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 vield (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 S2 S3 (Broothaerts et al., 1995), it would be expected that

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one of 'Anna's S-RNases would be either S2 or S3, while its second S-RNase, inherited from 'Hadassiva Red', remained to be identified. As shown in Fig. 1, PCR analysis, of DNA extracted from 'Anna', with specific primers for S2 and S3 (primers for S2 were OWB122 and OWB123, and for S3, 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 S3. For identification of 'Anna's second S-RNase, universal primers NSS2 and NSA1 (NSS2, 5'-CAC GGT/ATTG TGG CCT TC-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 S29 (accession number AY039702). Alignment of the exon regions (579bp) (Fig.

2) revealed that the highest degree of identity (89%) was to the S16 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 S29 and S24/Sh of apple and Sb of the European pear (Pyrus communis). When S29 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 Salleles predated speciation of the Rosaceae, but occurred shortly after the divergence into subfamilies (Ushijima et al., 1998).

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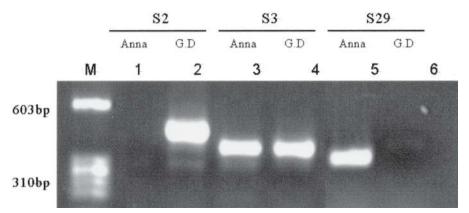


Fig. 1. Identification of the 'Anna' S-RNase alleles. PCR for S2, S3 and S29 RNase alleles was carried out on DNA from 'Anna'. DNA of 'Golden Delicious' served as the control. Lanes 1 and 2 = primers for S2; Lanes 3 and 4 = primers for S3: Lanes 5 and 6 = primers for S29. Lane 1,3 and 5 = 'Anna', lanes 2,4, and 6 = 'Golden Delicious' and lane M = size marker.

