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Identification and Characterization of *S*-RNases in Tetraploid Sour Cherry (*Prunus cerasus*)

Hisayo Yamane,[1] Ryutaro Tao,[1] and Akira Sugiura
Laboratory of Pomology, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan

Nathanael R. Hauck and Amy F. Iezzoni
Department of Horticulture, Michigan State University, East Lansing, MI 48824

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Abstract. **This report demonstrates the presence of *S*-ribonucleases (*S*-RNases), which are associated with gametophytic self-incompatibility (SI) in *Prunus* L., in styles of self-incompatible and self-compatible (SC) selections of tetraploid sour cherry (*Prunus cerasus* L.). Based on self-pollen tube growth in the styles of 13 sour cherry selections, seven selections were SC, while six selections were SI. In the SI selections, the swelling of pollen tube tips, which is typical of SI pollen tube growth in gametophytic SI, was observed. Stylar extracts of these selections were evaluated by two-dimensional polyacrylamide gel electrophoresis. Glycoproteins which had molecular weights and isoelectric points similar to those of *S*-RNases in other *Prunus* sp. were detected in all selections tested. These proteins had immunological characteristics and N-terminal amino acid sequences consistent with the *S*-RNases in other *Prunus* sp. Two cDNAs encoding glycoproteins from 'Erdi Botermo' were cloned. One of them had the same nucleotide sequence as that of S^4-RNase of sweet cherry (*Prunus avium* L.), while the amino acid sequence from the other cDNA encoded a novel *S*-RNase (named S^a-RNase in this study). This novel RNase contained two active sites of T2/*S* type RNases and five regions conserved among other *Prunus S*-RNases. Genomic DNA blot analysis using cDNAs encoding *S*-RNases of sweet cherry as probes indicated that three or four *S*-RNase alleles are present in the genome of each selection regardless of SI. All of the selections tested seemed to have at least one *S*-allele that is also found in sweet cherry. Genetic control of SI/SC in tetraploid sour cherry is discussed based on the results obtained from restriction fragment length polymorphism analysis.**

Gametophytic self-incompatibility (SI) is a widespread mechanism in flowering plants which prevents self-fertilization and promotes outcrossing (de Nettancourt, 1977). Most rosaceous tree fruit crops exhibit this SI system which is controlled by a single locus (*S*-locus) with multiple alleles (de Nettancourt, 1977). In this system, pollen tube growth is arrested if the pollen tube has a *S*-allele which is in common with the style. Recently, the molecular mechanism of gametophytic SI in *Prunus* sp. has been studied, and *S*-gene products in pistils of almond [*Prunus dulcis* (Mill.) D.A. Webb (syn. *P. amygdalus* Batsch) (Tao et al., 1997; Ushijima et al., 1998), apricot (*P. armeniaca* L.) (Burgos et al., 1998), Japanese plum (*P. salicina* Lindl.) (Yamane et al., 1999), and sweet cherry (*P. avium*) (Boskovic and Tobutt, 1996; Tao et al., 1999b) were shown to be *S*-RNases, as is the case with other rosaceous crops, such as apple [*Malus sylvestris* (L.) Mill. var. *domestica* (Borkh.) Mansf.] (Broothaerts et al., 1995; Sassa et al., 1994, 1996) and pear (*Pyrus* L. sp.) (Ishimizu et al., 1996; Sassa et al., 1992, 1996; Tomimoto et al., 1996).

One interesting aspect of gametophytic SI is that it commonly breaks down as a result of polyploidy resulting in self-compatible (SC) individuals. This breakdown of gametophytic SI in polyploid plants with SI diploid relatives has been documented in *Lycopersicon* Mill. (Chawla et al., 1997; de Nettancourt et al., 1974), *Nicotiana* L. (Pandy, 1968), *Petunia* Juss. (Entani et al., 1999; Stout and Chandler, 1942), *Solanum* L. (Livermore and Johnstone, 1940), *Trifolium* L. (Brewbaker, 1954), and *Pyrus* (Crane and Lewis, 1942). In *Prunus*, this phenomenon is exhibited in the diploid sweet cherry (2n = 2x = 16) and the tetraploid sour cherry (*P. cerasus* L., 2n = 4x = 32). *Prunus cerasus* (sour cherry) is thought to have resulted from hybridization between sweet cherry and the tetraploid ground cherry (*P. fruticosa* Pall., 2n = 4x = 32) (Brettin et al., 2000; Olden and Nybom, 1968). Sour cherry cultivars are predominately SC, however, SI types have been reported by pollination tests (Lansari and Iezzoni, 1990; Redalen, 1984a, 1984b). In addition, SI sour cherry selections can result from crosses between two SC parents (Lansari and Iezzoni, 1990). In contrast to sweet cherry, inheritance of SI in sour cherry has not been determined and no *S*-allele designations have been proposed. Since sour cherry is a *Prunus* sp. that has SI individuals, it is likely to possess *S*-alleles encoding pistil *S*-RNases. To date, no studies of *S*-RNases in sour cherry have been reported. In this study, evidence is presented that both SI and SC sour cherry selections have stylar RNases, some of which appear to be similar to the *S*-RNases already cloned from sweet cherry.

Table 1. Sweet cherry cultivars used as *S*_ allele standards and their *S*_ genotypes (Hauck et al., 2001).

Cultivar	*S*_ genotype
Early Rivers	S^1S^2
Bing	S^3S^4
Gaucher, Hedelfingen	S^3S^5
Gold	S^3S^6
Charger	S^1S^7
Guigne d'Annonay	S^2S^7
Burlat, Mona	S^3S^9
Inge	S^4S^9
Orleans 171	$S^{10}S^{11}$
Schneiders	S^3S^{12}

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[1]Research fellow of the Japanese Society for the Promotion of Science.
[2]Corresponding author; e-mail rtao@kais.kyoto-u.ac.jp.

