Self-incompatibility Alleles of Apple Cultivars and Advanced Selections

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Abstract. The *S*-alleles of 55 apple (*Malus ×domestica* Borkh.) cultivars and selections were determined using an allele-specific polymerase chain reaction (PCR) amplification system for 11 different *S*-alleles (S2, S3, S4, S5, S7, S9, S24, S26, S27, Sd, Sf). Four cultivars had *S*-alleles different than those predicted by their parentage. Three commercial cultivars of unknown pedigrees had *S*-genotypes that suggested 'Delicious' and 'Golden Delicious' were the parents. *S*-genotyping results supported controlled pollination test results. The genotypes of the five triploid cultivars examined were consistent with the unreduced gamete being contributed by the female parent. Although a large number of *S*-genotypes is available in apple, artificial selection or repeated use of the same cultivars as parents appears to have significantly restricted the number of compatibility groups associated with commercial clones. In controlled reciprocal crosses between two cultivars of known *S*-genotypes, the segregation of *S*-genotypes and *S*-alleles was 1:1:1:1, the ratio expected for random pairing of alleles.

Apple has a multi-allelic gametophytic incompatibility system controlled by a single *S*-gene (Frankel et al., 1977; Kobel et al., 1939). In order to produce commercial crops, most commercial cultivars must be grown with another cultivar or crabapple as a pollinizer. However, limited knowledge about the *S*-genotypes of commercial cultivars makes the selection of compatible pollinizers difficult.

Some apple cultivars are known to be cross-incompatible (Ishiyama et al., 1995; Way, 1995). Specific *S*-alleles have been designated on the basis of pollination tests (Kobel et al., 1939; Komori et al., 1996), isozyme markers (Battle et al., 1995; Manganaris and Alston, 1987), and *S*-glycoprotein profiles (Sassa et al., 1994). However, these methods are time-consuming, and environmental effects often make the results difficult to interpret.

Janssens et al. (1995) developed a molecular technique for *S*-allele genotyping in apple using an allele-specific polymerase chain reaction (PCR) amplification, followed by re-

striction enzyme digestion. The *S*-alleles of Japanese apple cultivars were determined using this system (Sakurai et al., 1997). The plant materials were limited to apple cultivars developed by Japanese breeders and the parents of these cultivars. Some of the cultivars investigated had an unknown *S*-allele that was distinct from the alleles categorized.

The nucleotide sequences of six additional *S*-alleles (S4, S24, S26, S27, Sd, and Sf) have been published (Katoh et al., 1997; Sassa et al., 1996; Verdoodt et al., 1998). In the present study, the allele-specific PCR method for these additional *S*-alleles was used to determine if the Japanese apple cultivars with an unknown *S*-allele in the previous study could be

genotyped further. Furthermore, cultivars and advanced selections useful in genetic improvement and cultivars of commercial interest were subjected to PCR analysis to determine their *S*-genotypes. The distribution of *S*-alleles among commercial cultivars and advanced selections was also analyzed. Finally, we examined the segregation of *S*-genotypes in progenies of reciprocal crosses between 'Golden Delicious' (S2S3) and 'Jonathan' (S7S9) to determine whether certain *S*-alleles had a selective advantage.

Materials and Methods

Plant material was obtained from collections at the New York State Agricultural Experiment Station and the U.S. Dept. of Agriculture's Plant Genetic Resources Unit in Geneva, N.Y. Extraction of DNA and the allele-specific PCR for S2, S3, S5, S7, and S9 were as described by Janssens et al. (1995) and modified by Sakurai et al. (1997). Procedures for S4, S24, S26, and S27 were as described by Verdoodt et al. (1998) (Table 1). Another set of primers for S24 were designed based on information provided by GenBank (AF016920). Primers for the Sd- and Sf-alleles were based on the Sd-RNase I cDNA from 'Delicious' (Katoh et al., 1997) and the Sf cDNA from 'Fuji' (Sassa et al., 1996), respectively (Table 1).

