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Identification of self-incompatibility alleles and pollen incompatibility groups in sweet cherry by PCR based s-allele typing and controlled pollination

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Summary

A total of 17 pollen incompatibility groups in sweet cherry (*Prunus avium* L.) were identified among 46 accessions by PCR based S-allele typing analysis and by controlled test pollinations. Two putative S-alleles different from S_1 to S_6, S_z and S_y were identified. Five S-genotypes, S_1S_5, S_1S_6, S_2S_6, S_4S_6, and S_5S_6, combinations of S_1 to S_6 alleles that had not previously been identified from cultivars in NYSAES, were positively confirmed by PCR based S-genotyping analysis. Also, the S-genotypes of cultivars in some pollen incompatibility groups that had previously been incorrectly reported have been clarified. Several popular cultivars, which were previously used as testers for S-allele typing analysis, were found to have been inaccurately genotyped. In addition, the S-genotypes and self-incompatibility groups of some relatively recently introduced cultivars were identified. The molecular typing system of S-genotypes based on PCR is a useful and rapid method for identifying new S-alleles and incompatibility groups in sweet cherry.

Introduction

Self-incompatibility is a widespread mechanism to promote out-crossing. In many species, self-incompatibility is controlled by a single locus, called the S-locus with multiple alleles (de Nettancourt, 1977). In the gametophytic self-incompatibility system the pollen/pistil interaction is genetically controlled by the haploid genome of each pollen grain and the diploid genome of the pistil tissue (Frankel & Galun, 1977). The gene products encoded by the S alleles are known to be ribonucleases (S-RNases) that are expressed in the pistil of Solanaceae (Anderson et al., 1986; McClure et al., 1989) and Rosaceae (Broothaerts et al., 1995; Janssens et al., 1995; Sassa et al., 1992, 1994, 1996; Tao et al., 1997, 1999). However, the complete process of specific pollen-pistil interaction is not well understood because of the lack of information on the function of the pollen part of the S locus. It has been postulated that the pistil's S-RNases in some plants with gametophytic self-incompatibility are able to enter into the cytoplasm of incompatible pollen tubes, where they degrade the RNA that is essential for protein translation, hence arresting of pollen tube growth (McClure et al., 1990; Matton et al., 1994).

Sweet cherry (*Prunus avium* L.) has a homomorphic, monofactorial, and multiallelic gametophytic incompatibility system similar to other self-incompatible fruit tree species of the Rosaceae (Crane and Lawrence, 1929). Sweet cherry requires accurate pollination group knowledge to assure fruit set in commercial orchards. Classifying sweet cherry S-allele genotypes and pollen incompatibility group information are also useful for breeding programs and may help selection. Certain S-alleles (S_1, S_2, and S_3) have a strong selective advantage in economic characters and may favor genetic improvement (William &

Brown, 1956; William & Gale, 1960). Furthermore, the operation of a gametophytic self-incompatibility system may lead to disturbed segregation ratios for some genes that are linked to the S-locus (Breiger & Mangelsdorf, 1926). Thus, correct determination of S-genotypes in choosing parents to know if they are completely or semi compatible is crucial to estimation of linkage and determination of progeny size(s).

The pollination compatibility problem in commercial orchards of sweet cherry was first recognized and studied in the state of Oregon in about 1914 (Gardner, 1946). Crane & Lawrence (1931) initially reported the existence of nine groups and this was extended to 11 in 1937 (Crane & Brown, 1937) and to 12 in 1955 (Crane & Brown, 1955). At that time, Brown (1955) had identified the S alleles present in 9 out of the 12 groups. Six specific S alleles responsible for pollen incompatibility were identified and designated S_1 to S_6 (Brown, 1955).

The identification of six specific S-alleles theoretically gives 15 different incompatibility groups. However, only ten genotyped incompatibility groups were reported (Knight, 1969). Additionally three groups with unknown S-alleles, plus group 'O,' were reported (Knight, 1969; Tehrani & Brown, 1992). Although many specific pollen incompatibility groups have been determined and their S-allele designations have been identified, further research is needed to expand scientific understanding of sweet cherry pollination biology.

Methods previously used for determination of pollen incompatibility groups and S-allele constitutions include controlled pollination tests, pollen tube growth tests, pedigree examination, and/or stylar ribonuclease detection on isoelectric focusing (IEF) gels. However, incompatibility groups based on such determinations are seldom clear-cut. The controlled pollination and pollen tube growth tests are sometimes poor in distinguishing compatible and incompatible genotypes because such determinations are affected by environmental and physiological factors (Tromp & Borsboom, 1994). Another factor that reduces the accuracy of such tests is their heavy reliance on previously identified test cultivars which may have had erroneous pollination group identifications. In the stylar RNase zymogram analysis, it is unclear whether the RNase activities are truly associated with S-allelic products.

A reasonably fast and unambiguous molecular method would greatly help for accurately determining incompatibility groups and S-alleles in sweet cherry. Recently, S-RNases have been shown to be involved in the monofactorial gametophytic self-incompatibility of many Rosaceous fruit crops i.e. almond (Tao et al., 1997), apple (Janssens et al., 1995; Sassa et al., 1996), and pear (Norioka et al., 1996, Sassa et al., 1996; Tomimoto et al., 1996). cDNAs for S-RNases in sweet cherry were cloned (Tao et al., 1999) and a system for typing alleles S_1-S_6 of sweet cherry was developed by using polymerase chain reaction (PCR) technology (Tao et al., 1999).

The purpose of this research was to determine the self-incompatibility groups of existing and new sweet cherry cultivars and sweet cherry seedlings in the Cornell Cherry Breeding Program by using PCR based S-allele typing plus controlled pollination tests. Another goal of this research was to identify the S-allele constitutions of some incompatibility groups, which have remained unknown until now.