Materials and Methods

Plant material. Thirteen sour cherry selections growing at the Michigan State University Clarksville Horticultural Experiment Station, Clarksville, Mich., were used for this research: 'Cigany 59', 'Crisana', 'Erdi Botermo', 'Erdi Nagygyumolcsu', 'Favorit', 'Montmorency', 'Pandy 38', 'Pandy 114', 'Rheinische Schattenmorelle', 'Surefire', 'Tschernokorka', 'Ujfehertoi Furtos', and MSU seedling selection I 20 (36). Twelve sweet cherry cultivars were used to provide standards for the previously reported *S*-alleles, S^1, S^7, S^9, S^{12} (Table 1). Leaves of 'Bing', 'Hedelfingen', and 'Gold' were collected from the Michigan State University North West Horticultural Research Station, Traverse City, Mich. Leaves of 'Early Rivers' and 'Burlat' were kindly provided by J. Soejima (Morioka, Japan) and leaves of 'Charger', 'Gaucher', 'Inge', and 'Orleans 171' were kindly provided by K. Tobutt (East Malling, United Kingdom) and leaves of 'Guigne d' Annonay', 'Schneiders', were kindly provided by C. Choi and R. L. Andersen (Geneva, N.Y.). Leaves of 'Mona' were obtained from the USDA National Clonal Repository, Davis, Calif. The leaf material was prepared for DNA isolation as described by Hauck et al. (2001).

Pollen tube growth assays. Pollination tests were performed based on Lansari and Iezzoni (1990) but with substantial modifications. Pollen from newly opened flowers was collected from both the cultivars being tested (self pollen) and from a bulk consisting of several cultivars (outcross pollen). Ten emasculated flowers were hand pollinated when receptive (24 h after emasculation) with self-pollen in the lab (25 °C). The other 10 flowers were pollinated with outcross pollen in the lab (25 °C). The outcross and self-pollinated pistils were collected 72 h after pollination and immersed in fixing solution [(1 chloroform : 3 (95%) ethanol : 1 glacial acetic acid) (v/v)] for 24 h, transferred to 100% ethanol, and stored at 4 °C until used. The pistils were washed thoroughly under running tap water and incubated in 10 N NaOH for 5 to 6 h to soften the tissues. The pistils were then soaked in 0.1 % aniline blue solution with 33 mM K_3PO_4 for 1 h. Pollen tubes were observed by ultraviolet fluorescent microscopy (BX60; Olympus, Tokyo, Japan).

Protein assay. Styles with stigmas were dissected from flower buds of 11 sour cherry selections at the balloon stage of development, frozen immediately in liquid nitrogen, and lyophilized. Acetone powder was prepared from the lyophilized samples as described previously (Tao et al., 1997) and used for protein assay. Crude extracts from the acetone powder were subjected to two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) using nonequilibrium pH gradient electrophoresis (NEPHGE) in the first dimension and sodium dodecyl sulfate (SDS)-PAGE in the second dimension (Tao et al., 1997). After electrophoresis, proteins in the gel were detected by silver staining using Sil-Best Stain for Protein/PAGE (Nacalai tesque, Kyoto, Japan).

For 'Rheinische Schattenmorelle' and 'Erdi Botermo', the proteins separated by 2D-PAGE were electroblotted onto a polyvinilidene difluoride (PVDF) membrane as described previously (Tao et al., 1997) and glycoproteins were detected with biotin-conjugated concanavalin A (Con A) and horseradish peroxidase-conjugated streptavidin. Peroxidase activity on the membrane was visualized using 4-chloro-1-naphthol. Immunodetection of proteins electroblotted onto PVDF membrane was also conducted for 'Rheinische Schattenmorelle' and 'Erdi Botermo' using the rabbit anti-S^c-serum prepared against recombinant S^c-RNase of almond (Ushijima et al., 2001). In addition, for 'Erdi Botermo', after the proteins were blotted onto the PVDF membrane and detected by Coomassie Blue staining, the portion of the PVDF membrane carrying the proteins of interest was cut out and used. This sample was divided further into two portions, one containing a higher molecular weight protein and one containing a lower molecular weight protein. The N-terminal amino acid sequence of each protein was determined using a gas-phase protein sequencer (476A, Applied Biosystems, Tokyo, Japan) as described by Tao et al. (1997).

cDNA library construction and screening. Total RNA was isolated from 'Erdi Botermo' styles with stigmas at the balloon stage of development as described by Tao et al. (1999b). Double-stranded cDNA was synthesized from the poly $(A)^+$ RNA isolated from the total RNA, cloned into Lambda ZAP II vector (Stratagene, La Jolla, Calif.), and packaged in vitro using MaxPlax Packaging Extract Kit (Epicentre Technologies, Madison, Wis.) as described by Tao et al. (1999b).

One microgram of total RNA from 'Erdi Botermo' was used for first strand cDNA synthesis by SUPER SCRIPT II RT (Life Technologies, Tokyo, Japan) with an Adp-dT primer (5'-CGACGTTGTAAAACGACGGCCAGTTTTTTTTTTTTTTT-3') that consisted of M13-20 sequence primer and oligo $(dT)_{16}$ (Tao et al., 1999b). Pru-T2 primer (5'-TSTTSTTGS-TTTTGCTTTCTTC-3') (Tao et al., 1999b) derived from the cDNA sequence corresponding to the signal peptide sequence of *S*-RNases of sweet cherry was used in 3' rapid amplification of cDNA ends (3' RACE) with M13-20 primer as the adapter primer. Polymerase chain reaction (PCR) condition was identical to that used by Tao et al. (1999b). The PCR products were subcloned into the T-A cloning vector (pGEM-T Easy Vector System; Promega, Madison, Wis.). DNA sequences of the inserts of several clones were determined using the Dye Terminator Cycle Sequencing Kit (Applied Biosystems, Tokyo, Japan) and the ABI PRISM™ 310 Genetic Analyzer (Applied Biosystems, Tokyo, Japan).

