Combined Analysis of S-Alleles in European Pear by Pollinations and PCR-based S-Genotyping; Correlation between S-Phenotypes and S-RNase Genotypes

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ABSTRACT. Pollen–pistil incompatibility in european pear (*Pyrus communis* L.) compromises adequate orchard pollination and fruit set and restricts cross-fertility between cultivars suitable as parents in breeding programs. Genetic control is simple, with a single locus expressed gametophytically in pollen controlling the rejection of the pollen tube in the style. Semicompatible pollination arises when only one allele of a pollen parent matches the pistil. Semicompatible test-crosses using partially S-genotyped european pear cultivars allowed the discrimination of 14 S-alleles (S₁ to S₁₄) at the phenotypic level and the assignment of 33 cultivars to 13 incompatibility groups. Partial genomic sequences of the S-RNase gene, spanning between the C1 and C5 conserved regions, were obtained for each new S-allele identified (S₆ to S₁₄). These sequences and those reported previously for the S₁ to S₅ RNases allowed a set of consensus primers amplifying all 14 S-RNase alleles to be designed. Allele-specific PCR allowed discrimination between those S-RNases giving amplification products of similar size with consensus primers. These two approaches provided a method for the molecular identification of all 14 S-alleles in european pear. With this methodology, we demonstrate that the S-RNase genotypes inferred from PCR exactly matches the S-phenotypes deduced from test-crosses. Comparison of the sequences obtained with those of S-RNases already published allowed us to relate S-alleles between studies. This will allow the prediction of cross-incompatibility among an even larger number of european pear cultivars.

European pear, like other fruit species of the Rosaceae, is impaired in effecting self-fertilization by a gametophytic self-incompatibility (GSI) system (Crane and Lewis, 1942). In the Rosaceae, GSI is inherited as a single multiallelic locus (S-locus) which controls both self-incompatibility and cross-incompatibility among cultivars (de Nettancourt, 2001). Because the specificity of the incompatibility reaction relies on genes encoded by the S-locus, the cross-sterility or cross-fertility of two diploid plants can be predicted in most cases

solely on the basis of their S-genotypes. S-genotyping is therefore a priority of cultivar characterization in many fruit species because this provides growers and breeders with valuable information to avoid the selection of incompatible combinations of cultivars when designing plantations or performing crosses in breeding programs (Kester et al., 1994; Tehrani and Lay, 1991). Test-crosses to characterize cross-(in)compatibilities among cultivars, together with the knowledge of their pedigrees, have traditionally been the basis for the assignment of S-genotypes in a number of species (Crane and Brown, 1937; Kester et al., 1994; Kobel et al., 1939). More recently, the finding that the S-locus encodes a ribonuclease (S-RNase) in the Rosaceae (Bošković and Tobutt, 1996; Broothaerts et al., 1995) has allowed further development of biochemical and molecular strategies for S-genotyping.

The majority of the molecular methods for S-genotyping are based on polymorphisms of the S-RNase gene. Initial attempts to identify S-RNases associated with S-alleles were based on the existing knowledge about the S-genotype constitutions of cultivars deduced from the assessment of their S-phenotypes after test-crosses. Classical descriptions of S-phenotypes were well known for apple (*Malus ×domestica* Borkh.) (Kobel et al., 1939), sweet cherry (*Prunus avium* L.) (Crane and Brown, 1937), japanese pear (*Pyrus pyrifolia* L.) [Terami et al., (1946),

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cited in Ishimizu et al. (1999)], and almond [*Prunus dulcis* (Mill.) Webb] (Kester et al., 1994). In European pear, similar studies have been lacking until recently, and reports on cross-incompatibilities between cultivars have been merely anecdotal (Crane and Lewis, 1942; Le Lézec, 1998; Modlibowska, 1945) and insufficient for the systematic identification of S-alleles. Recently, by means of crossing experiments we identified four S-alleles (S₁–S₄) in a group of cultivars on the basis of their pedigree and cross-(in)compatibility relationships (Sanzol and Herrero, 2002). With the identification of S-RNase genomic sequences associated with each S-allele, we were able to establish an initial correspondence between S-phenotypes as determined by crossing and S-RNase genotypes as detected by PCR and sequence analysis (Sanzol et al., 2006).

Since the first report characterizing S-RNases in European pear (Zuccherelli et al., 2002), sequences for 18 different alleles have been published (Moriya et al., 2007; Sanzol et al., 2006; Takasaki et al., 2006; Zisovich et al., 2004). With the exception of the S₀ allele, full-length cDNA and genomic sequences have now been obtained for all the S-RNases (Moriya et al., 2007; Takasaki et al., 2006). This information has been used for the S-genotyping of a large number of cultivars under the assumption that different S-RNase sequences are associated with different allelic specificities (Moriya et al., 2007; Takasaki et al., 2006; Zisovich et al., 2004; Zuccherelli et al., 2002). However, the extensive S-RNase-based genotyping developed in recent years contrasts with the limited information available on S-alleles characterized at the phenotypic level.

The aim of this work was to identify S-alleles in European pear by means of pollinations and subsequently analyze the correspondence between S-phenotypes and S-RNase genotypes. Following an approach based on the use of semicompatible cultivars, we were able to discriminate a total of 14 S-haplotypes and assign 33 cultivars to 13 incompatibility groups. S-RNase genomic sequences were identified for each S-allele. The correspondence between S-phenotypes and S-genotypes assigned to each cultivar was demonstrated by a combined use of consensus and allele-specific PCR.

Materials and Methods

EXPERIMENTAL DESIGN. To identify new S-alleles and cross-incompatibilities in European pear, we adopted the following strategy. First, we identified cultivars with S-genotypes sharing one S-allele (named as the common allele) while the second S-allele for each cultivar was unknown. This group of cultivars was designated as the testing group. Next, we identified cultivars bearing the common allele, and one of the other S-alleles we have characterized so far (S₁–S₅; Sanzol et al., 2006). Thus, if the S₁ allele was selected as the common allele, the four reference cultivars would have S-genotypes with a combination of S₁ and S₂–S₅ alleles, respectively. A particular characteristic of European pear breeding is the recurrent use of ‘Williams’ or its derivatives as a parent. As a consequence, a substantial number of cultivars developed over recent decades are direct descendants of this cultivar (Table 1). Thus, S-alleles present in ‘Williams’ (S₁S₂) were selected as candidates to be used as the common allele. Crossing cultivars belonging to the group of the testing cultivars with those belonging to the group of the reference cultivars provided us with the following information. If a testing cultivar was cross-incompatible with a reference cultivar, this was identified as sharing the same

S-phenotype. Alternatively, whenever a testing cultivar was cross-compatible with all the cultivars included in the reference group, this indicated the presence of a new S-allele. Moreover, this cultivar was incorporated as a new reference genotype combining the common allele and the newly identified allele.

PLANT MATERIAL. A full list of the 63 cultivars used in this work is presented in Table 1. Flowers, pollen, and leaf material for DNA extraction were obtained from trees located at the Campus de Aula Dei experimental orchards (Zaragoza, Spain). Because of a lack of overlap in the flowering time or an insufficient number of flowers, we were unable to use some of the cultivars as female parents for some of the crosses. Also, pollen inviability of Magness compelled this cultivar to be used only as a female parent (Thompson et al., 1976). ‘Beurré Alexander Lucas’ and ‘Merton Pride’ were not included in the test-cross experiments on account of their triploidy.