Materials and methods

Plant material

Plant material included a total of 46 accessions that were mainly obtained from collections at the New York State Agriculture Experiment Station (NYSAES) in Geneva, NY. Four sweet cherry cultivars, 'Knights Bigarreau,' 'Turkey Heart B,' 'Cryalls Seedling,' and 'Guigne d'Annonay' were obtained from the research station in Vineland, Ontario, Canada. 'Burlat' was double sampled from both NYSAES and Vineland Station. Thus, so far as the 15 possible groups (based on 6 known S-alleles) are concerned, all 13 previously reported pollen incompatibility groups were studied.

This research also sought to create through planned hybridization five S-allele combinations (S_1S_5, S_1S_6, S_2S_6, S_4S_6, and S_5S_6) which were not previously identified in cultivars in NYSAES. Progenies that were raised deliberately to create these groups are shown in Table 1. To minimize test cross work, semi-compatible crosses of parent S-genotypes were planned and implemented. In such crosses between cultivars with one allele in common, populations are created wherein one half of the seedlings have the same S-genotype as the pollen donor parent, and are thereby incompatible when backcrossed to it, and the other half have S-genotype sought and are all compatible with the initial pollen parent.

Also included were some cultivars and selections, the S-genotypes of which were previously unknown or ambiguous.

Table 1. Semi-compatible crossing plan employed in identifying S-genotypes of specific plants that have one of the five pairings: S_1S_5, S_1S_6, S_2S_6, S_4S_6 and S_5S_6

Seedlings[1]	Parents[2]	Parental S-genotype[2]	Expected S-genotypes in progeny[3]	No. of seedlings-compatible with male parent / Total No. of seedlings tested
RN004 R7T156-167	Rainier × Moreau	S_1S_4[4] × S_4S_5	$\mathbf{S_1S_5}$, S_4S_5	10 / 11
RN004 R13T264-265	Hartland × NY1725	S_3S_6 × S_1S_3	$\mathbf{S_1S_6}$, S_1S_3	Not tested (Dead)
RN004 R1T094-126	NY1507 × Hartland	S_2S_3 × S_3S_6	$\mathbf{S_2S_6}$, S_3S_6	13 / 30
RN004 R2T175-197	Hartland × Somerset	S_3S_6 × S_3S_4	$\mathbf{S_4S_6}$, S_3S_4	9 / 21
RN004 R4T120-185	NY 1625 × Hartland	S_3S_5[4] × S_3S_6	$\mathbf{S_5S_6}$, S_3S_6	48 / 50

[1] Seedling identification number: RN004 (Orchard identification) R7 (Row) T156-176 (Tree).
[2] According to Tehrani & Brown (1992) and NYSAES records (R. L. Andersen, unpublished data).
[3] Bold letters designate S-genotypes which are previously unknown and sought by this research.
[4] Incorrect S-genotypes (see text).

Table 2. Summary of controlled pollination test cross results for identification of S-genotype and incompatibility groups

Cultivars/ Selections	Testers S-genotype*									
	S_1S_2	S_1S_3	S_1S_4	S_1S_6	S_2S_3	S_2S_4	S_3S_4	S_3S_5	S_3S_6	S_4S_6
'Alma'										$-^{41}$
'BSRK'**						$+^{24,25}$	$-^{28,30,31,33}$			
'Chelan'							$+^{28,30,31}$	$-^{34,36}$		
'KB'***					$-^{19,20,21,22}$					
'NY1725'			$-^{7,9,10}$							
'NY518'		$+^{4,5,6}$		$-^{14,16}$			$+^{28,30,31}$			
'NY8182'	$+^{1}$	$+^{4,5,6}$	$+^{7,9}$	$-^{14,15}$	$+^{21,22}$	$+^{24,25}$	$+^{28,30,31}$	$+^{34,36}$	$+^{37}$	
'Regina'		$-^{4,5,6}$								
'Royalton'	$+^{1}$	$+^{4,6}$			$+^{21,22}$	$-^{24,25,26}$	$+^{28,30,31}$	$+^{34,36}$	$+^{37}$	
'Sam'						$-^{23,25,26}$				
'Summit'	$-^{1}$		$+^{7,9}$		$+^{18,20}$	$+^{25}$	$+^{28,30,31}$			
'Sylvia'			$-^{7,9,10}$							
'THB'***							$-^{28,30,31}$			
'Ulster'	$+^{1}$	$+^{4,6}$	$+^{7,9}$	$+^{14}$	$+^{21,22}$	$+^{25,26}$	$-^{28,30,31}$	$+^{34,36}$	$+^{37}$	
'Vega'					$-^{17,20,22}$	$+^{26}$				
'Vic'	$+^{1}$	$+^{4,6}$	$+^{7,9}$	$+^{14}$	$+^{21,22}$	$-^{23,24,25}$	$+^{28,30,31}$	$+^{34,36}$	$+^{37}$	
'Viscount'			$-^{7,9,10}$				$+^{28,30,31}$			

* Test cultivar(s) listed in Figure 1, compatible + or incompatible –.
** 'Büttners Späte Röte Knorpelkirsche'.
*** 'Knights Bigarreau' and 'Turkey Heart B'; These test crosses were made by William Lay in Vineland, Ontario for this present research.

Controlled pollination test

Field test confirmations of S-genotypes accomplished through controlled pollination test crosses were made between accessions with unknown S-genotypes or possibly mis-classified pollen incompatibility groups and a series of testers with previously reported, known S-alleles. Test crosses, which were made in this experiment, are listed in Table 2. Pollen was prepared by using the standard method used in many sweet cherry breeding programs as follows: pollen was collected by forcing cut branches indoors at room temperature and rubbing flowers over 2 mm mesh screen to separate anthers which were then allowed to dehisce by overnight exposure to light. Flowers on branches in the field were selected for crossing and were bagged and then hand-pollinated when the stigma was receptive (sticky). Each treatment was comprised of 100 to 300 flowers. Compatibility was arbitrarily classified as positive when 5% or greater final fruit set (Way, 1968) was achieved when averaged over three flowering seasons.