Putative 3'RACE clones for *S*-RNases obtained as described above were PCR amplified by Pru-C2 (5'-CTATGG-CCAAGTAATTATTCAAACC-3') and Pru-C4R (5'-GGATGT-GGTACGATTGAAGCG-3') primers (Tao et al., 1999b). PCR products obtained were labeled by random primer incorporation of DIG-dUTP (Boehringer Mannheim, Tokyo, Japan) and used as probes to screen the primary cDNA library consisting of 1×10^6 plaque forming units. The cDNA library was screened as described by Tao et al. (1999b), selected clones were converted to pBluescript plasmids, and DNA was sequenced as described above. The deduced amino acid sequences of two kinds of cDNAs obtained in this study and sweet cherry S^4-RNase were aligned using Clustal X (Thompson et al., 1997).

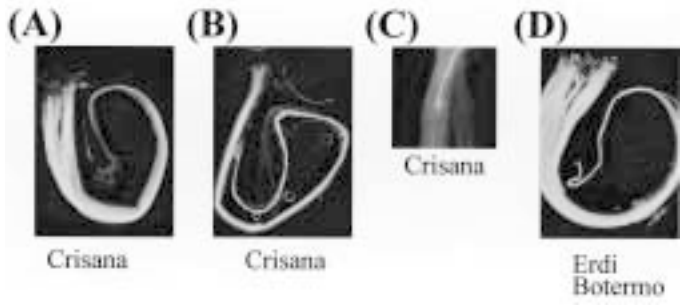


Fig. 1. Pollen tube growth in sour cherry pistils. The pistils were purposely curved before the photographs were taken. (**A**) Self-pollinated style of the self-incompatible selection, 'Crisana'. (**B**) Out-cross styles of 'Crisana'. (**C**) Swelling of the pollen tube tip in self-pollinated pistil of 'Crisana'. (**D**) Self-pollinated styles of the self-compatible selection, 'Erdi Botermo'.

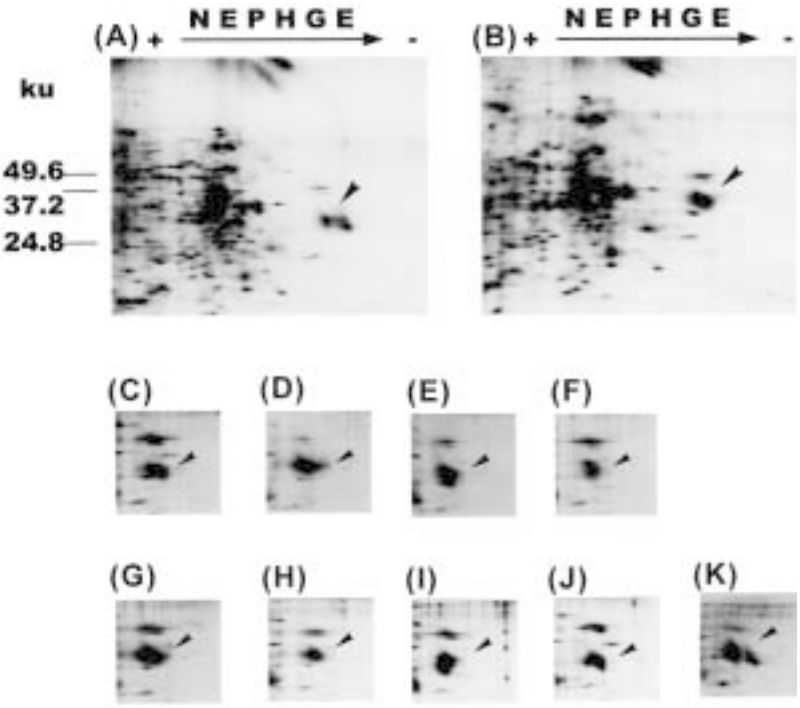


Fig. 2. Identification of *S*-RNases of 11 sour cherry selections. (**A–F**) stylar proteins from six self-compatible selections and (**G–K**) five self-incompatible selections were separated by 2D-PAGE and detected by silver staining. *S*-RNase spots are marked with arrows. (u = unified atomic mass unit). (**A**) 'Rheinische Schattenmorelle', (**B**) 'Erdi Botermo', (**C**) 'Montmorency', (**D**) 'Ujfehertoi Furtos', (**E**) 'Favorit', (**F**) 'Surefire', (**G**) 'Pandy 114', (**H**) 'Crisana', (**I**) 'Erdi Nagygyumolcsu', (**J**) 'Tschernokorka', and (**K**) MSU seedling selection I 20 (36).

Genomic DNA blot analysis. Total DNA was isolated from young leaves by the hexadecyltrimethylammonium bromide (CTAB) method described by Stockinger et al. (1996). Genomic DNA blot analysis was performed as described by Hauck et al. (2001). Probe cDNAs were prepared from PCR-amplified fragments of the S^4- and S^6-RNase cDNAs from sweet cherry and radiolabelled with ^{32}P-dCTP as described by Hauck et al. (2001).

