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

one of 'Anna's *S*-RNases would be either *S2* or *S3*, 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 *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/A TTG TGG CCT TC-3' and NSA1, 5'-GAC CTC AAC C/TAA TTC AG -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 *S*-alleles predated speciation of the Rosaceae, but occurred shortly after the divergence into subfamilies (Ushijima et al., 1998).

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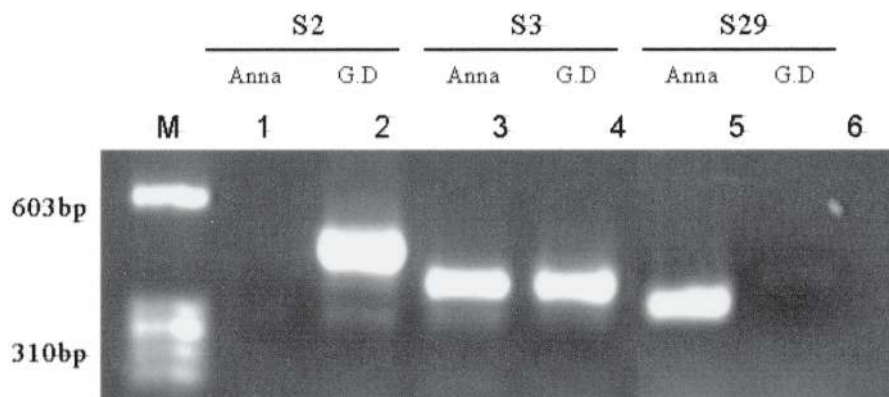Fig. 1. Identification of the 'Anna' *S*-RNase alleles. PCR for *S2*, *S3* and *S29* RNase alleles was carried out on DNA from 'Anna'. DNA of 'Golden Delicious' served as the control. Lanes 1 and 2 = primers for *S2*; Lanes 3 and 4 = primers for *S3*: Lanes 5 and 6 = primers for *S29*. Lane 1,3 and 5 = 'Anna', lanes 2,4, and 6 = 'Golden Delicious' and lane M = size marker.
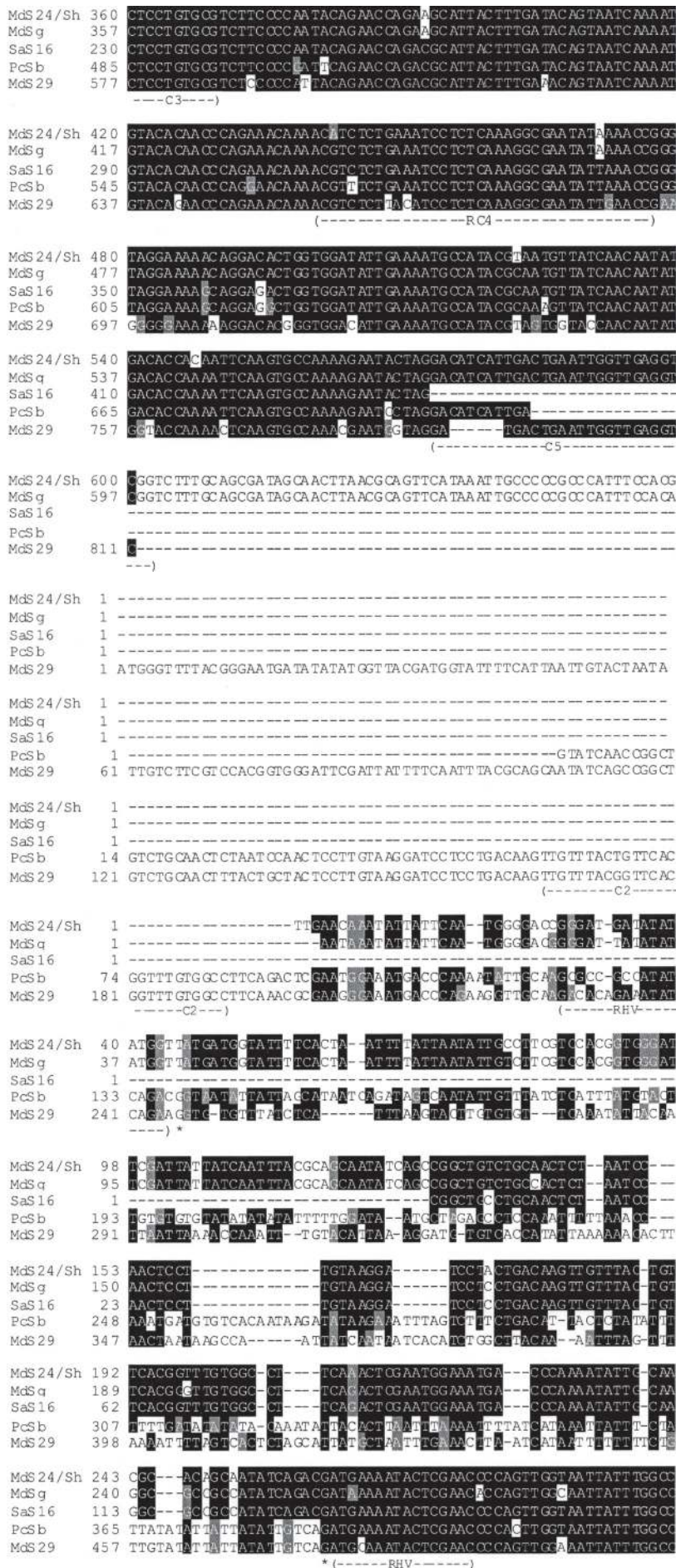
Fig. 2. Alignment of partial *S*-RNase gene sequences of apple *(Malus domestica)* S24/Sh (MdS24 Accession No. AF016920), apple *S*20/*S*g (MdSg–AB019184), rowan *(Sorbus aucuparia)* *S*16 (SaS16–AF504268), pear *(Pyrus communis)* *S*b (PcSb–AJ458182), and apple *S*29 (MdS29–AY039702). Black boxes indicate 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 *S*29 intron.