Isolation of genomic DNA

Total DNA was isolated from sweet cherry leaves according to the cetyltrimethylammonium bromide (CTAB) method (Doyle & Doyle, 1990) with the following modification. Polyvinylpyrrolidone (PVP) was used in the extraction buffer (100 mM Tris-HCl, pH 8.0, 20 mM sodium EDTA, 1.4 M NaCl and 2.0% (w/v) CTAB). The final concentration of PVP was 100 mg/g leaf tissue. The addition of 1/10 volume of 10% CTAB solution was used in the aqueous phase after the chloroform-isoamyl alcohol extraction step (for additional separation of polysaccharides from nucleic acids) when aged sweet cherry leaf samples were employed (Rogers & Bendich, 1994). At the end of the original protocol, the DNA was dissolved in 0.5 ml of TE (10 mM Tris-HCl, pH 8.0; 1 mM EDTA), 0.125 ml 4 M NaCl and 0.625 ml 13% polyethylene glycol (PEG) for purification of DNA according to Mak & Ho (1993). Tubes were incubated on ice water for 1 h. Pellets were collected by spinning at 14500 rpm in a microfuge for 10 min at 4 °C, washed with ice-cold 76% ethanol and resuspended in an adequate volume of TE. DNA concentration was determined spectrophotometrically.

PCR-based S-allele typing analysis

The PCR based S-allele typing was carried out under conditions based on the method of Tao et al. (1999). The PCR reaction mixture contained 10 mM Tris-HCl pH 9.0, 50 mM KCl, 0.1% Triton X-100, 1.5 mM $MgCl_2$, 200 μm each of dNTPs, 200 nM each of primers, 25 ng of template DNA, and an 0.5 unit of Taq polymerase (Promega, Madison, Wis.) in a 25 mL reaction volume. Prior to adding the Taq polymerase the mixture of buffer, oligos and template DNA was heated for 5 min to avoid enzyme activity during the heating up of the sample, which could cause low stringency priming or the formation of primer dimers (D'Aquila et al., 1991). PCR was performed in a PTC-100 Programmable Thermal Controller (MJ Research, Watertown, Mass.) programmed for 30 cycles at 94 °C for 1 min, 56 °C for 1 min, and 72 °C for 1 min 30 sec with an initial denaturing of 94 °C for 30 seconds and a final extension of 72 °C for 7 min. Two different primer sets were used. They were Pru-T2 (5′-TST TST TGS TTT TGC TTT CTT C-3′) and Pru-C4R (5′-GGA TGT GGT ACG ATT GAA GCG-3′); and Pru-C2 (5′-CTA TGG CCA AGT AAT TAT TCA AAC C-3′) and Pru-C4R. The location of these primers within the S-cDNA of sweet cherry is shown in Tao et al. (1999). After PCR, the amplified products were separated on 1.5% agarose gel. DNA bands were visualized after staining with ethidium bromide.

PCR products blot analysis (Southern Blotting)

After electrophoresis on a 1.5% agarose gel, the PCR products from S-alleles other than $S_1 - S_6$ were blotted to on Nytran® Plus nylon transfer membrane (Schleicher & Schuell, Grimsehlstrasse, Germany) by standard capillary blotting methods (Maniatis et al., 1982). The blots were hybridized with the Pru-T2 and Pru-C4R fragments of S_6 cDNA to confirm that the products other than S_1 to S_6 alleles were truly from the S-locus. The probes were prepared as follow: PCR was performed using the same program and reaction mixtures as described above except that the concentration of primers (Pru-T2 and -C4R) was increased to 400 nM and 10 ng of S_6 cDNA in pBluescript plasmids was used as a template. The amplified fragment of about 400 bp was excised from agarose gel and labeled by random primer incorporation of $^{32}\alpha$-CTP-labeling system (Prime-a-Gene®, Promega, WI). After prehybridization of the blots for 2 hrs at 65 °C, hybridization with $^{32}\alpha$-CTP-labeled probes was carried out

overnight at 65 °C. The blots were washed with 2X SSC and 0.1% SDS at room temperature (2 × 5 min), followed with 0.1% SSC and 0.1% SDS at 68 °C (2 × 15 min), prior to autoradiographing with Kodak Xomat-S film for varying lengths of time.

Results

In the PCR based S-allele typing analysis, all known $S_1 - S_6$ alleles were distinguished from each other. With the Pru-C2 and Pru-C4R primer set, S_1, S_2, S_3, S_4, S_5 and S_6 alleles had the following sizes; 750, 2100, 750, 1000, 680, and 500 bp, respectively (Figure 1). Since S_1 and S_3 alleles produced the same 750 bp size with this primer set, they were indistinguishable from one another. Hence, the Pru-T2 and Pru-C4R primers were used to distinguish S_1 and S_3 alleles where they gave different bands of 1220 and 1100 bp, respectively. Other S-alleles: S_2, S_4, S_5 and S_6 gave sizes of 2500, 1500, 1100, and 980 bp, respectively, while S_3 and S_5 alleles gave the same size band in the Pru-T2 and Pru-C4R primer set. Two newly identified putative alleles, S_z and S_y, showed the unique bands of 2700 and 2200 bp with the Pru-T2 and Pru-C4R, and 2200 and 1700 bp with Pru-C2 and Pru-C4R, respectively (Figure 2). Furthermore, PCR product blotting analysis showed that these two new S-alleles, S_z and S_y, hybridized with the T2-C4R fragment of S_6 cDNA (Figure 2 bottom). It was thus confirmed that S_z and S_y alleles belonged to the S-locus. These two primer sets could discriminate eight S-alleles in 46 sweet cherry accessions at Geneva, NY and Vineland, Ontario.