Each 35 µL PCR mixture contained 50 mM KCl, 10 mM Tris-HCl pH 9.0, 0.1% Triton X-100, 2 mM MgCl₂, 200 µM of each dNTP, 1 µM of each primer, 0.875 unit of Taq polymerase (Promega, Madison, Wis.), and 50 ng template DNA. Amplification was performed in a PTC-100 Programmable Thermal Controller (MJ Research, Inc., Watertown, Mass.) programmed for 30 cycles of denaturation (1 min at 94 °C), annealing (1 min at an optimal temperature for each primer combination)

Table 1. Oligonucleotide sequences and conditions for allele-specific polymerase chain reaction (PCR).

<i>S</i> -allele	Primers	Sense/antisense	Nucleotide sequence (5' → 3')	Annealing temp (°C)	Size (bp) of PCR products
Sf ²	FS1	Sense	caatcgaaacgatcatgaag	62	493
	FA1	Antisense	tccgtgtataggccatcgac		
Sd ³	DS2	Sense	atcgaaactgatcatgtaggc	62	355
	DA1	Antisense	tatcgtgaaccttgggtgg		
S4 and S27 ⁴	FTC5	Sense	atgaattcccaacaatacagaacgaga	58	278 ^w
	OWB249	Antisense	atgaattcaatcatgaaatgtgctctg		
S24a ^x	OWB234	Sense	atgaattcggatcctccgacaagttggtttacggttc	62	850
	FTC6	Antisense	atgaattctggggcagaaataccg		
S24b ^y	S24S1	Sense	gcaacgcacagcaaatcag	62	539
	S24A1	Antisense	tcgtcatccgtgtatagggc		
S26 ^z	FTC14	Sense	gaagatgccatacgcaatgg	55	193
	FTC9	Antisense	atgaattcttaataccgaatattggcc		
S2, S3, S5, S7, and S9 ^u					

²Primers designed based on sequence information from Sassa et al. (1996).

³Primers designed based on sequence information from Katoh et al. (1997).

⁴Primers designed based on sequence information from Verdoodt et al. (1998).

^wVerdoodt et al. (1998) reported that under these conditions, another *S*-allele that is presumed to be S4 is amplified yielding a PCR product the same size as S27. After Taq1 digestion, the S4 fragment is split into bands of about 190 and 90 base pairs, which can be discriminated from S27 digestion products that consist of 241 and 37 base pairs.

^xPrimers designed based on sequence information from GenBank (accession number AF016920).

^yPrimers and conditions for these *S*-alleles were published in Broothaerts et al. (1995), Janssens et al. (1995), and Sakurai et al. (1997).

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(Table 1), and extension (1 min at 72 °C). The PCR conditions for *Sd*- and *Sf*-alleles were optimized using DNA from 'Delicious' and 'Fuji', respectively. 'Gala' (*S2S5*), 'Golden Delicious' (*S2S3*), 'Jonathan' (*S7S9*), 'Braeburn' (*S9S24*), 'Baskatong' (*S26S27*), 'Delicious' (*S9Sd*) and 'Fuji' (*S9Sf*) were used as positive or negative controls. The PCR products were separated on 2% agarose gels. After staining with ethidium bromide, gels were observed and photographed under ultraviolet light.

Reciprocal crosses between 'Golden Delicious' and 'Jonathan', determined to be *S2S3* and *S7S9*, respectively (Janssens et al., 1995), were made in the orchard at Geneva to confirm segregation of *S*-genotypes. Blossoms were not bagged but were emasculated at the balloon stage. Two flowers were emasculated and pollinated per cluster and any nonpollinated blossoms were removed. Seeds were stratified at 2 °C for 3 months, placed at room temperature until radicle growth was observed, and then planted in an artificial soil mix.