POLLINATION PROCEDURES AND MICROSCOPIC PREPARATIONS. Test-crosses were performed as previously described (Sanzol and Herrero, 2002). Pollinations were performed during two different years, 2005 or 2006. Most of the crosses were performed during 2005, but some were repeated in 2006. Following fixation, pollinated pistils were prepared for the observation of pollen tubes growing into the ovules using the procedure described by Sanzol and Herrero (2002). A total of 50 ovules were scored for each cross under an Ortholux II microscope (Leitz Co., Wetzlar, Germany) with ultraviolet epifluorescence using a BP-355-425 exciter filter and an LP-460 barrier filter. Data were expressed as the percentage of ovules or carpels with pollen tubes present.

ISOLATION OF GENOMIC DNA AND PCR AMPLIFICATION. Total genomic DNA was prepared as described previously (Sanzol et al., 2006). DNA samples were diluted to a final concentration of 10 ng·μL⁻¹. PCR was performed using 50 ng of genomic DNA in a 30-μL reaction volume containing 1× reaction buffer (supplied with the enzyme), 2 mM MgCl₂, 0.2 mM dNTPs, 0.6 μM of each primer, and 0.8 U of *Taq* DNA polymerase (Invitrogen, Carlsbad, CA). PCR amplification was conducted in an iCycler thermal cycler (Bio-Rad, Hercules, CA) with the following program: 2 min of denaturation at 94 °C; 36 cycles of 30 s at 94 °C, 1 min at specific annealing temperature, and 2 min at 72 °C; and a final extension of 10 min at 72 °C. Previously described degenerate primers (Fig. 1) MPyC1F (5′-ATT ATT WTC AAT TTA CGC AKC ART ATC AG-3′) and MPyC5R (5′-CAA AKA SYR AYC TCR ACY AAT TCM G-3′), amplifying S-RNase products associated with alleles S₁, S₃, S₄, and S₅ and specific amplification of the S₁ and S₂ using the reverse primer PycomS2R (5′-GTA ATG GTT CTT GTC TAT TAT TGT GG-3′) were used as reported by Sanzol et al. (2006).

CLONING AND SEQUENCE ANALYSIS. PCR products were cloned using the pGEM T-Easy cloning kit (Promega, Madison, WI). Cloned PCR products were sequenced in a MegaBACE 1000 capillary sequencer (Amersham Pharmacia Biotech, Uppsala, Sweden) at the Genomics and Sequencing Service of the University of Zaragoza (Zaragoza, Spain). PCR amplifications obtained using the MPyC1F/MPyC5R degenerate primers for the S₆, S₇, S₈, S₉, S₁₁, and S₁₄ S-RNases were directly cloned and sequenced. However, degenerate primers did not amplify a PCR product putatively associated with alleles S₁₀, S₁₂, and S₁₃. Thus for these three alleles, the previous approach taken to identify the S₂-associated S-RNase was adopted (Sanzol et al., 2006). Briefly, the forward primer MPyBC3F (5′-TGR HAR TTA TTT GGC CSA AYG-3′) was used in combination with

MPyC5R to obtain a 300-bp sequence different from the corresponding sequence for the S₁-RNase in 'Espadona' (S₁S₁₀), 'Pierre Corneille' (S₁S₁₂), and 'Maxine' (S₁S₁₃). Based on these sequences, new reverse primers for each S-RNase were designed (5'-GAG ACC CAC AGA TGC CAT GTT TAG C-3' for S₁₀; 5'-ATG TCG TCC CGT GTC CTG AAT CTA CC-3' for S₁₂; and 5'-CTA TTG CGT ATG GCT ATT TCA AGA TC-3' for S₁₃) and used in combination with MPyC1F to complete an S-RNase sequence flanked by the C1 and C5 conserved regions for each S-allele, S₁₀, S₁₂, and S₁₃.

DESIGN OF CONSENSUS AND ALLELE-SPECIFIC PRIMERS. New consensus primers (Fig. 1) were thus designed amplifying all the S-RNase alleles reported in this work: forward orientation PycomC1F (5'-ATT TTC AAT TTA CGC AGC AAT ATC AGC-3') and reverse orientation PycomC5R (5'-CTG CAA AGW SHG ACC TCA ACC AAT TC-3'). The genomic sequences from the C1 to the C5 conserved regions of the 14 S-RNase alleles were aligned using ClustalX (Thompson et al., 1997). Variable regions were identified suitable for the design of reverse allele-specific primers which could be used together with the PycomC1F consensus primer and allowing the amplification of fragments not shorter than 300 bp. Allele-specific primers were thus designed for the S₅, S₆, S₇, S₈, S₉, S₁₁, S₁₂, and S₁₄ RNases (Table 2). This was possible for all the alleles with the exception of S₇ and S₈ for which the design of an alternative forward primer was necessary to achieve allele-specific PCR. The primers developed for allele-specific PCR are summarized in Table 2 together with the annealing temperatures used and the sizes of the PCR products amplified. The standard PCR conditions and concentrations described above were also used for allele-specific PCR.

Results and Discussion

IDENTIFICATION OF TESTING CULTIVARS FOR THE COMMON ALLELE (S₁). A group of 18 cultivars directly descended from cultivars genotyped as S₁S₂, Williams (Sanzol and Herrero, 2002; Sanzol et al., 2006), Max Red Bartlett (a sport of Williams), or Seckel, which was earlier shown to be cross-incompatible with Williams (Modlibowska, 1945) and later S-genotyped with the same S-RNase alleles (Takasaki et al., 2006), was initially selected to identify S-genotypes containing either the S₁ or S₂ alleles (Table 1; bold font). Genomic PCR amplifying S₁ and S₂ RNase indicated that 14 cultivars amplified the 1103-bp band associated with the S₁ allele, while three amplified the 1519-bp product associated with the S₂ allele and only Red Jewell amplified both (Table 1). Given the higher frequency of cultivars bearing S₁, this allele was selected for use as the common allele. The much greater proportion of these cultivars having the S₁ allele (15 out of 18 cultivars) compared with those having the S₂ allele, was a priori an unexpected result because the transmission of the S-alleles from Williams (Bartlett) to its descendants has been shown to fit the ratio expected under nondistorted Mendelian segregation (Yamamoto et al., 2002). A plausible hypothesis to explain this observation is that the S-locus is linked to other gene(s) encoding a trait important for pear cultivation; thus the S₁ allele has a selective advantage during breeding. Early work in sweet cherry showed that certain S-alleles were substantially more frequent, and selection for economic characters was suggested to explain this result (Williams and Brown, 1956). Recent genetic analyses in species belonging to distantly

Table 1. European pear cultivars analyzed in this study, parentages, and presence of S₁ (1103 bp) or S₂ (1519 bp) alleles after genomic PCR with primers MPyC1F and PycomS2R.