Both PCR based S-allele typing analysis and controlled pollination test crosses could determine pollen incompatibility groups of all subjected sweet cherry cultivars and unnamed seedling selections in this study. In most cases, the S-genotypes corresponded to the previously reported parents and/or reported S-genotypes. However, some accessions had S-alleles differing from that predicted by their published parentage and/or their previously reported S-genotypes. The results of controlled pollination test crosses are shown in Table 2 and these results were used as confirmation of the result of PCR based S-allele typing analyses.

Five specific S-genotype combinations from S_1 - S_6 alleles (S_1S_5, S_1S_6, S_2S_6, S_4S_6, and S_5S_6) which were not previously identified from cultivars in NYSAES

Five specific S-genotype combinations, S_1S_5, S_1S_6, S_2S_6, S_4S_6, and S_5S_6, which were combinations of 6 S-alleles that had not previously been identified from cultivars in NYSAES, were identified from our seedling selections and/or cultivars (Table 3). To gain efficiency in test crosses, semi-compatible crosses were applied as described in Material and Methods. For instance in S_2S_6 genotypes, a seedling population was made by a semi-compatible cross (sharing one S-allele in both parents) of 'NY 1507' (S_2S_3) with 'Hartland' (S_3S_6). In this semi-compatible cross, only two different S-allele genotypes, S_2S_6 and S_3S_6, were expected of which 50% are incompatible with the pollen parent, 'Hartland'. So all seedlings, which are compatible with pollen parent, 'Hartland,' would be S_2S_6. This strategy was applied for the identification of all other new S-genotypes from five purposely constructed seedling populations. However, S_1S_6 could not be identified in a semi-compatible cross population because the seedlings were not successfully grown out as living plants.

Previously unidentified genotypes S_2S_6 and S_4S_6 were identified from semi-compatible crosses of 'NY 1507' (S_2S_3) × 'Hartland' (S_3S_6), and 'Hartland' (S_3S_6) × 'Somerset' (S_3S_4), respectively. The progeny from 'NY 1507' × 'Hartland' segregated into two S-genotypes, 13 seedlings with S_2S_6 (compatible with 'Hartland') and 17 seedlings with S_3S_6 (incompatible with 'Hartland'), an approximately 1:1 ratio (χ^2 = 2.12). Similarly, the progeny from 'Hartland' × 'Somerset' segregated into two S-genotypes, 9 seedlings with S_4S_6 (compatible with 'Somerset') and 12 seedlings with S_3S_4 (incompatible with 'Somerset'), an approximately 1:1 ratio (χ^2 = 0.42). Those newly identified S-genotypes were confirmed by PCR based S-allele typing analysis.

In identification of S_1S_5 and S_5S_6 genotype combinations, unexpected results were encountered in two seedling populations, 'Rainer' × 'Moreau' and 'NY 1625′ × 'Hartland' (Table 1). The test crosses resulted in almost all seedlings in progeny from 'Rainier' × 'Moreau' being compatible with 'Moreau' (90.9%) and not 50%. Later we proved that 'Moreau' was S_3S_5 not S_4S_5 (see below). Hence, it was proven that this special seedling population was not from a semi-compatible cross but from a heterologous cross, which created four different genotypes, S_1S_3, S_1S_5, S_3S_4 and S_4S_5, all being fully compatible with both parents. PCR based S-genotyping analysis subsequently determined S_1S_5 and S_4S_5 genotypes for seedling RN4R7T160 and RN4R7T163 respectively from the seedling population, 'Rainer' × 'Moreau'.