Results and Discussion

S-genotyping of apple cultivars and selections. The apple cultivars and selections examined were either of interest in breeding or for genetic studies. *S*-genotypes determined for each of the cultivars/selections analyzed are presented in Table 2. The *S*-genotype of 'Northern Spy' (*S3Sf*) was the same as that of its sport, 'Red Spy' (*S3Sf*), supporting research showing sports to be cross-incompatible with the original cultivar (Ishiyama et al., 1995; Way, 1995; Yamada et al., 1971). 'Melrose' (*S9Sd*) has the same *S*-genotype as 'Delicious', and these cultivars were reported to be cross-incompatible based on controlled hybridizations (Way, 1995).

In most cases, the *S*-genotypes corresponded with the presumed parents. However, in four cases the data indicated that the accepted pedigree is incorrect (Table 2). 'Honeycrisp' and 'Kent' have *S*-alleles different from those predicted by their reported parentage. 'Honeycrisp' (*S2S24*) is listed as resulting from a cross of 'Macoun' (*S3S**) by 'Honeygold' (*S2S**), yet neither parent has the *S24*-allele (Table 2). The *S*-genotype of 'Kent' was determined to be *S3S9* by Janssens et al. (1995) and verified in this study, but its parents are listed as 'Cox's Orange Pippin' (*S5S9*) and 'Jonathan' (*S7S9*) (Alston and Watkins, 1975). Neither parent could have contributed the *S3*-allele, suggesting that 'Jonathan' is not the pollen parent. The other two cases in which *S*-genotypes contradicted established pedigrees are discussed below.

The *S*-alleles of 15 Japanese cultivars had been previously determined using the allele-specific PCR and protocols for *S2*, *S3*, *S5*, *S7*, and *S9* (Sakurai et al., 1997). Seven of these 15 cultivars had an unknown allele. The new primers were tested on these cultivars and the unknown allele was determined to be *S24* in 'Akane' and 'Beni No Mai', and *Sf* in 'Fuji', 'Hokuto', and 'Senshu' (Table 2). Previously unknown alleles were also identified in the

Table 2. *S*-allele genotyping and parentage of cultivars and selections.