Cultivar ^a	Parentage	Product size (bp) ^b
Agua de Aranjuez	Unknown	1103
Alexander Lucas	Unknown	1103
Alexandrine Douillard	Unknown	—
Aurore	Marguerite Marillat × Williams	1103
Bella di Giugno	Unknown	—
Beurré Bosc	Unknown	—
Beurré d'Anjou	Unknown	1103
Beurré Giffard	Unknown	1103
Beurré Hardenport	Unknown	—
Beurré Hardy	Unknown	—
California	Max Red Bartlett × Doyenne du Comice	1103
Carmen	Dr. Jules Guyot × Bella di Giugno	—
Cascade	Max Red Bartlett × Doyenne du Comice	1103
Charles Ernest	Unknown	—
Clapp's Favorite	Unknown	1103
Concorde	Conference × Doyenne du Comice	—
Condo	Conference × ?	—
Conference	Unknown	—
Cura	Unknown	—
Dawn	(Barseck × Williams) × Doyenne du Comice	—
Delbard Premiere	Unknown	1103
Dellette	Unknown	—
Devoe	Unknown	—
Director Hardy	Unknown	1519
Dr. Jules Guyot	Unknown	1103
El Dorado	Williams × Winter Nelis	1103
Epine du Mas	Unknown	—
Espadona	Unknown	1103
Etrusca	Coscia × Gentile	—
Gentil Bianca	Unknown	1103
Grand Champion	Williams × Josephine de Malines	1103
Harrow Delight	Williams × (Early Sweet × Old Home)	1103
Harrow Sweet	Williams × Purdue 80-51	1519
Highland	Williams × Doyenne du Comice	1103
Jeanne d'Arque	Beurré Diel × Doyenne du Comice	1103
Louise Bonne	Unknown	1103/1519
Magness	Seckel × Doyenne du Comice	1103
Maxine	Unknown	1103
Merton Pride	Glou Morceau × Williams	1103
Mondsallard	Unknown	—
Norma	Dr. Jules Guyot × Bella di Giugno	1103
Onwards	Laxton's Superb × Doyenne du Comice	1103
Packhams Triumph	Bella Angevina × Williams	1103

continued next page

Table 1. Continued.

Cultivar ^z	Parentage	Product size (bp) ^y
Pierre Corneille	Beurré Diel × Doyenne du Comice	1103
Precoco di Fiorano	Beurré Giffard × Coscia	1103
Precoco du Trévoux	Unknown	1103
Precoco Morettini	Coscia × Williams	1103
President Drouard	Unknown	—
President Heron	Unknown	—
Red Jewell	Max Red Bartlett × Dr. Jules Guyot	1103/1519
Rogue Red	Doyenne du Comice × (Seckel × Farmingdale)	—
Santa M^a Morettini	Williams × Coscia	1519
Sierre	Williams × Marguerite Marillat	1103
Sirrine	Williams × ?	1103
Spadona Estiva	Unknown	—
Star	Unknown	1103
Starkrimson	Flemish Beauty × Williams	1103
Supercomice Delbard	Unknown	1519
Tosca	Coscia × Williams	1519
Turandot	Dr. Jules Guyot × Bella di Giugno	—
Wilder	Unknown	1103
Williams	Unknown	1103/1519
Winter Nellis	Unknown	—

^zBold font refers to cultivars descended from a cultivar of genotype S₁S₂.

^yProduct sizes amplified using the primer pair MPyC1F and PycomS2R; “—” = no amplification.

related taxa are in clear agreement with this possibility (Bernacchi and Tanksley, 1997; Burke et al., 2002; Gandhi et al., 2005).

An additional group of 44 cultivars most having unknown parents or at least one parent of unknown S-genotype were evaluated for S₁. Nineteen amplified the 1103-bp product associated with the S₁ allele (Table 1). Among them we could ascertain that the 1103-bp PCR products amplified from Beurré Giffard (a cultivar with unknown parents; Table 1) and Norma, unequivocally corresponded to the S₁-associated S-RNase.

Our crossing results showed that ‘Precoco di Fiorano’ is cross-incompatible with ‘Agua de Aranjuez’ (S₁S₃). Table 3

Fig. 1. Diagram of the S-RNase gene of European pear. Boxes represent the two exons containing five conserved regions (C1–C3, RC4, and C5) and interrupted by the single intron of the gene (line). Arrows indicate the position of the consensus primers used by different authors and those reported in this study: (1) S1F/S1R (Zuccherelli et al., 2002), (2) FTQQYQ/anti-II(D/N)CP(H/R) (Zisovich et al., 2004), (3) MPyC1F/MPyC5R (Sanzol et al., 2006), (4) FTQQYQ/anti(I/T)IWPNV (Takasaki et al., 2006), (5) PycomC1F/PycomC5R (this study).

shows the results of pollen tube performance for reciprocal crosses, self-pollinations, and cross-compatible crosses using ‘Williams’ as a control. We knew that ‘Precoco di Fiorano’ (S₁S₃), came from a cross between the cultivars Beurré Giffard and Coscia (S₃S₄; Sanzol and Herrero, 2002). Because ‘Precoco di Fiorano’ inherited the S₃ allele from its male parent (‘Coscia’), it should have received the S₁ allele from its seed parent (‘Beurré Giffard’), confirming that the 1103-bp product from ‘Beurré Giffard’ corresponded to S₁. On the other hand, ‘Norma’ is the result of a cross between ‘Dr. Jules Guyot’ and ‘Bella di Giugno’, only the first one genotyped with S₁ (Table 1). Thus as the S₁-associated amplification present in ‘Norma’ was inherited from its female parent (‘Dr. Jules Guyot’), it was also genotyped with S₁.

SELECTION OF REFERENCE GENOTYPES (S₁S₂ TO S₁S₅). Our previous work (Sanzol and Herrero, 2002) provided us with reference cultivars bearing the allelic combinations S₁S₂ (Williams) and S₁S₃ (Precoco Morettini and Agua de Aranjuez). To identify reference cultivars with the allelic combinations S₁S₄ and S₁S₅, we selected four cultivars containing the S₁ allele (California, Cascade, Highland, and Magness), all of them descendants from a cross between an S₁S₂-genotyped cultivar and Doyenne du Comice (S₄S₅) (Table 1). Intercrossing the four cultivars showed that Highland, California, and Cascade were cross-incompatible and cross-compatible with Magness (Table 4). This indicated that the first three cultivars bear the same S-allele inherited from Doyenne du Comice, while Magness received the other allele from this parent. S-RNase genomic PCR using the primer combination (MPyC1F/MPyC5R) amplified the 756-bp product associated with S₄ from ‘California’, ‘Cascade’, and ‘Highland’ and the 651-bp associated with S₅ only from ‘Magness’, in agreement with the crossing results. Two independent studies have reported ‘Dr. Jules Guyot’ to have S-alleles S_e (= S_j and S₁) and S_a (Takasaki et al., 2006; Zisovich et al., 2004). Sanzol et al. (2006) speculated that S_a corresponded to S₅ characterized in ‘Doyenne du Comice’. Table 4 shows that the cultivar Dr. Jules Guyot is cross-incompatible with Magness (S₁S₅), thus demonstrating that the alleles denominated as S_a and S₅ are indeed of the same specificity. In summary, reference cultivars for the S-genotypes S₁S₄ and S₁S₅ are Cascade, Highland, and California, and Magness and Dr. Jules Guyot, respectively.

CROSS-(IN)COMPATIBILITIES AND IDENTIFICATION OF NEW S-ALLELES. Cultivars representing the four reference genotypes (S₁S₂ to S₁S₅) were crossed with the set of testing cultivars known to bear the S₁ allele (Table 3). A total of six cross-incompatibilities were detected. Red Jewell was cross-incompatible with the reference cultivar Williams (S₁S₂), Packhams Triumph with the reference cultivars Agua de Aranjuez/Precoco Morettini (S₁S₃), Grand Champion and Norma with the reference cultivar Highland (S₁S₄), and Aurore and Harrow Delight with the reference cultivar Dr. Jules Guyot (S₁S₅).

Beurré Giffard, El Dorado, Sierre, Sirrine, and Starkrimson were cross-compatible with all the reference cultivars, suggesting that they bear alleles different from S₂–S₅. A diallele cross with the five cultivars (Table 5) showed that Sierre and Starkrimson and that El Dorado and Sirrine were cross-incompatible while all other combinations were cross-compatible, indicating that three new S-alleles were present in this group of cultivars. We therefore assigned S-phenotypes S₁S₆ to ‘Beurré Giffard’, S₁S₇ to ‘El Dorado’ and ‘Sirrine’, and S₁S₈ to ‘Starkrimson’ and ‘Sierre’.