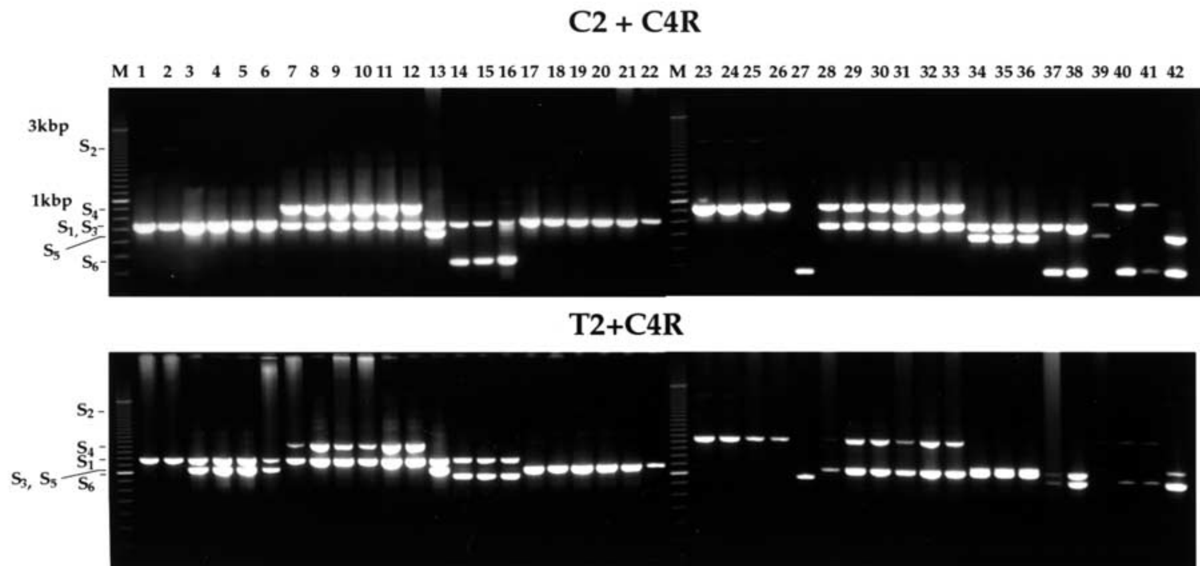

Figure 1. Analysis of PCR based S-allele typing of 14 S-genotypes. Genomic DNA was PCR amplified with two primer sets (Pru-C2 + Pru-C4R in upper and Pru-T2 + Pru-C4R in bottom). Fragments were electrophoresed in a 1.5% agarose gel and detected with ethidium bromide staining. M: 100 bp DNA ladder. S_1S_2: 1. 'Early Rivers,' 2. 'Summit,' S_1S_3: 3. 'Regina,' 4. 'Van,' 5. 'Venus,' 6. 'Windsor,' S_1S_4: 7. 'Hudson,' 8. 'NY1725,' 9. 'Rainier,' 10. 'Republican,' 11. 'Sylvia,' 12. 'Viscount,' S_1S_5: 13. 'RN4R7T160,' S_1S_6: 14. 'Noble,' 15. 'NY518,' 16. 'NY8182,' S_2S_3: 17. 'Knights Bigarreau,' 18. 'Vega,' 19. 'Velvet,' 20. 'Victor,' 21. 'Viva,' 22. 'Vogue,' M: 100 bp DNA ladder, S_2S_4: 23. 'Royalton,' 24. 'Sam,' 25. 'Schmidt,' 26. 'Vic,' S_2S_6: 27. 'RN4R1T102,' S_3S_4: 28. 'Bing,' 29. 'Büttners Späte Röte Knorpelkirsche,' 30. 'Emperor Francis,' 31. 'Napoleon,' 32. 'Turkey Heart B,' 33. 'Ulster,' S_3S_5: 34. 'Burlat,' 35. 'Chelan,' 36. 'Moreau,' S_3S_6: 37. 'Gold,' 38. 'Governor Wood,' S_4S_5: 39. 'RN4R7T163,' S_4S_6: 40. 'Alma,' 41. 'RN4R2T175,' and S_5S_6: 42. 'Early Lyons'.

Table 3. Identification of five new S-genotype combinations based on six S-alleles in sweet cherry

S-genotypes	Cultivars/Seedlings	Parentage	Previously reported pollen incompatibility group
S_1S_5	RN4R7T160	Rainier × Moreau	
S_1S_6	Noble		XII (Unknown)[1]
	NY 518	Germersdorf open pollinated	
	NY 8182	Yellow Glass × Emperor Francis	
S_2S_6	RN4R1T102	NY 1507 × Hartland	
S_4S_6	Alma	Rube × Allers Späte	S_1S_5[2]
	RN4R2T175	Hartland × Somerset	
S_5S_6	Early Lyons		× (Unknown)[1]

[1] Tehrani & Brown (1992).
[2] Schmidt & Timmann (1997).

Similarly other unexpected results were found in the progenies from 'NY 1625′ (previously genotyped as S_3S_5) × 'Hartland' (S_3S_6) when we sought to create the S_5S_6 genotype. We failed to identify the S_5S_6 genotype from semi-compatible crossing population strategies. If the previously reported parentage of 'NY 1625′ and its previously reported S-genotype had been correct, the progeny would have given 50% compatibility with 'Hartland'. However, almost all of the seedlings were compatible with the pollen donor, 'Hartland' (96.0%). So this special seedling population might also not be from a semi-compatible cross but from a heterologous cross which created four different genotypes, all fully compatible with both parents. Furthermore, PCR based S-allele typing analysis for some selected seedlings and 'NY 1625′ showed there seemed to be no S_5 genotype in this population