Cultivar/selection	<i>S</i> -genotype	Seed parent	<i>S</i> -genotype	Pollen parent	<i>S</i> -genotype
Akane	<i>S7S24</i>	Jonathan	<i>S7S9</i> ^z	Worcester Pearmain	<i>S24S</i> ^{xx}
Amanishiki	<i>SfS*</i>	Ralls Janet	<i>S2Sf</i>	Indo	No data
Ambitious	<i>S2S9</i>	Toko	No data	Unknown	
Beni No Mai	<i>S7S24</i> ^w	Fuji	<i>S9Sf</i>	Unknown	
Cameo	<i>S2Sd</i>	Unknown		Unknown	
Empire	<i>SdS*</i>	McIntosh	<i>S*S*</i>	Delicious	<i>S9Sd</i>
Freedom	<i>S5S*</i>	NY 18492	No data	NY 49821-46	No data
Fuji	<i>S9Sf</i>	Ralls Janet	<i>S2Sf</i>	Delicious	<i>S9Sd</i>
Ginger Gold	<i>S3Sd</i>	Unknown		Unknown	
Golden Supreme	<i>S3Sd</i>	Unknown		Unknown	
Goldrush	<i>S2Sd</i>	Golden Delicious	<i>S2S3</i> ^z	Co-op 17	No data
Hacine (triploid)	<i>S3S9Sf</i>	Fuji	<i>S9Sf</i>	Tsugaru	No data
Hokuto (triploid)	<i>S7S9Sf</i> ^w	Fuji	<i>S9Sf</i>	Mutsu	<i>S2S3S*</i>
Honeycrisp	<i>S2S24</i> ^w	Macoun	<i>S3S*</i>	Honeygold	<i>S2S*</i>
Kent	<i>S3S9</i> ^{zw}	Cox's Orange Pippin	<i>S5S9</i>	Jonathan	<i>S7S9</i> ^z
Kitanosachi	<i>S7S*</i>	Tsugaru	No data	American Summer Pearmain	<i>SfS*</i>
Korei	<i>S3Sde</i>	Golden Delicious	<i>S2S3</i> ^z	Indo	No data
Liberty	<i>S3S5</i>	Macoun	<i>S3S*</i>	P54-12	No data
McIntosh	<i>S*S*</i>	Unknown		Unknown	
Melrose	<i>S9Sd</i>	Jonathan	<i>S7S9</i> ^z	Delicious	<i>S9Sde</i>
Murasaki	<i>S9Sd</i>	Jonathan	<i>S7S9</i> ^z	Delicious	<i>S9Sde</i>
Nebuta	<i>S3S9</i>	Kitakami	No data	Tsugaru	No data
North Queen	<i>S3Sf</i>	Fuji	<i>S9Sf</i>	Tsugaru	No data
Northern Spy	<i>S3Sf</i>	Unknown		Unknown	
NY 489	<i>S3S9</i>	NW Greening	No data	Delicious	<i>S9Sd</i>
NY 65707-19	<i>S*S*</i>	Spartan	<i>S9S*</i>	NY 55140-9	No data
NY 674	<i>S3S*</i>	Golden Delicious	<i>S2S3</i> ^z	Monroe	<i>S7S*</i>
NY 73334-35	<i>S3S5S9</i>	Liberty	<i>S3S5</i>	Delicious	<i>S9Sd</i>
NY 75413-30	<i>S3S5Sd</i>	Liberty	<i>S3S5</i>	Delicious	<i>S9Sd</i>
NY 75414-1	<i>S5S*</i>	Liberty	<i>S3S5</i>	Macspur	<i>S*S*</i>
NY 79507-72	<i>S3Sd</i>	Empire	<i>SdS*</i>	Redfree	<i>S3S7</i>
NY 79510-91	<i>S7S*</i>	Empire	<i>SdS*</i>	NY 70712-53	No data
Orei	<i>S2Sd</i>	Golden Delicious	<i>S2S3</i> ^z	Delicious	<i>S9Sd</i>
Red Spy	<i>S3Sf</i>	Sport of Northern Spy	<i>S3Sf</i>	NA ^a	
Redfree	<i>S3S7</i>	Raritan	No data	PRI 1018-101	No data
Rome Beauty	<i>S24S*</i>	Unknown		Unknown	
Russian sdg. ^y (R12740-7A)	<i>S*S*</i>	Unknown		Unknown	
Senshu	<i>S7Sf</i>	Toko	No data	Fuji	<i>S9Sf</i>
Shamrock	<i>S2S*</i>	McIntosh	<i>S*S*</i>	Golden Delicious	<i>S2S3</i> ^z
Shinsei	<i>S3S5</i>	Golden Delicious	<i>S2S3</i> ^z	Early McIntosh ^w	No data
Spartan	<i>S9S*</i>	McIntosh	<i>S*S*</i>	Unknown	
Spigold (triploid)	<i>S2S3Sf</i>	Red Spy	<i>S3Sf</i>	Golden Delicious	<i>S2S3</i> ^z
Spijon	<i>S3S7</i>	Red Spy	<i>S3Sf</i>	Monroe	<i>S7S*</i>
Suncrisp	<i>S3S*</i>	NJ 303955	No data	Golden Delicious	<i>S2S3</i> ^z
Tohoku #2	<i>S24S*</i>	McIntosh	<i>S*S*</i>	Worcester Pearmain	<i>S24S*</i>
Toyo	<i>S5Sd</i>	Delicious	<i>S9Sd</i>	Unknown	
White Angel	<i>S5S*</i>	Unknown		Unknown	

^zDetermined by Janssens et al. (1995) and verified by Sakurai et al. (1997).

^y*S*-allele that is underlined is the allele contributed to the offspring.

^x*S**, *S*-allele unknown, *S*-allele is not *S2*, *S3*, *S4*, *S5*, *S7*, *S9*, *S24*, *S26*, *S27*, *Sd*, or *Sf*.