Table 2. Primers and reaction conditions for allele-specific PCR of S-RNases in european pear.

S-RNase	Primers	Orientation	Sequence (5'–3')	Annealing temp. (°C)	Product size (bp)
S ₅	PycomC1F	Forward	ATTTTCAATTTACGCAGCAATATCAGC	65	438
	PycomS5R	Reverse	GTCGTTCTGTATTGCGGGTCG		
S ₆	PycomC1F	Forward	ATTTTCAATTTACGCAGCAATATCAGC	54	462
	PycomS6R	Reverse	GCGTGTCTGTTTATTATTCGC		
S ₇	PycomS7F	Forward	CGACCCGGAATATTGCAAGATAAGG	60	438
	PycomS7R	Reverse	TTTCAATGTCCACCAGCCCT		
S ₈	PycomS8F	Forward	CTTGTAACGATCGTCCTGAACAA	54	613
	PycomS8R	Reverse	CCTCAACTAATTCAGTCGTCGTC		
S ₉	PycomC1F	Forward	ATTTTCAATTTACGCAGCAATATCAGC	62	546
	PycomS9R	Reverse	CTACCAGTGGCCTTTTCCAC		
S ₁₁	PycomC1F	Forward	ATTTTCAATTTACGCAGCAATATCAGC	54	560
	PycomS11R	Reverse	TTGTCCCGTTCGGTTGAATAGC		
S ₁₂	PycomC1F	Forward	ATTTTCAATTTACGCAGCAATATCAGC	55	1177
	PycomS12R	Reverse	GCGCATGAAAATCTATGTTGAGC		
S ₁₄	PycomC1F	Forward	ATTTTCAATTTACGCAGCAATATCAGC	62	297
	PycomS14R	Reverse	CAATGTAACATTACAATGGAAGAACTC		

A similar analysis was extended to the remaining group of cultivars S-genotyped with S₁. Reference cultivars now representing genotypes S₁S₂ to S₁S₈, were crossed with the testing cultivars (Table 6) identifying seven cross-incompatibilities: *Precoce du Trevoux* and *Louise Bonne* with the reference cultivar *Williams* (S₁S₂) in agreement with an earlier report (Le Lézec, 1998; Osterwalder, 1910), *Gentil Bianca* with the reference cultivar *Beurré Giffard* (S₁S₆), *Jeanne d'Arque* and *Onwards* with the reference cultivar *Highland* (S₁S₄), and *Star* and *Clapp's Favorite* with the reference cultivar *Starkrimson* (S₁S₈). The cultivars *Delbard Premiere*, *Wilder*, *Beurré d'Anjou*, *Espadona*, *Maxine*, and *Pierre Corneille* were cross-compatible with all the reference genotypes, indicating the presence of alleles different from S₁–S₈. A diallele cross showed that the six cultivars were cross-compatible with each other, suggesting that the second allele for each cultivar was different (Table 7). We assigned S-phenotypes S₁S₉ to 'Delbard Premiere', S₁S₁₀ to 'Espadona', S₁S₁₁ to 'Wilder', S₁S₁₂ to 'Pierre Corneille', S₁S₁₃ to 'Maxine', and S₁S₁₄ to 'Beurré d'Anjou'.

The deduced S-phenotypes were in agreement with those expected after the parentage analysis of the cultivars, with the sole exceptions of *Red Jewell* and *Pierre Corneille*. The case of *Red Jewell* is of special interest because it is the result of the cross *Max Red Bartlett* (S₁S₂) × *Dr. Jules Guyot* (S₁S₅), and so it was expected to have S-genotype S₁S₅ or S₂S₅. However, crossing results have shown 'Red Jewell' to have S-genotype S₁S₂. If 'Red Jewell' received the S₂ allele from its female parent, the S₁ allele should have been inherited from its male parent 'Dr. Jules Guyot', indicating that an incompatible pollen tube overcame the rejection reaction and effected fertilization. The chance for a pollen tube in such a case to avoid the self-incompatibility reaction is low if we consider that in a semi-compatible cross, incompatible pollen tubes are competing with pollen tubes bearing a compatible S-allele. Such events have been observed for sweet cherry (a species for which the self-incompatibility reaction is considered to be more sharply defined than for pear) after the analysis of progeny coming from a semicompatible cross (Sonneveld et al., 2003). It follows that the chances for this phenomenon to be observed in pear

are higher, particularly in a cross involving 'Williams' as a female, a cultivar considered to be partially self-incompatible (Griggs and Iwakiri, 1954). Indeed this is evident in terms of its pollen tube performance with levels of selfing substantially higher than any other cultivar analyzed so far by us (Table 3; Sanzol and Herrero, 2002).

IDENTIFICATION OF S-RNASE GENOMIC SEQUENCES ASSOCIATED WITH THE ALLELES S₆–S₁₄. Putative genomic S-RNase sequences associated with the S-alleles S₆–S₁₄, were identified from 'Beurré Giffard' (S₁S₆), 'El Dorado' (S₁S₇), 'Starkrimson' (S₁S₈), 'Delbard Premiere' (S₁S₉), 'Espadona' (S₁S₁₀), 'Wilder' (S₁S₁₁), 'Maxine' (S₁S₁₂), 'Pierre Corneille' (S₁S₁₃), and 'Beurré d'Anjou' (S₁S₁₄). Cultivars representing reference genotypes with known S-RNases [*Williams* (S₁S₂), *Precoce Morettini* (S₁S₃), *Highland* (S₁S₄), and *Magness* (S₁S₅)] gave the expected PCR products corresponding to each allele (Fig. 2A) using the C1/C5 (MPyC1F/MPyC5R) pair of degenerate primers with the exception of the S₂-RNase from *Williams*, which is not amplified by this primer pair (Sanzol et al., 2006). Cultivars with S-phenotypes S₁S₆ to S₁S₁₄ all amplified the 1306-bp band corresponding to S₁ (Fig. 2A). In addition, 'El Dorado' (S₁S₇), 'Delbard Premiere' (S₁S₉), and 'Beurré d'Anjou' (S₁S₁₄), amplified a second PCR product of ≈650 bp, similar in size to the PCR product for S₅. 'Beurré Giffard' (S₁S₆), 'Starkrimson' (S₁S₈), and 'Wilder' (S₁S₁₁) also amplified a second novel band of ≈675 bp. 'Espadona' (S₁S₁₀), 'Pierre Corneille' (S₁S₁₂), and 'Maxine' (S₁S₁₃) amplified only the PCR amplification for S₁ (Fig. 2A).

These PCR products putatively associated with alleles S₆, S₇, S₈, S₉, S₁₁, and S₁₄ were cloned and sequenced. The novel allelic S-RNase sequence from 'Espadona', 'Maxine', and 'Pierre Corneille' was obtained following the approach used previously to characterize the S-RNase associated with the S₂ allele (Sanzol et al., 2006). Sequence analysis between the C1 and the C5 conserved regions confirmed that all the sequences obtained had the conserved and variable regions characteristics of the Maloideae S-RNase. As expected, all were different, consistent with cross-compatibility of all the cultivars.