PCR amplification, cloning, and DNA sequencing of PCR-amplified fragments of the *S*-RNase fragments. As described above for the genomic DNA blot analysis, total DNA was isolated from 11 sour cherry selections and used as template DNA for PCR. PCR procedures were identical to those used by Tao et al. (1999b). PCE-R primer (5'-TGTTTGTTCCATTCGCYTTCCC-3'), which corresponds to the previously identified C3 conserved domain of rosaceous *S*-RNases (Ushijima et al., 1998), was designed based on the nucleotide sequences of obtained sour cherry *S*-RNase cDNAs. After PCR with Pru-C2 (Tao et al., 1999b) and PCE-R primer, the PCR mixture was run on 1.5% agarose gel and the DNA bands were visualized by ethidium bromide staining. Furthermore, PCR products from 'Erdi Botermo' were subcloned into the T-A cloning vector (pGEM-T Easy Vector System; Promega, Madison, Wis.) and their sequences were determined as described above.

Results and Discussion

Evaluation of self-incompatibility or self-compatibility in sour cherry selections. Six selections, 'Erdi Nagygyumolcsu', 'Crisana', 'Pandy 38', 'Pandy 114', 'Tschernokorka' and MSU seedling selection I 20 (36), were SI and no self-pollen tubes reached the ovule 72 h after pollination in any of the 10 pistils observed (Fig. 1A), while outcross pollen tubes reached the ovule (Fig. 1B). Self-pollen tube growth of these selections was inhibited in the middle part of stylar tissue (Fig. 1A), and swelling of pollen tube tips, which is a typical reaction of gametophytic SI, was observed in the SI sour cherry selections (Fig. 1C). Seven selections, 'Cigany 59', 'Erdi Botermo', 'Montmorency', 'Favorit', 'Rheinische Schattenmorelle', 'Surefire', and 'Ujfehertoi Furtos' were SC because self-pollen tubes successfully reached the ovule (Fig. 1D). These results were consistent with previous observations (Iezzoni, 1996; Lansari and Iezzoni, 1990; Redalen, 1984a, 1984b).

Protein assay. After 2D-PAGE and silver staining of stylar extracts from six SC selections ('Erdi Botermo', 'Montmorency', 'Favorit', 'Rheinische Schattenmorelle', 'Surefire', and 'Ujfehertoi Furtos') and five SI selections ('Erdi Nagygyumolcsu', 'Crisana', 'Pandy 114', 'Tschernokorka', and MSU seedling selection I 20 (36)), protein spots were detected (Fig. 2), that had similar molecular weights and isoelectric points to those of other *S*-RNases in other SI *Prunus* sp., such as almond (Tao et al., 1997), sweet cherry (Tao et al., 1999b) and Japanese plum (Yamane et al., 1999). Furthermore, for 'Rheinische Schattenmorelle' and 'Erdi Botermo', these stylar proteins were shown to be glycoproteins that contained sugar chains reacting with Con A (Fig. 3A) and exhibited an immunoreaction with anti S^c-serum raised against S^c-RNase of almond (Fig. 3B). This result indicates that they are immunologically similar to *S*-RNases of other *Prunus* sp. The putative *S*-RNase spot from 'Erdi Botermo' was divided into two regions of higher (spot EB_H) and lower (spot EB_L) molecular weight. The N-terminal amino acid sequences of these proteins were homologous to those of *S*-RNases of other *Prunus* sp. (Fig. 4) (Tao et al., 1997, 1999b; Yamane et al., 1999).

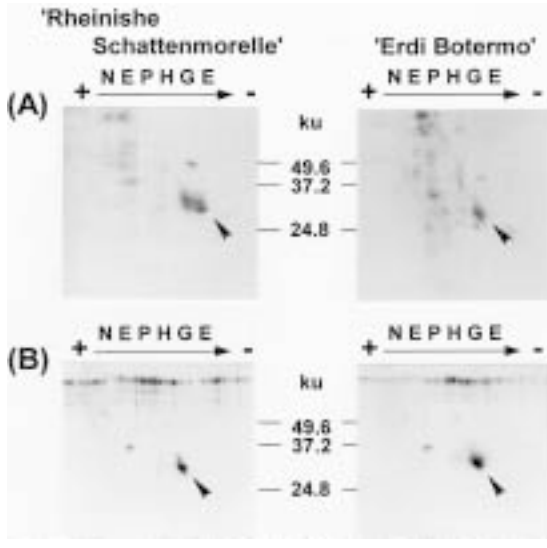


Fig. 3. *S*-RNase proteins from 'Rheinische Schattenmorelle' and 'Erdi Botermo'. Proteins in the 2D-PAGE gel were blotted to a PVDF membrane and (**A**) detected by glycoprotein staining with Con A and (**B**) immunodetection with the anti-S^c-serum prepared from almond S^c-RNase (u = unified atomic mass unit). *S*-RNase spots are marked with arrows.