^w*S*-genotype contradicts that suggested by the presumed parents.

^ysdg. = seedling.

^aNA = not applicable; cultivar/selection is a bud mutation.

parents of these Japanese cultivars, including *S24* in 'Worcester Pearmain', *Sd* in 'Delicious', and *Sf* in 'Ralls Janet' (Table 2). The *S*-genotype of 'Beni No Mai' (*S7S24*) indicates that 'Fuji' (*S9Sf*) was not its seed parent.

S-genotypes of 'Ginger Gold', 'Goldrush', and 'Honeycrisp'. 'Ginger Gold', 'Goldrush', and 'Honeycrisp' were determined to be *S3Sd*, *S2Sd*, and *S2S24*, respectively (Table 2). Pollination tests were conducted to clarify the *S*-genotype designation (Table 3). Crosses between cultivars presumed to have the same *S*-genotype often failed to set fruit. However, 'Superchief Delicious' x 'Goldrush' and 'Redgold' x 'Honeycrisp' produced little fruit

set, although their *S*-genotypes were different. These results emphasize the problem of pollination tests to determine incompatibility. Tests must be repeated over several years and the sometimes deleterious effects of emasculation must be considered in the design of crosses.

S-genotypes of 'Akane', 'Kitanosachi', and 'Monroe'. These three cultivars were first assigned the genotype *S7S24* using the primers designed according to Verdoodt et al. (1998). 'Akane' x 'Kitanosachi' and the reciprocal cross averaged 12% fruit set. 'Monroe' x 'Akane' produced 37.8% fruit set (Table 3). Although these three cultivars were all designated *S7S24*, the occurrence of fruit set sug-

gests that an error in *S* genotyping may have occurred. Hence, new primers for the S24-allele, designated S24S1 and S24A1, were designed based on the S24-allele sequence provided by GenBank (Table 1). Allele-specific PCR was performed again on these three cultivars using these primers. 'Akane' produced a predictive band for the S24-allele. However, 'Kitanosachi' and 'Monroe' did not produce the PCR products. Therefore, the *S*-genotype of 'Akane' was determined to be S7S24 and 'Kitanosachi' and 'Monroe' had the S7-allele and an unknown *S*-allele. These unknown *S*-alleles of 'Kitanosachi' and 'Monroe' are probably identical, because 'Monroe' did not fertilize 'Kitanosachi' (Table 3). However, the existence of distinct *S*-alleles with a high degree of homology is a possibility.

S-genotyping of triploid cultivars and selections. Two triploids, 'Hacnine' and 'Spigold', were determined to be S3S9Sf and S2S3Sf, respectively (Table 2). 'Hacnine' was reported to be a hybrid of 'Fuji' (S9Sf) by 'Tsugaru' (Watanabe et al., 1990), and 'Spigold' resulted from the cross of 'Red Spy' (S3Sf) by 'Golden Delicious' (S2S3) (Way et al., 1968). The *S*-genotyping using the allele-specific PCR provided evidence that the female parent contributed the 2n gamete in both cultivars. This finding parallels studies on 'Jonagold' (Chyi and Weeden, 1984; Harada et al., 1993; Janssens et al., 1995; Sassa et al., 1994) that identified the seed parent as the contributor of the 2n gamete. Controlled pollination tests showed that 'Hacnine' (S3S9Sf) was cross-incompatible with both 'Fuji' (S9Sf) and 'North Queen' (S3Sf) (Watanabe et al., 1990), and 'Spigold' (S2S3Sf) was not compatible with 'Golden Delicious' (S2S3) (Ishiyama et al., 1995). These test results support the proposed genotypes.