To be consistent with the denomination of the S-alleles discriminated in this study at the phenotypic level and with the

Table 3. Pollen tube performance and cross-(in)compatibility relationships, between reference european pear cultivars representing S-genotypes S₁S₂, S₁S₃, S₁S₄, and S₁S₅ and testing cultivars descended from an S₁S₂-genotyped cultivar. Data refer to the evaluation of 50 ovules per cross.

Genotype	Reference cultivar	Testing cultivar											
		Precoce di Fiorano (O = 0, C = 0) ^z			Beurré Giffard (O = 4, C = 8)			Aurore (O = 6, C = 12)			El Dorado (O = 6, C = 8)		
		O (%) ^y	C (%) ^x	C/I ^w	O (%)	C (%)	C/I	O (%)	C (%)	C/I	O (%)	C (%)	C/I
S ₁ S ₂	Williams (O = 26, C = 44)	NS ^v (85)	NS (92)	+	93 (93)	100 (95)	+	96 (93)	96 (96)	+	98 (88)	100 (96)	+
S ₁ S ₃	Agua de Aranjuez (O = 8, C = 12)	2 (8)	4 (16)	–	NS (88)	NS (92)	+	NS (84)	NS (96)	+	NS (92)	NS (100)	+
	Precoce Morettini (O = 4, C = 4)	NS (NS)	NS (NS)		81 (NS)	100 (NS)	+	NS (NS)	NS (NS)		NS (NS)	NS (NS)	
S ₁ S ₄	Highland (O = 18, C = 27)	NS (NS)	NS (NS)		98 (69)	100 (80)	+	73 (91)	83 (100)	+	83 (84)	100 (91)	+
S ₁ S ₅	Dr. Jules Guyot (O = 7, C = 9)	NS (NS)	NS (NS)		92 (98)	100 (100)	+	7 (10)	10 (15)	–	96 (90)	100 (92)	+
											94 (98)	100 (100)	+
											84 (88)	92 (92)	+

Genotype	Reference cultivar	Testing cultivar											
		Packhams Triumph (O = 7, C = 8)			Sierra (O = 2, C = 5)			Red Jewell (O = 4, C = 4)			Harrow Delight (O = 6, C = 12)		
		O (%)	C (%)	C/I	O (%)	C (%)	C/I	O (%)	C (%)	C/I	O (%)	C (%)	C/I
S ₁ S ₂	Williams	95 (94)	100 (100)	+	89 (100)	96 (100)	+	4 (6)	8 (8)	–	100 (88)	100 (92)	+
S ₁ S ₃	Agua de Aranjuez	NS (5)	NS (9)	–	NS (100)	NS (100)	+	NS (NS)	NS (NS)		NS (NS)	NS (NS)	+
	Precoce Morettini	10 (6)	12 (12)	–	NS (NS)	NS (NS)		NS (98)	NS (100)		96 (76)	96 (84)	+
S ₁ S ₄	Highland	98 (88)	100 (95)	+	95 (82)	100 (93)	+	87 (NS)	96 (NS)	+	96 (83)	100 (96)	+
S ₁ S ₅	Dr. Jules Guyot	96 (92)	100 (96)	+	96 (72)	100 (74)	+	69 (NS)	80 (NS)	+	15 (4)	20 (8)	–
											86 (83)	100 (96)	+
											88 (96)	96 (100)	+

^zRecords for self-pollinations are annotated beside the name of each cultivar within brackets.

^yO = percentage of ovules with pollen tube.

^xC = percentage of carpels with at least one ovule with pollen tube.

^wC/I = compatible cross (+) or incompatible cross (–).

^vFor each cross, first value corresponds to results from using the reference cultivar as female. Reciprocal crosses using the reference cultivar as pollinator are indicated within brackets; NS = cross not scored.

Table 4. Pollen tube performance and cross-(in)compatibility relationships between european pear cultivars, for the identification of S₁S₄ and S₁S₅ reference genotypes (data refer to the evaluation of 50 ovules per cross).

Pollinated cultivar	Pollinator cultivar											
	Highland			Cascade			California			Dr. Jules Guyot		
	O (%) ^z	C (%) ^y	C/I ^x	O (%)	C (%)	C/I	O (%)	C (%)	C/I	O (%)	C (%)	C/I
Magness	78	95	+	83	92	+	NS ^w			3	6	-
Highland	18	27	-	10	16	-	10	20	-	83	96	+
Cascade	14	16	-	20	28	-	15	20	-	90	93	+
California	14	20	-	19	29	-	18	24	-	83	91	+
Dr. Jules Guyot	94	100	+	88	96	+	94	96	+	7	9	-

^zO = percentage of ovules with pollen tube.

^yC = percentage of carpels with at least one ovule with pollen tube.

^xC/I = compatible cross (+) or incompatible cross (-).

^wNS = cross not scored.

original work identifying S-alleles in european pear by Sanzol and Herrero (2002), the nine S-RNases we characterized were annotated following the same numerical series. On the basis of the sequences characterized, we correlated the nomenclature with the other annotation system used to denominate S-RNase alleles which is based on letters (Moriya et al., 2007; Takasaki et al., 2006; Zisovich et al., 2004; Zuccherelli et al., 2002) as shown in Table 8. In a previous paper we proposed a unified nomenclature based on numbers to denominate S-alleles in european pear (Sanzol et al., 2006). Numbers are used for the annotation of S-alleles in the majority of the fruit tree species, including apple, japanese pear, almond, and sweet cherry. A numbering system is not limited by the number of labels available (a-z), and in the case of european pear it would resolve the problem of double-annotations already existing with the letter-based nomenclature [discussed by Sanzol et al. (2006)].

CORRESPONDENCE BETWEEN S-PHENOTYPES AND S-GENOTYPES.

We attempted to confirm an association between the S-alleles we identified through pollinations and the S-RNase sequences we obtained. Genomic PCR using the primer combination (PycomC1F/PycomC5R) amplified characteristic PCR product sizes for S₁ (≈1300 bp), S₂ (≈1700 bp), S₄ (≈750 bp), S₁₀ (≈2200 bp), and S₁₃ (≈2000 bp); thus they could be accurately evaluated in terms of their amplification sizes. However, the S-RNase groups S₃/S₁₂, S₅/S₇/S₉/S₁₄, and S₆/S₈/S₁₁ amplified products of similar size using consensus primers, and they could not be easily distinguished after visualization of their PCR products on agarose gels (Fig. 2). For convenience, these product sizes were annotated under the general denominations

of 1300 bp for S₃/S₁₂, 675 bp for S₆/S₈/S₁₁, and 650 bp for S₅/S₇/S₉/S₁₄, although actual sizes amplified for each S-RNase allele within each group were slightly different. We designed allele-specific primers to distinguish between the S-RNase alleles within these groups (Table 2). Allele-specific PCR for S₅, S₆, S₉, S₁₁, S₁₂, and S₁₄ used a reverse specific primer in combination with the PycomC1F consensus primer. However, allele-specific PCR for S₇ and S₈ used both forward and reverse specific primers. The specific primer pairs under the optimal annealing temperatures gave amplification products of the expected size from the cultivar used as reference for each S-allele (data not shown). No amplification products were obtained when cultivars containing the other S-alleles were tested.