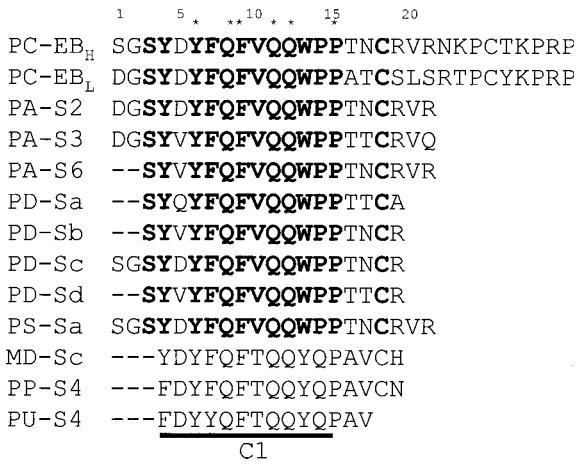


Fig. 4. Alignment of N-terminal amino acid sequences of *S*-RNases of sour cherry 'Erdi Botermo' and other rosaceous *S*-RNases. Amino acid sequences corresponding to the C1, a previously reported conserved domain of *S*-RNases in Rosaceae (Ishimizu et al., 1998; Ushijima et al., 1998), are indicated at the bottom. Amino acid residues conserved in all *Prunus S*-RNases are indicated by bold face and those conserved in all rosaceous *S*-RNases are marked with asterisks. Sequence data for the *S*-RNases included are as follows: PC-EB$_{H, L}$ [corresponding to the spot EB$_H$ and EB$_L$, respectively, from *Prunus cerasus* ('Erdi Botermo' sour cherry) (this study)]; PA-S2, S3, and S6 [S^2, S^3, and S^6-RNases of *P. avium* (sweet cherry) (Tao et al., 1999b)]; PD-Sa, Sb, Sc, and Sd [S^a, S^b, S^c, and S^d-RNases of *P. dulcis* (almond) (Tamura et al., 2000; Ushijima et al., 1998)]; PS-Sa [S^a-RNase of *P. salicina* (Japanese plum) (Yamane et al., 1999)]; MD-Sc [S^c-RNase of *Malus sylvestris* var. *domestica* (apple) (Sassa et al., 1996)]; PP-S4 [S^4-RNase of *Pyrus pyrifolia* (Japanese pear) (Sassa et al., 1996)]; PU-S4 [S^4-RNase of *Pyrus ussuriensis* (Chinese pear) (Tomimoto et al., 1996)].

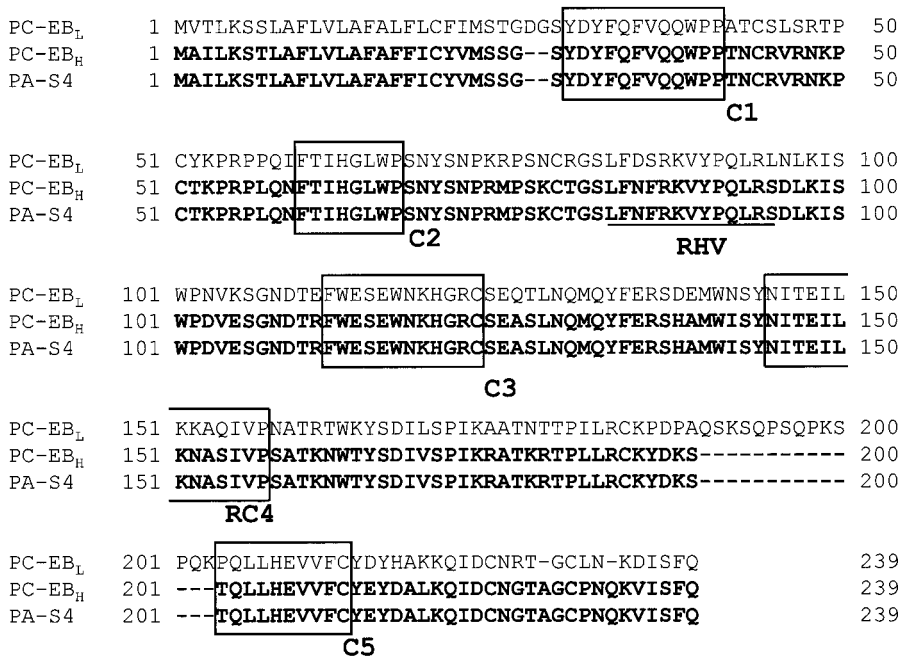
cDNA CLONING. For 3'RACE using the Pru-T2 primer corresponding to the signal peptide sequence of *S*-RNases of sweet cherry, 10 clones that could be nested PCR-amplified by the AS1 (5'-TATTTTCAATTTGTNCAGCAATGG-3') and Pru-C4R primer set that were designed based on conserved regions of *S*-RNases of *Prunus* (Tao et al., 1999b), were obtained. Nine clones had the same nucleotide sequence and their deduced amino acid sequence contained the N-terminal amino acid sequence of the spot EB$_H$ from 'Erdi Botermo' (Fig. 4). The remaining one clone contained the N-terminal amino acid sequence encoding the spot EB$_L$ from 'Erdi Botermo' (Fig. 4). These two 3'RACE clones were further PCR amplified by Pru-C2 and Pru-C4R primers and used as probes to screen the cDNA library. About 5000 plaques were screened, and several positive plaques were converted to pBluescript and DNA sequenced. Two different kinds of full-length cDNA clones corresponding to two spots, EB$_H$ and EB$_L$, were obtained. The coding region of one cDNA clone encoding the spot EB$_H$ was exactly the same as the S^4-cDNA of sweet cherry (Fig. 5) (Tao et al., 1999a). The other cDNA clone encoding the spot EB$_L$ contained five conserved regions in rosaceous *S*-RNases (Ushijima et al., 1998) but did not share the same DNA sequence with any of known *S*-RNase sequences, suggesting that it encodes a novel *S*-RNase (named S^a-RNase in this study) in sour cherry (Fig. 5).

***S*-RNASE ALLELES IN TWELVE SOUR CHERRY SELECTIONS.** Hybridization signals with the cDNA probes encoding S^4-, and S^6-RNases of sweet cherry were observed with all 12 sour cherry selections tested (Fig. 6). With *Hin*dIII and *Xba*I digestions, four different *S*-alleles seemed to be present in three selections, 'Cigany 59', 'Erdi Nagygyumolcsu', and 'Rheinische Schattenmorelle', and three different *S*-alleles were present in all other selections (Table 2; Fig. 6). Three selections, 'Crisana', 'Pandy 38', and 'Pandy 114', yielded the same banding pattern for both blots, which is consistent with the fact that 'Crisana' and 'Pandy' represent selections from the same landrace (Iezzoni, 1996).