Two breeding selections, NY 73334-35 and NY 75413-30, both hybrids of 'Liberty' (S3S5) by 'Delicious' (S9Sd), were determined to be triploid (Table 2). Their *S*-genotypes were S3S5S9 and S3S5Sd, respectively, suggesting that both arose from an unreduced gamete of 'Liberty'. Because their pollen could fertilize other apple cultivars, these selections were initially believed to be diploid. However, examination of breeding records revealed poor fruit set in crosses involving both selections, and their seedlings resembled aneuploids. Their large fruit size, large leaves, and strong growth suggest triploid vigor.

Classifying of cultivars and selections by S-genotype. With 11 *S*-alleles established (Table 1), 55 cross-incompatibility groups are possible. There are additional *S*-alleles that need to be defined, so the actual number of groups will be greater than 55. The diploid cultivars and selections classified have been placed into only 21 groups (Table 4) and eight of these groups have only one cultivar assigned. The S3S9 group had the largest number of diploid cultivars and selections (five) and also had three triploid cultivars and selections, 'Jonagold', NY 73334-35, and 'Hacnine', with both S3 and S9 (Table 2). S2S9, S3S7, and S3Sd were the next largest groups, with four clones each.

Table 3. Controlled pollination tests of apple cultivars in 1997.

Seed parent	Proposed <i>S</i> -genotype	Pollen parent	Proposed <i>S</i> -genotype	No. flowers pollinated	No. fruit set	Fruit set (%)	No. seeds per fruit
Golden Supreme	S3Sd	Ginger Gold	S3Sd	126	0	0.0	---
Kent	S3S9	Ginger Gold	S3Sd	117	18	15.4	7.9
Superchief							
Delicious	S9Sd	Ginger Gold	S3Sd	124	17	13.7	6.5
Goldrush	S2Sd	Redgold	S2S9	119	36	30.3	6.5
Orei	S2Sd	Goldrush	S2Sd	133	0	0.0	---
Redgold	S2S9	Goldrush	S2Sd	109	36	33.0	7.0
Superchief							
Delicious	S9Sd	Goldrush	S2Sd	110	8	7.3	4.6
Honeycrisp	S2S24	Redgold	S2S9	113	30	26.5	7.5
Honeycrisp	S2S24	Tohoku #4	S9S24	110	17	15.5	6.9
Redgold	S2S9	Honeycrisp	S2S24	121	5	4.1	4.3
Tohoku #4	S9S24	Honeycrisp	S2S24	108	43	39.8	9.4
Akane	S7S24	Kitanosachi	S7S*	114	12	10.5	9.6
Kitanosachi	S7S*	Akane	S7S24	129	17	13.2	7.4
Monroe	S7S*	Akane	S7S24	135	51	37.8	7.7
Monroe	S7S*	Kitanosachi	S7S*	116	0	0.0	---

Table 4. Classification of diploid cultivars and selections by *S*-genotype.

<i>S</i> -genotype	Cultivar
S2S3	Golden Delicious ^z
S2S5	Gala ^z
S2S7	Arlet ^y , Orin ^y
S2S9	Ambitious, Kinsei ^y , Megumi ^y , Redgold ^y
S2S24	Honeycrisp
S2Sd	Cameo, Goldrush, Orei
S2Sf	Ralls Janet
S3S5	Elstar, Liberty, Shinsei
S3S7	Idared ^z , Kogetsu ^y , Redfree, Spijon
S3S9	Kent ^z , Nebuta, NY 489, Sayaka ^y , Sekai-ichi ^y
S3Sd	Ginger Gold, Golden Supreme, Korei, NY 79507-72
S3Sf	North Queen, Northern Spy
S5S7	Sansa ^y
S5S9	Cox's Orange Pippin
S5Sd	Toyo
S7S9	Himekami ^y , Jonathan ^z
S7S24	Akane, Beni No Mai
S7Sf	Amanishiki, Senshu
S9S24	Braeburn ^x , Tohoku #4
S9Sd	Delicious, Melrose, Murasaki
S9Sf	Fuji

^z*S*-genotypes determined by Janssens et al. (1995) and verified by Sakurai et al. (1997).

^y*S*-genotypes determined by Sakurai et al. (1997).