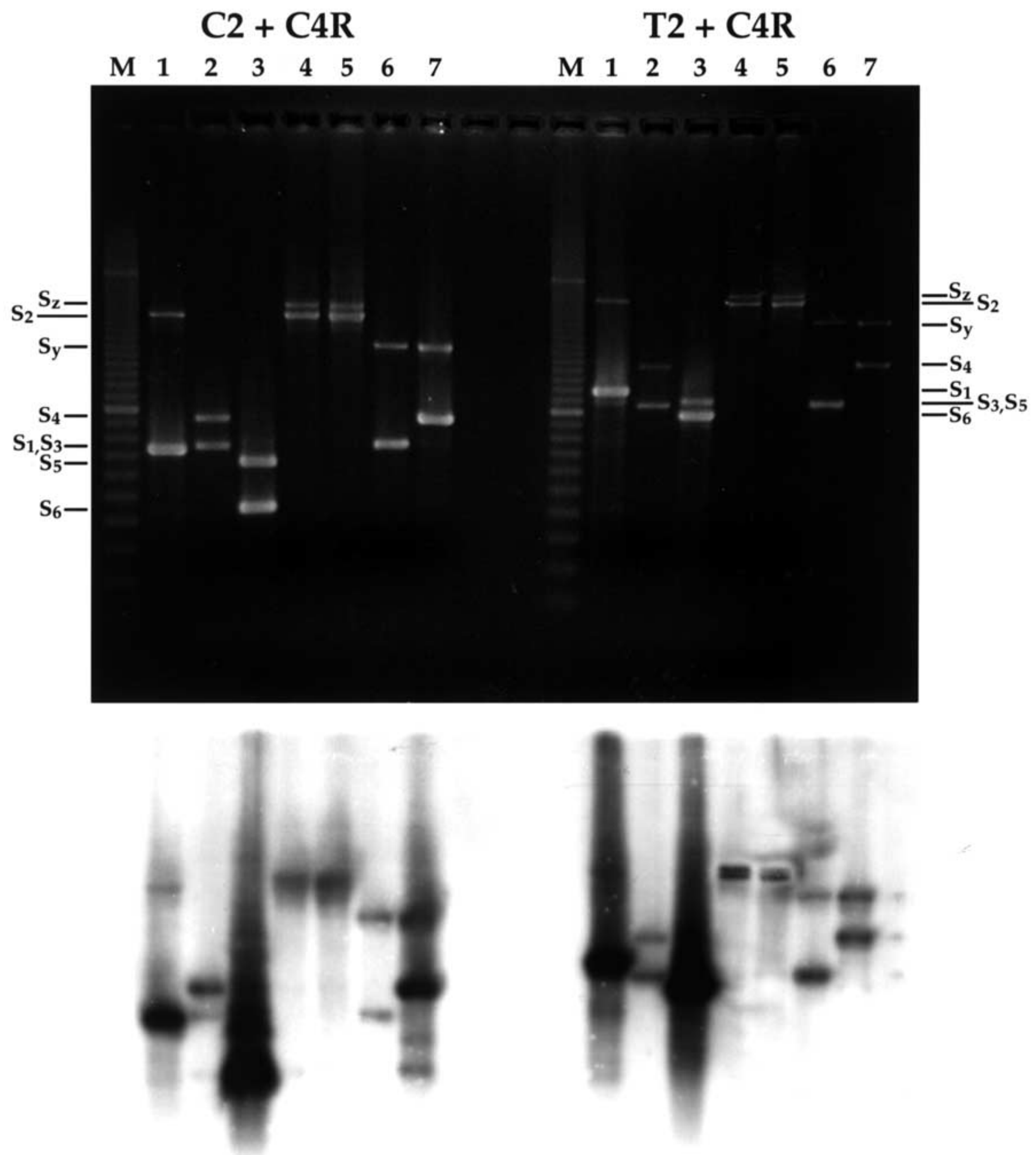

Figure 2. Analysis of PCR based S-allele typing of two new (S_z and S_y) identified S-alleles. Genomic DNA was PCR amplified with two primer sets (Pru-T2 + Pru-C4R and Pru-C2 + Pru-C4R). Fragments were electrophoresised in a 1.5% agarose gel and detected with ethidium bromide staining (upper photograph). The gel was then blotted onto a nylon membrane, and probed and detected with T2-C4R fragment of S_6 cDNA (lower photograph) M: 100 bp DNA ladder. 1. 'Early Rivers' (S_1S_2), 2. 'Bing' (S_3S_4), 3. 'Early Lyons' (S_5S_6), 4. 'Cryalls Seedling' (S_2S_z), 5. 'Guigne d'Annonay' (S_2S_z), 6. 'Schneiders' (S_3S_y) and 7. 'NY 9801' (S_4S_y).

and/or 'NY 1625′ (data not shown). It may be that 'NY 1625′ does not carry the S_5 allele.

The S-genotype combinations of S_1S_6 and S_5S_6 were found in NY selections/cultivars as summarized in Choi et al. (2000). The S-genotype of 'Noble,' 'NY 518' ('Germersdorf' open pollination) and 'NY 8182,' were determined as S_1S_6 by PCR based S-allele typing analysis. However, 'NY 8182' did not correspond to its presumed parentage of record at NYSAES. Its reported parents, 'Yellow Glass' (S_1S_4) and 'Emperor Francis' (S_3S_4), do not possess the S_6 allele. Controlled pollination tests showed 'NY 8182' was incompatible with 'Noble' and 'NY 518' but compatible with all other testers in Table 2. So, the previously recorded parentage of 'NY 8182' is probably wrong. The S-genotype of S_5S_6 was identified in 'Early Lyons' by PCR based S-allele typing analysis. This cultivar was previously classified as Group 'X' with unknown S-genotype (Tehrani & Brown, 1992).

The S-genotype of 'Alma,' a hybrid of 'Rube' and 'Allers Späte' (Schmidt & Timmann, 1997) was suggested to be S_4S_6 by controlled pollination test against S_4S_6 genotype of seedling (RN4R2T175) and with PCR based S-allele typing analysis. However, Schmidt & Timmann (1997) concluded 'Alma' was S_1S_5 based on incompatibility with 'Valera,' which they considered to be S_1S_5. However, the S-genotype of 'Valera' was not clearly substantiated in previous studies. The identification of the incompatibility group 'XIV,' known as S_1S_5 (Way, 1968), could not be verified and thus 'Valera' was assigned to the universal donor Group 'O' (Tehrani & Brown, 1992). Supporting data came from the controlled pollination which showed 'Valera' was compatible with our seedling RN4R7T160, mentioned above as S_1S_5. Furthermore, other research with group 'O' suggested that 'Valera' is neither S_1S_5 nor S_4S_6 (Choi, 1999). If our accession of 'Alma' is correctly identified, the S-genotype of 'Alma' should be S_4S_6.

S-genotypes of cultivars which were previously untyped or incorrectly reported

The S-genotypes for cultivars which were not listed or incorrectly reported in Knight (1969) or Tehrani & Brown (1992) are given in Table 4.

'NY1725,' a selection from NYSAES, resulted from a cross of 'Giant' by 'Emperor Francis'. The S-genotype of 'NY 1725′ was previously assigned to S_1S_3 (Knight, 1969). However, the S-genotype of this selection was typed to be S_1S_4 and confirmed by test cross with 'Hudson,' 'Rainier' and 'Viscount,' which set no fruit. Also 'Sylvia' which was reportedly from 'Compact Lambert' (S_3S_4) × 'Van' (S_1S_3) (D. Lane, personal communication) and was previously of unknown S-genotype, was determined to be S_1S_4.

'Knights Bigarreau' and 'Vega' cultivars were previously assigned to Group 'XI' and Group 'O' respectively, both with unknown S-genotype (Tehrani & Brown, 1992). However, inconsistent results were obtained with these cultivars in controlled pollination tests. 'Knights Bigarreau' was compatible with 'Cryalls Seedling' and 'Guigne d'Annonay,' which were previously assigned to the same Group 'XI'. However, 'Knights Bigarreau' was incompatible with 'Velvet,' 'Victor,' 'Viva' and 'Vogue' which were shown to be S_2S_3 by PCR based S-genotype analysis and by previous assignment. As with controlled pollination tests, the S-genotype of 'Knights Bigarreau' was determined to be S_2S_3 by PCR based S-allele typing analysis.

'Vega' was reported to be a result of the cross 'Bing' (S_3S_4) × 'Victor' (S_2S_3) (Brooks & Olmo, 1997). Therefore, its S-genotypes should be S_2S_3 or S_2S_4, so it should not be in Group 'O'. The S_2S_3 designation for 'Vega' was typed by PCR based S-genotype analysis. Supporting data came from controlled pollination tests of 'Vega' with 'Knights Bigarreau,' 'Victor,' and 'Vogue'; and with cultivars assigned by Knight (1969) to Group 'O'.