Based on the RFLP analysis, 'Erdi Botermo' appears to have three different *S*-alleles: S^4, S^a, and S^6 (Table 2; Fig. 6). In the *Xba*I digest, 'Erdi Botermo' exhibited the fragment that corresponds to the sweet cherry S^6-allele, however, the fragment identified from the *Hin*dIII digest showed different size from that of the sweet cherry S^6-allele. To resolve this discrepancy, the 'Erdi Botermo' fragment that was associated with the S^6-allele was amplified by PCR (Fig. 7) and sequenced. Sequencing data showed this fragment contained a partial DNA sequence unique to the S^6-RNase gene (data not presented). Therefore, we propose that 'Erdi Botermo' has a S^6-allele that has an altered *Hin*dIII cut site. The mutation might affect transcription of the allele as cDNA encoding S^6-RNase could not be obtained by screening of the stylar cDNA library or 3'RACE from the mRNA from the style of 'Erdi Botermo'. This conclusion is also indicated by the fact that the protein spot corresponding to S^6-RNase was absent in the 2D-PAGE profile of 'Erdi Botermo'.

The band corresponding to the S^1-allele of sweet cherry was found in four SI selections, 'Erdi Nagygyumolcsu', 'Crisana', 'Pandy 38', and 'Pandy 114'. The band corresponding to the S^4-allele was found in three SC selections ('Erdi Botermo', 'Sure-

Fig. 5. Amino acid sequence alignment of two *S*-RNases, EB$_L$ and EB$_H$, from 'Erdi Botermo' sour cherry and sweet cherry S^4-RNase. The alignment was generated by CLUSTAL X (Thompson et al., 1997). Gaps are marked by dashes. The five conserved regions, C1, C2, C3, RC4, and C5 (Ushijima et al., 1998) are shown and boxed, and hypervariable region, RHV (Ushijima et al., 1998), reported in rosaceous *S*-RNases are shown under the alignment and underlined. Sequence data for the *S*-RNases included are as follows: PC-EB$_L$, EB$_H$ [corresponding to the spot EB$_L$ and EB$_H$, respectively, from *Prunus cerasus* ('Erdi Botermo' sour cherry) (this study)], PA-S4 [S^4-RNase of *P. avium* (sweet cherry) (Tao et al., 1999a)].



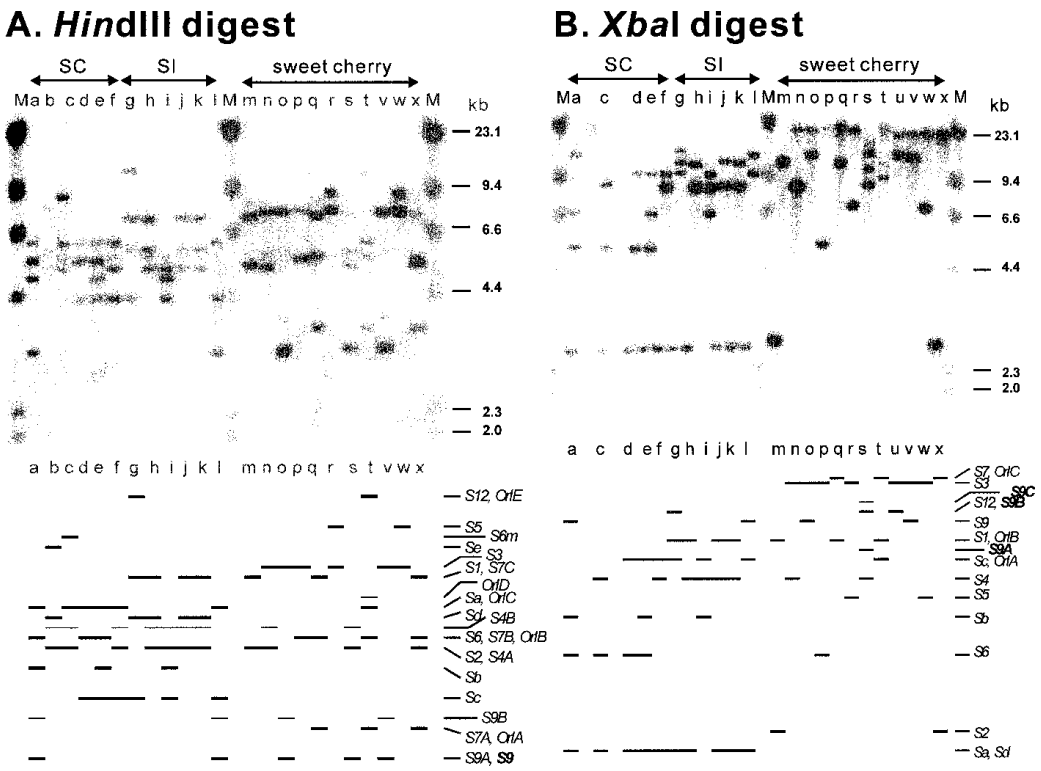


Fig. 6. Genomic DNA blot analysis of 12 sour cherry selections. Six micrograms of genomic DNA were digested by (A) *Hin*dIII or (B) *Xba*I and hybridized to the cDNAs encoding (A) S^6-RNase or (B) S^4-RNase, respectively. M: Lambda/*Hin*dIII marker. (**a**) 'Cigany 59', (**b**) 'Ujfehertoi Furtos', (**c**) 'Erdi Botermo', (**d**) 'Montmorency', (**e**) 'Rheinische Schattenmorelle', (**f**) 'Surefire', (**g**) 'Erdi Nagygyumolcsu', (**h**) 'Crisana', (**i**) MSU seedling selection I 20 (36), (**j**) 'Pandy 38', (**k**) 'Pandy 114', (**l**) 'Tschernokorka', (**m**) 'Early Rivers' (S^1S^2), (**n**) 'Bing' (S^3S^4), (**o**) 'Burlat' (S^3S^9), (**p**) 'Gold' (S^3S^6), (**q**) 'Charger' (S^1S^7), (**r**) 'Gaucher' (S^3S^5), (**s**) 'Inge' (S^4S^9), (**t**) 'Orleans 171' ($S^{10}S^{11}$), (**u**) 'Schneiders' (S^3S^{12}), (**v**) 'Mona' (S^3S^9), (**w**) 'Hedelfingen' (S^3S^5), and (**x**) 'Guigne d'Annonay' (S^2S^7). *Orl* represents S^{10} and S^{11} found in 'Orleans 171'. If more than one fragment corresponds to an allele, A is used to designate the smallest fragment, B the next smallest, etc. The fragments corresponding to the S^9-allele from 'Inge' are bold, whereas the fragments corresponding to the S^9-allele from 'Burlat' are not bold.