^x*S*-genotype determined by Verdoodt et al. (1998) and verified in this study.

Table 5. Segregation for *S*-alleles in crosses between 'Golden Delicious' and 'Jonathan'.

Cross	No. of seedlings	No. of seedlings tested (%)					χ^2	Significance
(<i>S</i> -genotype)		Total	S2S7	S2S9	S3S7	S3S9		
Golden Delicious x Jonathan (S2S3 x S7S9)	111	105 (100)	18 (17)	23 (22)	28 (27)	36 (34)	6.73	NS
(Jonathan x Golden Delicious) (S7S9 x S2S3)	33	25 (100)	9 (36)	4 (16)	6 (24)	6 (24)	2.04	NS

^zChi-square tests were adjusted to compensate for having three degrees of freedom.

^{NS}Nonsignificant.

Distribution of S alleles among cultivars. The origin of the S2- and/or the S3-allele in many cultivars was 'Golden Delicious' (S2S3), which has been used widely in breeding (Janick et al., 1996). The use of 'Jonathan' and 'Delicious' in breeding may account for the prevalence of genotypes with either S7, S9, or Sd alleles. The large acreage of 'Delicious' and

'Golden Delicious' in the United States appears to be reflected in the genotypes of chance seedlings. 'Ginger Gold' (S3Sd), 'Golden Supreme' (S3Sd), and 'Cameo' (S2Sd) are all chance seedlings with the suspected parents being 'Golden Delicious' (S2S3) and 'Delicious' (S9Sd).

Six out of 10 cultivars with the Sf-allele

were Japanese cultivars and descendants of 'Ralls Janet' (S2Sf), but this cultivar did not originate in Japan. 'Ralls Janet' was first noted in Virginia in the early 17th century. It was thought to be a chance seedling, but some argued that it was brought to America from France (Beach et al., 1905). Both 'Fuji' (S9Sf), a hybrid of 'Ralls Janet' x 'Delicious', and 'Ralls Janet' have been used widely in apple breeding in Japan to impart good fruit quality, late ripening, and long storage.

Although S-genotypes appear to be diverse in apple, artificial selection or use of the same cultivars as parents could restrict the number of compatibility groups. Noiton and Alspach (1996) examined genetic diversity in apple clones used in breeding and found that 64% of 439 cultivars and selections studied were descended from only five founding clones: 'Cox's Orange Pippin' (S5S9), 'Golden Delicious' (S2S3), 'Delicious' (S9Sd), 'Jonathan' (S7S9), and 'McIntosh' (S*S*). Careful consideration of pedigrees and an increased genetic base were suggested for future apple breeding.

Controlled crosses to determine segregation of S-genotypes. 'Golden Delicious' x 'Jonathan' and its reciprocal cross produced 111 and 33 seeds, respectively, of which 105 and 25 were studied (Table 5). Both parents are heterozygous for the pale green lethal (PGL) gene (Way et al., 1976; Yoshida et al., 1964) and 25% of the seedlings died due to PGL. Therefore, DNA from 6 and 8 seedlings in the 'Golden Delicious' x 'Jonathan' and its reciprocal cross, respectively, could not be extracted before they died.

In cross-compatible pollination, each S-allele of the parents is presumed to have an equal probability of being transmitted to the offspring. However, in sweet cherry (*Prunus avium* L.) certain S-alleles (S1, S2, S3) were found to have a strong selective advantage, and selection on economic characters was suggested (Williams and Brown, 1956). In our controlled pollination tests, the segregation of S-genotypes did not differ significantly from the expected 1:1:1:1 ratio (Table 5). This result is in agreement with results reported by Manganaris and Alston (1987) and Batlle et al. (1995).

The information obtained on incompatibility alleles in apples will be useful in planning

breeding strategies, controlled hybridizations, and orchard designs to ensure adequate pollination. Development of primers to detect new S-alleles will further our understanding of the incompatibility system in apple, and may allow us to examine whether self-fertility alleles exist within this system.

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