCTGTGCGTCTTC OCC AA TACAG AAC CA GACGC ATT AC TTTGA TAC AG TAATC AAA AT
 ---C3---)

MdS 24/Sh 420 GTACA CAA CC CAGAA ACA AA AC TC TCT GAAATCC TCT CAAAGGC GAA TA TAAAA CCG GG
 MdS g 417 GTACA CAA CC CAGAA ACA AA ACGTC TCT GAATCC TCT CA AAGGC GAA TA TAAAA CCG GG
 SaS 16 290 GTACA CAA CC CAGAA ACA AA ACGTC TCT GAATCC TCT CA AAGGC GAA TA TAAAA CCG GG
 PcS b 545 GTACA CAA CC CAGAA ACA AA ACGTC TCT GAATCC TCT CA AAGGC GAA TA TAAAA CCG GG
 MdS 29 637 GTACA CAA CC CAGAA ACA AA ACGTC TCT GAATCC TCT CA AAGGC GAA TA TAAAA CCG GG
 (-----RC4-----)

MdS 24/Sh 480 TAGGA AAA AC AGGAC ACT GG TGGAT ATT GA AAATG OCA TA CGTAA TGT TATCAAC AAT AT
 MdS g 477 TAGGA AAA AC AGGAC ACT GG TGGAT ATT GA AAATG OCA TA CGCAA TGT TA TCAAC AAT AT
 SaS 16 350 TAGGA AAA AC AGGAC ACT GG TGGAT ATT GA AAATG OCA TA CGCAA TGT TA TCAAC AAT AT
 PcS b 605 TAGGA AAA AC AGGAC ACT GG TGGAT ATT GA AAATG OCA TA CGCAA TGT TA TCAAC AAT AT
 MdS 29 697 TAGGA AAA AC AGGAC ACT GG TGGAT ATT GA AAATG OCA TA CGTAA TGT TACCAAC AAT AT

MdS 24/Sh 540 GACAC CAA AATTCAA GTGCCAAAAG AAT ACTAGGA CAT CATTGAC TGA ATTGGTT GAG GT
 MdS q 537 GACAC CAA AA TTCAA GTGCCAAAAG AAT AC TAGGA CAT CA TTGAC TGA AT TGGTT GAG GT
 SaS 16 410 GACAC CAA AA TTCAA GTGCCAAAAG AAT AC TAGG-----
 PcS b 665 GACAC CAA AA TTCAA GTGCCAAAAG AAT CC TAGGA CAT CA TTGA-----
 MdS 29 757 GGTAC CAA AA TTCAA GTGCCAAAAG AAT CC TAGGA-----TGAC TGA AT TGGTT GAG GT
 (-----C5-----)

MdS 24/Sh 600 CGGTC TTT GC AGCGA TAG CA ACTTA ACG CA GTTCA TAA AT TGCCC CCG CC CATTT OCA GG
 MdS g 597 CGGTC TTT GC AGCGA TAG CA ACTTA ACG CA GTTCA TAA AT TGCCC CCG CC CATTT OCA CA
 SaS 16
 PcS b
 MdS 29 811 C-----
 ---)

MdS 24/Sh 1 -----
 MdS g 1 -----
 SaS 16 1 -----
 PcS b 1 -----
 MdS 29 1 A TGGGT TTT AC GGGAA TGA TA TATAT GGT TA CGATG GTA TT TTCAT TAA TT GTACT AAT A

MdS 24/Sh 1 -----
 MdS q 1 -----
 SaS 16 1 -----
 PcS b 1 -----G TA TCAAC CGG CT
 MdS 29 61 TTGTC TTC GT CCACG GTG GG ATT CG ATT AT TTTCA ATT TA CGCAG CAA TA TCAGC CGG CT

MdS 24/Sh 1 -----
 MdS g 1 -----
 SaS 16 1 -----
 PcS b 14 GTCTG CAA CT CTAAT CCA AC TCCTT GTA AG GATCC TCC TG ACAAG TTG TT TACTG TTC AC
 MdS 29 121 GTCTG CAA CT TTAAT GCT AC TCCTT GTA AG GATCC TCC TG ACAAG TTG TT TACGG TTC AC
 (-----C2-----)

MdS 24/Sh 1 -----TTGAAC AAT ATTATTC AA--TGGGGA CCGGAT--GAATAT
 MdS q 1 -----AATAAAT ATTATTC AA--TGGGGA CCGGAT--TAATAT
 SaS 16 1 -----
 PcS b 74 GGT TT GTG GC CTTCA GAC TC GAATGGAATGACCC AAT ATTGCAAGCC--CCCATAT
 MdS 29 181 GGT TT GTG GC CTTCA AAC GC GAAGGGAATGACCC AAGGTTGCAAGACACAGATAT
 -----C2---) (-----RHV-----)