fire', and 'Ujfehertoi Furtos') and in four SI selections ('Crisana', 'Pandy 38', 'Pandy 114', and MSU seedling selection I 20 (36)) (Table 2; Fig. 6). Similarly, four SC selections ('Cigany 59', 'Erdi Botermo', 'Montmorency', and 'Rheinische Schattenmorelle') were shown to have the S^6-allele of sweet cherry. One SC selection ('Cigany 59') and one SI selection ('Tschernokorka') were shown to have the S^9-allele of the sweet cherry cultivars 'Burlat' and 'Mona' (Tables 1 and 2; Fig. 6). 'Erdi Nagygyumolcsu' exhibited the band corresponding to the S^{12}-allele of the sweet cherry cultivar 'Schneiders' (Tables 1 and 2; Fig. 6).

Five SC selections ('Cigany 59', 'Erdi Botermo', 'Montmorency', 'Rheinische Schattenmorelle', and 'Surefire') and one SI selection ('Tschernokorka') yielded the band that was considered to encode the novel putative *S*-RNase from 'Erdi Botermo', named S^a-RNase in this study (Table 2; Fig. 6). The other four novel putative *S*-alleles, named S^b-, S^c-, S^d-, and S^e-alleles of sour cherry, were observed in several selections (Table 2, Fig. 6). These putative *S*-alleles were assigned a letter, as opposed to numerical designations, because they have yet to be confirmed to act as SI alleles through crossing studies. PCR amplification of the sour cherry *S*-alleles was consistent with the RFLP analysis (Fig. 7). Recently, at least 14 *S*-alleles, S^1- to S^7-, S^9- to S^{13}-, and two new putative *S*-alleles, both found in 'NY1625', have been confirmed (Hauck et al., 2001). Although the five novel *S*-alleles, S^a- to S^e-, identified in this study were different from the sweet cherry S^1- to S^7-, S^9- to S^{12}-alleles, it is possible that these novel *S*-alleles are present in other sweet cherry clones.

The ultimate goal of this research is to determine genetic control of SI/SC in tetraploid sour cherry. Lewis (1947) proposed that heterogenic pollen loses its SI phenotype, which causes breakdown of SI in polyploid plants with diploid relatives possessing a gametophytic SI system. To date, some observations that are consistent with this hypothesis have been obtained. As demonstrated in a tissue culture-derived *Lycoper-sicon peruvianum* Mill. selection, when an $S^1S^1S^2S^2$ individual was self-pollinated, only S^1S^2 pollen achieved fertilization (Chawla et al., 1997). In artificially induced tetraploid lines of *Petunia ×hybrida* Hort. Vilm.-Andr., the obtained $S^{B1}S^{B1}S^{B1}S^{B1}$ homoallelic tetraploid remained self-incompatible, whereas the $S^{B1}S^{B1}S^{B2}S^{B2}$ heteroallelic tetraploid became self-compatible (Entani et al., 1999). In addition, Golz et al. (1999) found breakdown of SI in mutated diploid plants with an extra *S*-allele generated by irradiation. The hypothesis of Lewis (1947) and the observations of Chawla et al. (1997), Entani et al. (1999), and Golz et al. (1999) indicate that tetraploid plants with more than two *S*-alleles are SC, and SI is found only when all of the four *S*-alleles are the same. In this study, however, we identified three or four different *S*-alleles in each SI sour cherry selection. If these *S*-alleles are functional in both pollen and stylar parts, it appears that our results are inconsistent with the hypothesis of Lewis (1947). It is possible that SI/SC in sour cherry is controlled by not only *S*-alleles but also other factors, such as the HT-protein of *Nicotiana* sp. (McClure et al., 1999). Alternatively, it is possible that the identified *S*-alleles could be mutated such that *S*-proteins of pollen part and/or stylar part are nonfunctional. The genetic control of SI/SC in naturally occurring tetraploid sour cherry is apparently more complicated than that in artificially produced tetraploid tomato (*Lycopersicon peruvianum*) (Chawla et al., 1997) and petunia (Entani et al.,1999).

In conclusion, it appears that *S*-RNases including sweet cherry *S*-RNases are present in the styles of each SI or SC sour cherry selections. A project to determine genetic control of SI/SC in tetraploid sour cherry is currently underway, utilizing a sour cherry cross, 'Rheinische Schattenmorelle' x 'Erdi Botermo', in which the progeny are segregating for SI.

Table 2. Putative *S*_ alleles of 12 sour cherry selections predicted by their RFLP kb size on Southern blots following *Hin*dIII and *Xba*I digests. S^1, S^4, S^6, S^9, and S^{12} refer to *S*-alleles already identified in sweet cherry (Hauck et al., 2001) while S^a, S^b, S^c, S^d, and S^e are assigned to the novel putative *S*-alleles identified in sour cherry.