The S-genotypes of 'Royalton,' 'Sam,' 'Schmidt' and 'Vic' were identified to be S_2S_4 as summarized in Choi et al. (2000). Sam was previously reported to be a result of open pollination of 'Windsor' (S_1S_3) (Brooks & Olmo, 1997). However, 'Windsor' does not possess either S_2 or S_4. Therefore, our study suggests that 'Windsor' is not the true seed parent of 'Sam'.

'Büttners Späte Röte Knorpelkirsche' and 'Schmidt' cultivars were assigned to Group VIII (S_2S_5) by Knight (1969). However, controlled pollination tests shown here demonstrate cross compatibility between these two cultivars. Further, 'Büttners Späte Röte Knorpelkirsche' was shown to be incompatible with 'Bing,' 'Emperor Francis,' and 'Napoleon' which were all previously known to be S_3S_4 and with 'Ulster' which is now shown to be S_3S_4. With controlled pollination tests, 'Büttners Späte Röte Knorpelkirsche' appeared to be S_3S_4 not S_2S_4 (tested with 'Schmidt' and shown to be compatible).

'Turkey Heart B,' previously assigned to Group V (S_3S_5) along with 'NY 1625′ (Knight, 1969), proved in the controlled pollination tests here to be incompatible

Table 4. S-genotypes identified from PCR based S-allele typing method and controlled pollination test of cultivars with genotypes previously reported by Knight (1969), and Tehrani & Brown (1992).

S-genotypes from this study	Cultivars	S-genotypes according to Knight (1969); and Tehrani & Brown (1992)	
S_1S_2	Summit*[1]		
S_1S_3	Regina*[2]		
S_1S_4	NY 1725	II	(S_1S_3)
	Sylvia		
S_2S_3	Knights Bigarreau	XI	(Unknown)
	Vega	O	(Unknown)
S_2S_4	Royalton		
	Sam*[3]		
	Schmidt	VIII	(S_2S_5)
	Vic*[1]	XII	(S_2S_4)**[1];
		O	(Unknown)
S_3S_4	Büttners Späte Röte Knorpelkirsche	VIII	(S_2S_5)
	Turkey Heart B	V	(S_3S_5)
	Ulster*[1]	XII	(S_2S_4)
S_3S_5	Chelan		
	Burlat	VII	(S_4S_5); (S_3S_x)**[2]
	Moreau	VII	(S_4S_5)
S_4S_5	RN4R7T163		
S_2S_z	Cryalls Seedling	XI	(Unknown)
	Guigne d'Annonay	XI	(Unknown)
S_3S_y	Schneiders	III	(S_3S_4)
S_4S_y	NY 9801		

* S-genotyped after Tehrani & Brown (1992).
*[1] Boskovic et al. (1997).
*[2] Schmidt & Timmann (1997).
*[3] Schmidt et al. (1999).
**[1] Matthews & Dow (1966).
**[2] Schmidt et al. (1999).

with 'Bing' (S_3S_4). Results of PCR based S-genotype analysis agreed with controlled pollination data in current research. Both pieces of evidence suggest it to be S_3S_4 not S_3S_5. So, the clone of 'Turkey Heart B' that is in NYSAES is S_3S_4, not S_3S_5. Mistaken identity of 'Turkey Heart B' is another possibility.

'Burlat' and 'Moreau' were previously assigned to S_4S_5 (Group VII) (Knight, 1969). However, the cultivars were shown here to be S_3S_5 in PCR based S-genotyping analysis. This correction was indirectly supported by the controlled pollination test in the semi-compatible cross seedling population (mentioned above in S_1S_5 genotype identification). If 'Moreau' were S_4S_5, the semi-compatible cross progeny ('Rainier' × 'Moreau') should have given only 50% compatibility with pollen parent ('Moreau'). Also, 'Bradbourne Black,' which was previously placed in the same group as 'Burlat' and 'Moreau,' was concluded to carry the genotype S_3SY (Boskovic et al., 1997) where SY was considered to be S_5. So, compiling all evidence together, 'Burlat' and 'Moreau' are suggested to be S_3S_5. 'Chelan,' resulted from the cross 'Stella' by 'Beaulieu' (Brooks & Olmo, 1997) and was previously assigned to be S_3S_5 (Choi et al., 2000). It gave the same band positions as 'Burlat' and 'Moreau' and confirmed was to the S_3S_5 genotype as well. This assignment was supported with controlled pollination tests which showed incompatibility between 'Moreau' and 'Chelan'. 'Chelan' was compatible with 'Bing,' 'Emperor Francis' and 'Napoleon'.

S-alleles other than S_1 to S_6

A new putative S-allele, S_z, was identified from 'Cryalls Seedling' and 'Guigne d'Annonay'. These two cultivars were previously assigned to Group 'XI' with unknown S-genotypes (Knight, 1969; Tehrani & Brown, 1992) along with 'Knights Bigarreau'. As mentioned before, the S-genotype of 'Knights Bigarreau' was shown by research reported here to be S_2S_3. 'Cryalls Seedling' and 'Guigne d'Annonay' gave PCR evidence of the S_2 allele and another allele which is revealed to carry a long fragment of about 2200bp and 2700bp in Pru-C2 and Pru-C4R primer set, and Pru-T2 and Pru-C4R primer set, respectively. This unexpected band is attributed to S_z. It was different from known PCR fragments derived from the S_1-S_6 alleles. So, the S-genotypes of the 'Cryalls Seedling' and 'Guigne d'Annonay' cultivars were assigned to a new genotype, S_2S_z.