MdS 24/Sh 40 ATGGT TAT GA TGGTA TTT TCACTA--AT TT TATTAATA TT GCCTT CGT GCACGGT GGGAT
 MdS g 37 ATGGT TAT GA TGGTA TTT TCACTA--AT TT TATTAATA TT GCCTT CGT GCACGGT GGGAT
 SaS 16 1 -----
 PcS b 133 CAGTC GGT AAT TTTAAGCATATTC GATAGTCAATA TT GTTATA TCT CATTTAT TACT
 MdS 29 241 CAGTGGTG--TGTTATCTCA-----TTAAGTACCTGCTGCT--TCAATAT TCA
 -----) *

MdS 24/Sh 98 TCGAT TAT TATCAAT TTACGACGAATA TCAGCCGGCT GTCTGCAACT CT--AATCC--
 MdS q 95 TCGAT TAT TATCAAT TTACGACGAATA TCAGCCGGCT GTCTGCAACT CT--AATCC--
 SaS 16 1 -----CGGCTGCTGCAACT CT--AATCC--
 PcS b 193 TGTGT GTG TATATATATATTTTGTGATA--ATGTTGAGCCCTCAATTTTAAAA CC--
 MdS 29 291 TTAAT TAA AACCAATT--TGTACATTA--AGGAT C-TGTACCA TAT TAAAAA CACTT

MdS 24/Sh 153 AACTCCT-----TGTAAGGA-----TCC TACTGAC AAG TT GTTAC--TGT
 MdS g 150 AACTCCT-----TGTAAGGA-----TCC TACTGAC AAG TT GTTAC--TGT
 SaS 16 23 AACTCCT-----TGTAAGGA-----TCC TACTGAC AAG TT GTTAC--TGT
 PcS b 248 AATATGATGTG TCACAATAAGATATAAGATTTAGTCTTCTGACAT--TACTCTATATTT
 MdS 29 347 AACTATAAGCCA-----ATATCATTA TCACA TGT GCTTACAA--AATTATA--TGT

MdS 24/Sh 192 TCACGGTTTG TGGC--CT--TCAACTCGAATGGAAA TGA--CCCAAAATATT C--CAA
 MdS q 189 TCACGGTTTG TGGC--CT--TCAGACT CGAATGGAAA TGA--CCCAAAATATT C--CAA
 SaS 16 62 TCACGGTTTG TGGC--CT--TCAGACT CGAATGGAAA TGA--CCCAAAATATT C--CAA
 PcS b 307 TTTTGTATATATA--CAAATATTACACTTATTTAAAA TT TATCATAAATATT T--CAA
 MdS 29 398 AATATTTAGTCACTCAGCATATCTCTAATTTTGAACCTA--ATCATAATTTTCTC

MdS 24/Sh 243 GGC--ACAGCATA TCAGATGAAAAT ACTCGAAC CC CAGTT GGT AA TTATT TGGC
 MdS g 240 GGC--GC CGCATA TCAGATGAAAAT ACTCGAAC CC CAGTT GGT AA TTATT TGGC
 SaS 16 113 GGC--GC CGCATA TCAGATGAAAAT ACTCGAAC CC CAGTT GGT AA TTATT TGGC
 PcS b 365 TTGTA TAT TATATA TTGTCAGATGAAAAT ACTCGAAC CC CAGTT GGT AA TTATT TGGC
 MdS 29 457 TTGTA TAT TATATA TTGTCAGATGAAAAT ACTCGAAC CC CAGTT GGT AA TTATT TGGC
 * (-----RHV-----)

Fig. 2. Alignment of partial *S*-RNase gene sequences of apple (*Malus domestica*) S24/Sh (MdS24 Accession No. AF016920), apple S20/Sg (MdSg-AB019184), rowan (*Sorbus aucuparia*) S16 (SaS16-AF504268), pear (*Pyrus communis*) Sb (PcSb-AJ458182), and apple S29 (MdS29-AY039702). Black boxes indicate identical residues of at least three sequences. Half-tone boxes indicate conservative substitutions. The conserved regions (C2, C3, RC4, and C5) and the hyper variable region (RHV) are indicated (Ushijima et al., 1998). Asterisks denote the borders of the S29 intron.