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MdS24/Sh 360 MdSg 357 SaS16 230 PcSb 485 MdS29 577	CTCCT GTG OG TCTTC OCC AN TACAG AAC CA GA <mark>A</mark> GC ATT AC TTTGA TAC AG TAATC AAA AT CTCCT GTG OG TCTTC OCC AN TACAG AAC CA GAAGC ATT ACTTTGA TAC AG TAATC AAA AT CTCCT GTG OG TCTTC OCC AN TACAG AAC CA GACGC ATT AC TTTGA TAC AG TAATC AAA AT CTCCT GTG OG TCTTC OCC AN TTCAG AAC CA GACGC ATT AC TTTGA TAC AG TAATC AAA AT CTCCT GTG OG TCTTC OCC ATTCAG AAC CA GACGC ATT AC TTTGA TAC AG TAATC AAA AT CTCCT GTG OG TCTTC OCC ATTACAG AAC CA GACGC ATT AC TTTGA TAC AG TAATC AAA AT CTCCT GTG OG TCTTC OCC ATTACAG AAC CA GACGC ATT AC TTTGA AC AG TAATC AAA AT
MdS24/Sh 420 MdSg 417 SaS16 290 PcSb 545 MdS29 637	$\begin{array}{l} GTACACACCCCAACCCCAAAAAAAAAAAAAAAAAAAAAA$
MdS24/Sh 480 MdSg 477 SaS16 350 PcSb 605 MdS29 697	TAGGA AAA AC AGGAC ACT GO TGGAT ATT GA AAATG CCA TA CG <mark>T</mark> AA TGT TATCAAC AAT AT TAGGA AAA AC AGGAC ACT GO TGGAT ATT GA AAATG CCA TA CGCAA TGT TA TCAAC AAT AT TAGGA AAA CC AGGAC ACT GO TGGAT ATT GA AAATG CCA TA CGCAA TGT TA TCAAC AAT AT TAGGA AAA CC AGGAC ACT GO TGGAT ATT GA AAATG CCA TA CGCAA AGT TA TCAAC AAT AT GGGCG AAA A AAGGAC ACT GO TGGAT ATT GA AAATG CCA TA CGCAA <mark>A</mark> GT TA TCAAC AAT AT GGGCG AAA A A
MdS24/Sh 540 MdSq 537 SaS16 410 PcSb 665 MdS29 757	GACAC CA <mark>C</mark> AATTCAA GTG CCAAAAG AAT ACTAGGA CAT CATTGAC TGA ATTGGTT GAG GT GACAC CAA AA TTCAA GTG CC AAAAG AAT AC TAGGA CAT CA TTGAC TGA AT TGGTT GAG GT GACAC CAA AA TTCAA GTG CC AAAAG AAT AC TAG GACAC CAA AA TTCAA GTG CC AAAAG AAT GO TAGGA CAT CA TTGA GGTAC CAA AACTCAA GTG CC AAAAG GAAT GO TAGGA
MdS24/Sh 600 MdSg 597 SaS16 PcSb MdS29 811	GGTC TTT & AGCGA TAG CA ACTTA ACG CA GTTCA TAA AT TGCCC & G & CATTT & AG GGTC TTT & AGCGA TAG CA ACTTA ACG CA GTTCA TAA AT TGCCC & G & CATTT & AG
MdS 24/Sh 1 - MdS g 1 - SaS 16 1 - PcS b 1 - MdS 29 1 A	TGGGT TTT AC GGGAA TGA TA TA TAT AT GTT TA CGATG GTA TT TTCAT TAA TT GTACT AAT A
MdS24/Sh 1 - MdSq 1 - SaS16 1 - PcSb 1 MdS29 61	
MdS 24/Sh 1 MdS g 1 SaS 16 1 PcS b 14 MdS 29 121	GTCTG CAA CT CTAAT CCA MC TCCTT GTA MG GATCC TCC TG ACAAG TTG TT TACTG TTC MC GTCTG CAA CT TTACT GCT MC TCCTT GTA MG GATCC TCC TG ACAAG TTG TT TACGG TTC MC GTCTG CAA CT TTACT GCT MC TCCTT GTA MG GATCC TCC TG ACAAG TTG CAAG TCC TCC C2
MdS 24/Sh 1 MdS q 1 SaS 16 1 PcS b 74 MdS 29 181	TTCAACA AATAT ATTATTCAATGGGGACCGGGAT-GA TATAT
MdSg 37 SaS16 1 PcSb 133	ATGCT TAT CATCGTA TTT TCACTA - AT TT TATTA ATA TT CCCTT CCTCC ACCGT GGGAT ATGCT TAT CATCGTA TTT TCACTA - AT TT TATTA ATA TT GTCTT CCTC ACCGT GGGAT CACAC CCT DA TATTA TTA TCACTA - AT TT TATTA ATA TT GTCTT AT TCTC ATTTAT CTACT CACAC GGT DA TATTATA TCACTA CATAT CAGA TA TT GTCTTAT TTATTATA CACAC GGT DA TATTATA CCATAT TACTA CACAC GGT DA TATTATA CCATAT CAGA TA CTACTT CTGTCT - TCAAATAT TACCA ATATTACCA
MdS q 95	TCEAT TAT FATCAAT TTA CGCACCA ATA TCAGCCG GCT GTCTGCA ACT CT AAT OC TCEAT TAT FATCAAT TTA CGCACCA ATA TCAGC CG GCT GTCTGC ACT CT AAT OC CG GCT CCCTGCA ACT CT AAT OC TGTT GTG FATATAT ATA TTTTTCEATA ATGCT HGA CCCTC CA AAT TTTTAAAOC TTAAT TAAFACCAAATTT GTACATTAA-AGGATC-T GTCACCA TAT TAAAAAACAC TT
SaS16 23 PcSb 248 MdS29 347	AACTCCT AACTCCT AACTCCT AAATGATGTGTCACAATAAGATATAAGAAATTTAGTCTCTGACAAGTTGTTTACTGT AAATGATGTGTCACAATAAGATATAAGAAATTTAGTCTGTCT
MdSq 189 SaS16 62 PcSb 307 MdS29 398	$ \begin{array}{l} \mathrm{TCACGGFT} \ \mathrm{TGTGGC} & \mathrm{CI} - \cdots - \mathrm{TCARACT} \ \mathrm{GSAATGGAAA} \ \mathrm{TGA} & - \cdots - \mathrm{CCCRAAATATT} \ \mathrm{G-CAA} \\ \mathrm{TCACGGC} \ \mathrm{TGTGGC} & \mathrm{CI} - \cdots - \mathrm{TCARACT} \ \mathrm{GSAATGGAAA} \ \mathrm{TGA} & - \cdots - \mathrm{CCCRAAATATT} \ \mathrm{C-CAA} \\ \mathrm{TCACGGCT} \ \mathrm{TGTGGC} & \mathrm{CI} - \cdots - \mathrm{TCARACT} \ \mathrm{GSAATGGAAA} \ \mathrm{TGA} & - \cdots - \mathrm{CCCRAAATATT} \ \mathrm{C-CAA} \\ \mathrm{TTTGGATA} \ \mathrm{TGTATA} - \mathrm{CAAATATT} \ \mathrm{C-CAA} \\ \mathrm{TTTGGATA} \ \mathrm{TATAT} \ \mathrm{CAAATATT} \ \mathrm{C-CAA} \\ \mathrm{TTTGGATA} \ \mathrm{TATAT} \ \mathrm{CAAATATT} \ \mathrm{TC} \ \mathrm{CAAATG} \ \mathrm{TAAAAT} \ \mathrm{TTTACATAAATATT} \ \mathrm{CAAAATATT} \ \mathrm{C-CAA} \\ \mathrm{AAAATTTTACATAAATATTA \ \mathrm{CAAATATT} \ \mathrm{CCAAATATT} \ \mathrm{CCAAAC} \ \mathrm{TA-AACATATATC} \ \mathrm{CAAATATTT} \ \mathrm{CCAAATC} \ \mathrm{TAAAAT} \ \mathrm{TTAAAATTTTACATAAATTTT \ \mathrm{CAAATTTT} \ \mathrm{CAAATC} \ \mathrm{TAAATTT} \ \mathrm{CAAAATATTT} \ CAAAATTTTACATAATTTTACATAAATTTTACATAATTTACATAATTTTACATAATTTATCATAATTTACATAATTTACATAATTTATCATAATTTATATCATAATTTATATCATAATTTATATTTATATTTATTTATTTTTT$
MdSg 240 SaS16 113 PcSb 365	$\begin{array}{c} cgc = & - Ac P g c A At A t ca ca c gat g a A at a ctcg a a c c cagt g t a ttatt t g c c g g c = & - Ac P g c a c c t a t c a c a c g t A At A t a ctcg a a c c c c a g t g c at t a t t t t g c c g g c = & - Ac P g c c c t t t t c a c a c g t A At A t a ctcg a a c P c c a g t g c At t a t t g c c c g t t t t t t t g c c g g c = & - Ac P g c c c t t t t c a c a c g t a At A t a ctcg a a c C c c c f t g t a t t a t t t g c c c t t t t a t t t t t g c c c c c c c c c c c c c c$

SHORT COMMUNICATION

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.