Another new S-allele, S_y, was identified from 'Schneiders' and 'NY 9801,' a seedling from 'Schneiders' open pollination. The PCR amplified fragments derived from S_y were 2200 and 1700 bp from Pru-T2 and Pru-C4R, and Pru-C2 and Pru-C4R primer set, respectively. 'Schneiders' gave S_3 and S_y alleles and 'NY 9801' gave S_4 and S_y alleles. Thus, 'Schneiders' contributed the S_y allele to 'NY9801'. Both S_z and S_y bands were hybridized with T2-C4R fragments of the S_6 cDNA; so this hybridization result confirmed that they were derived from the S-locus.

Discussion

In this study, the number of pollen incompatibility groups in sweet cherry were extended to 17 groups where previously only 13 incompatibility groups had been well characterized (reviews in Knight, 1969; Tehrani & Brown, 1992). Research reported here shows findings that include identification of two putative S-alleles, in addition to the previously known S_1 to S_6. If these results withstand the challenge of further research one can conclude that at least 28 different incompatibility groups are possible among common commercial cultivars with eight S-alleles confirmed. Also, some of the previously known pollen incompatibility groups and their S-genotypes have been clarified. As mentioned before, S-genotypes of many popular cultivars, which were previously used as testers for controlled pollination, were found to be inaccurate and it is suggested here that they should be corrected.

'NY 1625,' which was previously reported as Group 'V,' probably is not S_3S_5. Supporting data were obtained in a seedling population of 'NY 1625′ crossed by 'Hartland'. If 'NY 1625′ were S_3S_5, only 50% of progeny would be compatible with pollen donor, 'Hartland'. However, data showed almost all progeny were compatible with 'Hartland'. Additionally, the S_5 allele was not shown to be present in PCR based S-allele typing analysis form some selected seedlings and 'NY 1625′. Further, the S-genotype of 'NY 1625′ suggests it to be S_4S_x and not S_3S_5, where S_x is neither S_1-S_6 nor S_z or S_y (Choi, 1999). This hypothesis was also supported by the parentage of 'NY 1625,' 'Hedelfingen' (S_3S_x) × 'Emperor Francis' (S_3S_4). Based on this parentage, 'NY 1625′ would not carry the genotype S_3S_5. 'Hedelfingen' and 'Emperor Francis' contributed S_x and S_4, respectively, to 'NY 1625′. We conclude that the previously reported S-genotype of 'NY 1625′ (S_3S_5) was probably incorrect.

Results reported here show that 'Burlat' and 'Moreau' should be assigned the genotype S_3S_5. They were previously reported to belong to Group 'VII' (S_4S_5). Our S-allele typing data were supported through the semi-compatible cross tests in a seedling population from 'Rainier' × 'Moreau'. Furthermore, 'Bradbourne Black' and 'Hedelfingen' were also previously typed to be S_3SY where SY was interpreted to be S_5 through S-RNase zymogram analysis (Boskovic et al., 1997). However, our results from PCR based S-allele typing analysis and controlled pollination of 'Hedelfingen' with 'Moreau' and/or 'Burlat,' has shown that 'Hedelfingen' is not in the same group as 'Burlat' and 'Moreau' (data not shown). Further, no S_5 allele was found in several progenies from 'Hedelfingen' (Choi, 1999).

As with 'Sam,' S-allele genotyping was useful for checking the pedigree of some other cultivars. 'Vogue' was genotyped as S_2S_3 by PCR based S-genotype results in this research as well as previously reported (Tehrani and Dickson, 1974), but its parents, 'Hedelfingen' (S_3S_x) and 'Windsor' (S_1S_3) (Tehrani & Dickson, 1974), do not possess the S_2 allele. The reported parentage should be reconsidered. Similarly, 'Viscount' was genotyped as S_1S_4 by PCR typing, the same genotype as previously reported (Tehrani, 1984), but its reported parents 'V 35024' (Hedelfingen × Bing = S_3S_4 or S_4S_x) and 'V 35029' ('Hedelfingen' × 'Bing' = S_3S_4 or S_4S_x) (Tehrani, 1984), do not possess the S_1 allele.

'Rube' was one parent of both 'Regina' and 'Alma,' which were determined from results shown in this research to be S_1S_3 and S_4S_6, respectively. Based on the information that 'Regina' (S_1S_3) was a result of 'Schneiders' (S_3S_y) × 'Rube,' 'Rube' would have to carry the S_1 allele. Also, because 'Alma' (S_4S_6), is derived from a cross of 'Rube' (unknown) × 'Allers Späte' (unknown), 'Rube' is expected to possess either the S_4 or the S_6 allele. Therefore, the genotype of 'Rube' can be predicted to be either S_1S_4 or S_1S_6. 'Rube' was recently confirmed to be S_1S_4 (Schmidt et al., 1999).

This study provides strong evidence for the existence of two additional S-alleles, increasing the number of S-alleles from 6 to 8 and increasing the number of possible pollen incompatibility groups from 15 to 28. Also, the PCR based S-allele typing system could be useful for determining pollen compatibility groups of commercially important new cultivars to elucidate their incompatibility relationships. S-genotype information for sweet cherry cultivars could aid in the selection of parents for hybridization and genetic studies.

Recently, Boskovic et al. (1997) proposed the existence of new S-alleles of sweet cherry, S_7 to S_{11}, by investigating stylar RNase zymograms. S_y and S_z, new S-alleles occurring in the present study may or may not be one or two of the S_7 to S_{11}. Further research is needed to compare cultivars that carry S_y and S_z to those with S_7 to S_{11}. Use of DNA based technology and controlled pollinations are suggested.

The PCR based S-allele typing system yielded results consistent with controlled pollination tests. The molecular typing system of S-genotypes based on PCR is a useful and rapid method for indicating new S-alleles and incompatibility groups in sweet cherry in the absence of pollination tests, which require a series of test cultivars confirmed previously. Such pollination tests can be expected to be quite cumbersome when eight or more S-alleles exist in sweet cherry and would require 28 or more incompatibility testers.

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