By combining consensus and allele-specific PCR, we obtained the S-RNase genotypes for the 33 cultivars that were assigned to 13 incompatibility groups by pollination tests. Table 9 shows the amplification products obtained for each cultivar using consensus primers. As expected, all cultivars amplified two bands, one of which was common to all cultivars corresponding to S₁. The product size (1700 bp) corresponding to S₂ was present exclusively in ‘Williams’, ‘Precoc du Trevoix’, ‘Red Jewell’, and ‘Louise Bonne’, all identified as S₁S₂. Similarly, Highland, Cascade, Jeanne d’Arc, Onwards, California, Grand Champion, and Norma assigned to S₁S₄ were the only cultivars to amplify a product of 750 bp, corresponding to S₄. Espadona and Maxine were single members of the incompatibility groups S₁S₁₀ and S₁S₁₃, respectively, and they were the only cultivars amplifying bands of 2200 and 1950 bp, respectively corresponding to S₁₀ and S₁₃. Using consensus

Table 5. Diallele cross involving the european pear cultivars descended from a cultivar genotyped as S₁S₂ that were shown to be cross-compatible with all the reference cultivars representing genotypes S₁S₂, S₁S₃, S₁S₄, and S₁S₅ (data refer to the evaluation of 50 ovules per cross).

Pollinated cultivar	Pollinator cultivar											
	Beurré Giffard			El Dorado			Starkrimson			Sierre		
	O (%) ^z	C (%) ^y	C/I ^x	O (%)	C (%)	C/I	O (%)	C (%)	C/I	O (%)	C (%)	C/I
Beurré Giffard	Table 3			90	92	+	98	100	+	85	80	+
El Dorado	86	92	+	Table 3			89	95	+	85	88	+
Starkrimson	97	100	+	89	91	+	Table 3			2	4	-
Sierre	85	90	+	94	94	+	3	7	-	Table 3		
Sirrine	96	96	+	10	13	-	100	100	+	92	96	+

^zO = percentage of ovules with pollen tube.

^yC = percentage of carpels with at least one ovule with pollen tube.

^xC/I = compatible cross (+) or incompatible cross (-).

^wNS = cross not scored.

Table 6. Pollen tube performance and cross-(in)compatibility relationships, between reference european pear cultivars representing S_1S_2 to S_1S_8 and testing cultivars having unknown pedigree or parent with unknown S -genotype (data refer to the evaluation of 50 ovules per cross).

Genotype	Reference cultivar	Testing cultivar											
		Precoce Trevoux ($O = 10, C = 16$) ^z			Gentil Bianca (NS)			Star ($O = 21, C = 29$)			Jeanne d'Arque ($O = 0, C = 0$)		
		O	(%) ^y	C (%) ^x	C/I ^w	O (%)	C (%)	C/I	O (%)	C (%)	C/I	O (%)	C (%)
S_1S_2	Williams (Table 3)	16 [*] (9)	28 (12)	-	93 (NS)	100 (NS)	+ 98 (98)	100 (100)	+ 96 (100)	100 (100)	+ 92 (98)	100 (100)	+ 96 (100)
S_1S_3	Agua de Aranjuez (Table 3)	98 (78)	100 (88)	+ 82 (NS)	88 (NS)	+ NS (NS)	NS (NS)	NS (NS)	NS (NS)	NS (NS)	100 (92)	100 (96)	+ NS (NS)
	Packhams Triumph (Table 3)	NS (NS)	NS (NS)	NS (NS)	NS (NS)	NS (NS)	96 (98)	100 (100)	+ 94 (86)	100 (92)	+ NS (NS)	NS (NS)	81 (85)
S_1S_4	Highland (Table 3)	93 (82)	95 (96)	+ 85 (NS)	96 (NS)	+ 100 (100)	100 (100)	+ 5 (0)	9 (0)	97 (92)	97 (92)	+ 4 (4)	8 (8)
S_1S_5	Aurore (Table 3)	92 (NS)	96 (NS)	+ 90 (NS)	96 (NS)	+ NS (NS)	NS (NS)	NS (NS)	NS (NS)	NS (NS)	NS (NS)	NS (NS)	NS (NS)
	Dr. Jules	NS (68)	NS (84)	+ NS (NS)	NS (NS)	90 (98)	100 (100)	+ 83 (85)	83 (96)	+ 100 (88)	100 (96)	+ 96 (93)	100 (100)
	Guyot (Table 3)	88 (90)	96 (100)	+ 4 (NS)	8 (NS)	- 86 (100)	92 (100)	+ 82 (90)	92 (96)	+ 98 (82)	100 (88)	+ 92 (90)	96 (100)
	Beurré Giffard (Table 4)	82 (93)	92 (100)	+ 84 (NS)	92 (NS)	+ 84 (98)	96 (100)	+ 80 (100)	100 (100)	+ 98 (90)	100 (100)	+ 86 (90)	96 (100)
S_1S_7	El Dorado (Table 4)	NS (98)	NS (100)	+ 92 (NS)	95 (NS)	+ 3 (16)	6 (37)	- NS (100)	NS (100)	+ NS (96)	NS (100)	+ 93 (84)	100 (88)
S_1S_8	Starkrimson (Table 4)	NS (98)	NS (100)	+ 92 (NS)	95 (NS)	+ 3 (16)	6 (37)	- NS (100)	NS (100)	+ NS (96)	NS (100)	+ 93 (84)	100 (88)

Testing cultivar

Genotype	Reference cultivar	Testing cultivar											
		Louise Bonne ($O = 0, C = 0$)			Clapp's Favorite ($O = 4, C = 8$)			Wilder ($O = 29, C = 42$)			Beurré d'Anjou ($O = 4, C = 8$)		
		O (%)	C (%)	C/I	O (%)	C (%)	C/I	O (%)	C (%)	C/I	O (%)	C (%)	C/I
S_1S_2	Williams	25 (0)	44 (0)	- 82 (90)	100 (96)	+ 96 (82)	100 (92)	+ 92 (78)	96 (88)	+ 98 (NS)	100 (NS)	+ 100 (92)	100 (92)
S_1S_3	Agua de Aranjuez	57 (90)	74 (92)	+ NS (NS)	NS (NS)	NS (NS)	NS (NS)	NS (82)	NS (92)	+ 85 (91)	100 (96)	+ NS (NS)	NS (NS)
	Packhams Triumph	NS (NS)	NS (NS)	76 (71)	92 (91)	+ 86 (98)	92 (100)	+ 86 (NS)	92 (NS)	+ NS (NS)	NS (NS)	90 (94)	96 (96)
S_1S_4	Highland	NS (NS)	NS (NS)	95 (NS)	100 (NS)	+ 94 (94)	100 (100)	+ 93 (78)	100 (92)	+ 80 (NS)	88 (NS)	+ 88 (98)	96 (100)
S_1S_5	Aurore	NS (NS)	NS (NS)	NS (NS)	NS (NS)	88 (NS)	96 (NS)	+ 84 (NS)	92 (NS)	+ 92 (NS)	92 (NS)	+ 98 (NS)	100 (NS)
	Dr. Jules	NS (NS)	NS (NS)	82 (NS)	96 (NS)	+ NS (91)	NS (92)	+ NS (82)	NS (88)	+ NS (NS)	NS (NS)	NS (86)	NS (88)
	Guyot	75 (NS)	83 (NS)	+ 95 (70)	100 (80)	+ 94 (96)	92 (96)	+ 90 (88)	96 (96)	+ 90 (NS)	96 (NS)	+ 84 (82)	92 (84)
S_1S_6	Beurré Giffard	80 (NS)	88 (NS)	+ 78 (NS)	96 (NS)	+ 70 (86)	92 (96)	+ 82 (96)	96 (100)	+ 74 (NS)	96 (NS)	+ 76 (94)	96 (96)
S_1S_7	El Dorado	70 (NS)	85 (NS)	+ 2 (0)	4 (0)	- NS (83)	NS (92)	+ 88 (80)	96 (92)	+ 93 (NS)	96 (NS)	+ 100 (90)	100 (96)
S_1S_8	Starkrimson	70 (NS)	85 (NS)	+ 2 (0)	4 (0)	- NS (83)	NS (92)	+ 88 (80)	96 (92)	+ 93 (NS)	96 (NS)	+ 100 (90)	100 (96)

^zRecords for self-pollinations are annotated beside the name of each cultivar within brackets.