	Sweet cherry *S* alleles								Novel *S* alleles						
	S1	S4[z]	S4[z]	S6m[y]	S6	S9[x]	S9[x]	S12	Sa	Sb	Sc	Sd	Se		
	kb size								kb size					No. of	Identified
Digest	8.7	5.6	6.1	9	5.8	3.1	4	12.1	6.4	5.1	4.6	6.2	9.6	*S*_ alleles	*S*_ alleles
*Hin*dIII															
Self-compatible cultivars															
Cigany 59					x	x	x		x	x				4	S6, S9, Sa, Sb
Ujfehertoi Furtos		x	x									x	x	3	S4, Sd, Se
Erdi Botermo		x	x	x					x					3	S4, S6m, Sa
Montmorency					x				x		x			3	S6, Sa, Sc
Rheinische Schattenmorelle					x				x	x	x			4	S6, Sa, Sb, Sc
Surefire		x	x						x		x			3	S4, Sa, Sc
Self-incompatible cultivars															
Erdi Nagygyumolcsu	x							x			x	x		4	S1, S12, Sc, Sd
Crisana	x	x	x										x	3	S1, S4, Sd
MSU seedling I 20 (36)		x	x							x	x			3	S4, Sb, Sc
Pandy 38	x	x	x									x		3	S1, S4, Sd
Pandy 114	x	x	x									x		3	S1, S4, Sd
Tschernokorka						x	x		x		x			3	S9, Sa, Sc

	Sweet cherry *S*_ alleles					Novel *S*_ alleles				
	S1	S4	S6	S9	S12	Sa, Sd	Sb	Sc		
	kb size					kb size			No. of	Identified
Digest	13	8.8	5.5	15	16	2.4	5	9.4	*S*_ alleles	*S*_ alleles
*Xba*I										
Self-compatible cultivars										
Cigany 59			x	x		x	x		4	S6, S9, Sa, Sb
Erdi Botermo		x	x			x			3	S4, S6, Sa
Montmorency			x			x		x	3	S6, Sa, Sc
Rheinische Schattenmorelle			x			x	x	x	4	S6, Sa, Sb, Sc
Surefire		x				x		x	3	S4, Sa, Sc
Self-incompatible cultivars										
Erdi Nagygyumolcsu	x				x	x		x	4	S1, S12, Sc, Sd
Crisana	x	x				x			3	S1, S4, Sd
MSU seedling I 20 (36)		x					x	x	3	S4, Sb, Sc
Pandy 38	x	x				x			3	S1, S4, Sd
Pandy 114	x	x				x			3	S1, S4, Sd
Tschernokorka				x		x		x	3	S9, Sa, Sc

[z]The S^4_ allele band is identified by two fragments of unequal intensity. The 6.1 kb band is faint and sometimes not found because of short exposure time as described by Hauck et al. (2001).

[y]'Erdi Botermo' has a mutated S^6_ allele which has an altered *Hin*dIII cut site (see text).

[x]The S^9_ allele band is identified by two fragments of unequal intensity. The 4-kb band is faint and sometimes not found because of short exposure time as described by Hauck et al. (2001).

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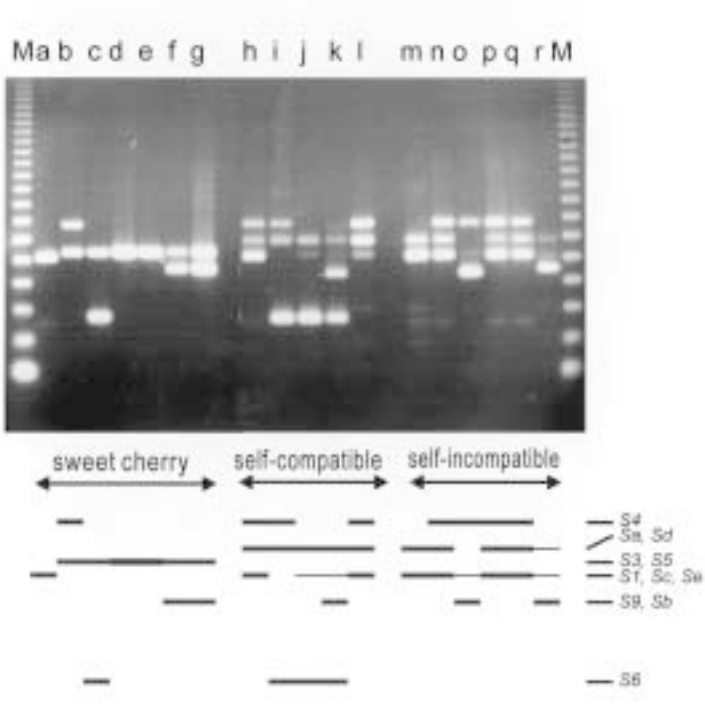

Fig. 7. PCR analysis for *S*-alleles of 11 sour cherry selections. Genomic DNA was PCR amplified with Pru-C2 (Tao et al., 1999b) and PCE-R primer set, separated on an agarose gel and detected with ethidium bromide staining. M: 123 bp DNA ladder. (**a**) 'Early Rivers' (S^1S^2), (**b**) 'Bing' (S^3S^4), (**c**) 'Gold' (S^3S^6), (**d**) 'Hedelfingen' (S^3S^5), (**e**) 'Gaucher' (S^3S^5), (**f**) 'Burlat' (S^3S^9), (**g**) 'Mona' (S^3S^9), (**h**) 'Ujfehertoi Furtos', (**i**) 'Erdi Botermo', (**j**) 'Montmorency', (**k**) 'Rheinische Schattenmorelle', (**l**) 'Surefire', (**m**) 'Erdi Nagygyumolcsu', (**n**) 'Crisana', (**o**) MSU seedling selection I 20 (36), (**p**) 'Pandy 38', (**q**) 'Pandy 114', and (**r**) 'Tschernokorka'.

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