^yO = percentage of ovules with pollen tube.

^xC = percentage of carpels with at least one ovule with pollen tube.

^wC/I = compatible cross (+) or incompatible cross (-).

^yFor each cross, first value corresponds to results from using the reference cultivar as female. Reciprocal crosses using the reference cultivar as pollinator are indicated within brackets; NS = cross not scored.

Table 7. Diallele cross involving the european pear cultivars that were shown to be cross-compatible with all the reference cultivars representing genotypes S₁S₂ to S₁S₈ (data refer to the evaluation of 50 ovules per cross).

Pollinated cultivar	Pollinator cultivar																	
	Delbard Premiere			Espadona			Wilder			Pierre Corneille			Maxine			Beurré d' Anjou		
	O	C	C/I*	O	C	C/I	O	C	C/I	O	C	C/I	O	C	C/I	O	C	C/I
	(%) ^z	(%) ^y		(%)	(%)		(%)	(%)		(%)	(%)		(%)	(%)		(%)	(%)	
Delbard Premiere	Table 6			90	96	+	88	96	+	74	92	+	76	88	+	92	96	+
Espadona	81	92	+	Table 6			NS			72	84	+	NS			NS		
Wilder	94	96	+	90	100	+	Table 6			86	92	+	90	100	+	88	92	+
Pierre Corneille	84	84	+	100	100	+	96	100	+	Table 6			NS			100	100	+
Maxine	92	100	+	100	100	+	94	100	+	90	96	+	Table 6			96	100	+
Beurré d' Anjou	96	100	+	90	92	+	92	92	+	90	100	+	84	88	+	Table 6		

^zO = percentage of ovules with pollen tube.
^yC = percentage of carpels with at least one ovule with pollen tube.
*C/I = compatible cross (+) or incompatible cross (-).
^wNS = cross not scored.

Fig. 2. PCR amplification of reference european pear cultivars representing the 13 S-genotypes discriminated in this study, using (A) degenerate primers MPyC1F/MPyC5R and (B) consensus primers PycomC1F/PycomC5R. M = 1-kb molecular marker, W = ‘Williams’ (S₁S₂), PM = ‘Precoce Morettini’ (S₁S₃), Hi = ‘Highland’ (S₁S₄), Mg = ‘Magness’ (S₁S₅), BG = ‘Beurré Giffard’ (S₁S₆), ED = ‘El Dorado’ (S₁S₇), St = ‘Starkrimson’ (S₁S₈), DP = ‘Delbard Premiere’ (S₁S₉), Es = ‘Espadona’ (S₁S₁₀), WI = ‘Wilder’ (S₁S₁₁), PC = ‘Pierre Corneille’ (S₁S₁₂), Mx = ‘Maxine’ (S₁S₁₃), BA = ‘Beurré d’Anjou’ (S₁S₁₄).

primers, a product of 1600 bp was expected for both S₃ and S₁₂ (Fig. 2B). All the cultivars belonging to the incompatibility group S₁S₃ amplified this product size, as did Pierre Corneille, the only cultivar assigned to the incompatibility group S₁S₁₂. The S-genotypes from these cultivars were further explored with allele-specific PCR for S₁₂. No amplification was obtained from ‘Agua de Aranjuez’, ‘Precoce Morettini’, ‘Precoce di Fiorano’, or ‘Packhams Triumph’, while the expected 1177-bp amplification (Table 2) was obtained for ‘Pierre Corneille’ (Table 9).

Cultivars amplifying the 675-bp product with consensus primers were scored with specific PCR for S₆, S₈, and S₁₁ (Table 2). The banding pattern (Fig. 3) was consistent with the S-phenotypes assigned to each cultivar (Table 9). ‘Beurré Giffard’ and ‘Gentile Bianca’, both assigned to S₁S₆, amplified the 472-bp product corresponding to S₆. Similarly, ‘Starkrimson’,

‘Sierra’, ‘Clapp’s Favorite’, and ‘Star’, all with S-phenotypes S₁S₈, gave the S₈-specific amplification. Wilder was the only cultivar assigned to S₁S₁₁ and was the only cultivar amplifying the 560-bp product expected for S₁₁. Finally, cultivars amplifying the 650-bp product with consensus primers were tested with allele-specific PCR for S₅, S₇, S₉, and S₁₄ (Fig. 4). Results from this analysis were also consistent with the grouping of cultivars according to their S-phenotypes (Table 9).

CONCLUDING REMARKS. For 30 out of the 33 cultivars analyzed in this study, the S-phenotypes are assigned for the first time. The S-phenotype for ‘Williams’, ‘Precoce Morrettini’, and ‘Agua de Aranjuez’ had been previously reported (Sanzol and Herrero, 2002). From these 30 cultivars, the S-RNase genotypes of 14 have been published (Table 9). Following the correspondence reported between S-RNase alleles (Table 8), the S-phenotypes and S-genotypes assigned in this work are in accordance with the S-genotypes published by other groups, with the exception of ‘Pierre Corneille’. The S-RNase associated with the S₁₂ allele of this cultivar differs by six amino acids from the sequence reported for the S_q RNase (Takasaki et al., 2006). The S-allele constitutions for the other 16 cultivars are described in this work for the first time.

During the last years the S-genotype for a substantial number of cultivars has been published (Moriya et al., 2007; Sanzol and Herrero, 2002; Sanzol et al., 2006; Takasaki et al., 2006; Zisovich et al., 2004; Zuccherelli et al., 2002). This information promises to assist nurseries, growers, and breeders in selecting compatible combinations of cultivars. Obviously, this work has been facilitated by the availability of molecular tools that exploit the sequence diversity present at the S-RNase gene. Here we have developed consensus and allele-specific PCR methods for the S-genotyping of 14 S-alleles. Consensus and allele-specific PCR methods are being widely used for the discrimination of S-RNase alleles in the Rosaceae (Janssens et al., 1995; Sonneveld et al., 2003; Wu et al., 2007), including european pear (Sanzol et al., 2006; Zisovich et al., 2004). The main advantage of this methodology over restriction analysis is that it provides a simple plus/minus assay that is easily scored in a PCR reaction.

Molecular genotyping has several intrinsic limitations that should not be ignored. First, it may be insensitive toward unknown S-alleles; therefore, it is important to complement these methods with a reliable and efficient in vivo test to control

Table 8. Correspondence between the S-RNases associated with alleles S₁ to S₁₄ and S-RNases previously reported for european pear cultivars.

Our work			Letter-based labeling system		
S-RNase	Reference cultivar	Sequence report	S-RNase	Reference cultivar	Sequence report
S ₁	Williams	Sanzol et al., 2006	S _e , S _j	Spadona	Zisovich et al., 2004
S ₂	Williams	Sanzol et al., 2006	S ₁	Spadochina	Zisovich et al., 2004
S ₃	Precoco Morettini	Sanzol et al., 2006	S _k	Spadona	Zisovich et al., 2004
S ₄	Doyenne du Comice	Sanzol et al., 2006	S _b	Doyenne du Comice	Zuccherelli et al., 2002
S ₅	Doyenne du Comice	Sanzol et al., 2006	S _a	Doyenne du Comice	Zuccherelli et al., 2002
S ₆	Beurré Giffard	This work	S _i	Gentile	Zisovich et al., 2004
S ₇	El Dorado	This work	S _h	Beurré Bosc	Zuccherelli et al., 2002
S ₈	Starkrimson	This work	S _d	Beurré Hardy	Zuccherelli et al., 2002
S ₉	Delbard Premiere	This work	S _p	Delbard Premiere	Zisovich et al., 2004
S ₁₀	Espadona	This work	S _g	Passe Crassane	Morilla et al., 2007
S ₁₁	Wilder	This work	S _s	Dona's Hovay	Morilla et al., 2007
S ₁₂	Pierre Corneille	This work			
S ₁₃	Maxine	This work	S _t	Maxine	Morilla et al., 2007
S ₁₄	Beurré d'Anjou	This work	S _c	Beurré Hardy	Zuccherelli et al., 2002

Table 9. S-genotype analysis of the 33 european pear cultivars assigned to 13 incompatibility groups.

S-phenotype	Cultivars ^z	Consensus primers	Allele-specific PCR ^y								S-genotype
			S ₅	S ₆	S ₇	S ₈	S ₉	S ₁₁	S ₁₂	S ₁₄	
S ₁ S ₂	Williams	1700/1300	–	–	–	–	–	–	–	–	S ₁ S ₂
	Precoco du Trevous^x	1700/1300									S ₁ S ₂
	Red Jewell	1700/1300									S ₁ S ₂
	Louise Bonne	1700/1300									S ₁ S ₂
S ₁ S ₃	Agua de Aranjuez	1600/1300	–	–	–	–	–	–	–	–	S ₁ S ₃
	Precoco Morettini	1600/1300							–		S ₁ S ₃
	Precoco di Fiorano	1600/1300							–		S ₁ S ₃
	Packhams Triumph	1600/1300							–		S ₁ S ₃
S ₁ S ₄	Highland	750/1300	–	–	–	–	–	–	–	–	S ₁ S ₄
	Cascade	750/1300									S ₁ S ₄
	Jeanne d'Arque	750/1300									S ₁ S ₄
	Onwards	750/1300									S ₁ S ₄
	California	750/1300									S ₁ S ₄
	Grand Champion	750/1300									S ₁ S ₄
	Norma	750/1300									S ₁ S ₄
S ₁ S ₅	Magness	650/1300	+	–	–	–	–	–	–	–	S ₁ S ₅
	Aurora	650/1300	+		–		–			–	S ₁ S ₅
	Dr. Jules Guyot	650/1300	+		–		–			–	S ₁ S ₅
	Harrow Delight	650/1300	+		–		–			–	S ₁ S ₅
S ₁ S ₆	Beurré Giffard	675/1300	–	+	–	–	–	–	–	–	S ₁ S ₆
	Gentile Bianca	675/1300		+		–		–	–		S ₁ S ₆
S ₁ S ₇	El Dorado	650/1300	–	–	+	–	–	–	–	–	S ₁ S ₇
	Sirrine	650/1300	–		+		–			–	S ₁ S ₇
S ₁ S ₈	Starkrimson	675/1300	–	–	–	+	–	–	–	–	S ₁ S ₈
	Sierra	675/1300		–		+		–	–		S ₁ S ₈
	Clapp's Favorite	675/1300		–		+		–	–		S ₁ S ₈
	Star	675/1300		–		+		–	–		S ₁ S ₈
S ₁ S ₉	Delbard Premiere	650/1300	–	–	–	–	+	–	–	–	S ₁ S ₉
S ₁ S ₁₀	Espadona	2200/1300	–	–	–	–	–	–	–	–	S ₁ S ₁₀
S ₁ S ₁₁	Wilder	675/1300	–	–	–	–	–	+	–	–	S ₁ S ₁₁
S ₁ S ₁₂	Pierre Corneille	1600/1300	–	–	–	–	–	–	+	–	S ₁ S ₁₂
S ₁ S ₁₃	Maxine	1950/1300	–	–	–	–	–	–	–	–	S ₁ S ₁₃
S ₁ S ₁₄	Beurré d'Anjou	650/1300	–	–	–	–	–	–	–	+	S ₁ S ₁₄

^zThe S-RNase genotype of 14 cultivars has been previously reported: 'Williams' and 'Cascade' (Zuccherelli et al., 2002); 'Dr. Jules Guyot' and 'Gentile' (Zisovich et al., 2004); 'Agua de Aranjuez' and 'Precoco Morettini' (Sanzol et al., 2006); 'Aurora', 'Magness', 'Harrow Delight', 'Highland', 'California', 'Clapp's Favorite', 'El Dorado', and 'Pierre Cornelle' (Takasaki et al., 2006); 'Beurré Giffard', 'Maxine', and 'Beurré d'Anjou' (Moriya et al., 2007).

^yPresence (+) or absence (–) of specific amplification after allele-specific PCR.

^xCultivars with S-RNase genotype described in this work for the first time are indicated in **bold**.

Fig. 3. Allele-specific PCR for European pear cultivars amplifying an S-RNase product of ≈ 675 bp with consensus primers (A) allele-specific for S_6 , (B) allele-specific PCR for S_8 , and (C) allele-specific for S_{11} . Lanes from the left are BG = 'Beurré Giffard' (S_1S_6), GB = 'Gentile Bianca' (S_1S_6), St = 'Starkrimson' (S_1S_8), Si = 'Sierra' (S_1S_8), CF = 'Clapp's Favorite' (S_1S_8), S = 'Star' (S_1S_8), and Wl = 'Wilder' (S_1S_{11}).

putative new specificities. In this study we have reported on the use of semicompatible reference S-genotypes allowing a putative new S-allele to be scored whenever it is found in combination with the S_1 allele. Second, recent findings in other species of the Rosaceae suggest that sharing the same S-RNase protein does not guarantee the identity between two S-alleles (Šurbanovski et al., 2007). Finally, the S-genotype of a European pear cultivar does not strictly predict its performance in terms of self-fertility or cross-fertility with other cultivars sharing the same S-genotype. Results in this study present a wide analysis of the fertility of European pear cultivars under self- and cross-incompatible pollinations. The data obtained confirm previous observations from fruit and seed set data

Fig. 4. Allele-specific PCR for European pear cultivars amplifying an S-RNase product of ≈ 650 bp with consensus primers (A) allele-specific PCR for S_5 , (B) allele-specific PCR for S_7 , (C) allele-specific PCR for S_9 , and (D) allele-specific PCR for S_{14} . Lanes from the left are Mg = 'Magness' (S_1S_5), Au = 'Aurora' (S_1S_5), Dr = 'Dr. Jules Gujot' (S_1S_5), HD = 'Harrow Delight' (S_1S_5), ED = 'El Dorado' (S_1S_7), Sr = 'Serrine' (S_1S_7), DP = 'Delbard Premiere' (S_1S_9), and BA = 'Beurré d'Anjou' (S_1S_{14}).

(Crane and Lewis, 1942) and pollen tube performance (Lewis and Modlibowska, 1942; Sanzol and Herrero, 2002) showing that the intensity of the incompatibility reaction in European pear varies strongly among cultivars, probably due to the action of one or several modifier genes (Zuccherelli et al., 2002). Recently, we have shown how slight differences in the level of selfing can have a great impact on the variation of cropping for the cultivar Agua de Aranjuez (Sanzol and Herrero, 2007). Therefore two self-incompatible cultivars can behave differently regarding their cropping ability under self-pollination. Similarly, two cultivars sharing the same S-alleles may substantially differ regarding their ability to be crossed.

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