Determining the Self-incompatibility Alleles of Japanese Apple Cultivars

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Additional index words. *Malus ×domestica*, polymerase chain reaction

Abstract. The S alleles of 15 Japanese apple cultivars were determined by using the allele-specific polymerase chain reaction amplification and restriction enzyme digestion system developed by Janssens et al. (1995). Both S alleles were identified in eight diploid cultivars, two S alleles in three triploid cultivars, and one S allele in the remaining four diploid cultivars. Two cultivars had S alleles different than those predicted by their parentage, and in one comparison of a cultivar with its sport, an identity problem was discovered. The technique helped to indicate the parent contributing the unreduced gamete in triploids.

Apple (*Malus ×domestica* Borkh) exhibits gametophytic self-incompatibility controlled by a single locus, the *S* locus, with multiple alleles (Frankel et al., 1977; Kobel et al., 1939). Thus, apple cultivars will not set fruit unless they are pollinated with cultivars from genetically distinct pollination groups. However, most commercial cultivars have not been characterized as to their incompatibility groups.

Cross-fertility between cultivars is determined by conducting controlled pollination followed by an assessment of fruit set. The use of fluorescence microscopy to measure pollen tube growth in styles generally supports field pollination results (Spiegel-Roy and Alston, 1982). However, demonstration of a clear distinction between compatibility and incompatibility often is difficult because environmental and physiological factors affect pollen tube growth (Tromp and Borsboom, 1994).

Isozymes and S-glycoproteins have been used in the analysis of incompatibility groups (Batlle et al., 1995; Manganaris and Alston, 1987; Sassa et al., 1994). However, these methods are time consuming and the results are not always clear. In 1995, Janssens et al. (1995) developed an S allele genotyping method in apple using allele-specific polymerase chain reaction (PCR) amplification followed by restriction enzyme digestion. This method was used on apple cultivars with known S phenotypes, and the S-allele designations matched those suggested by controlled pollination tests (Janssens et al., 1995).

The purpose of our research was to use the allele-specific PCR method to determine the S genotypes of Japanese apple cultivars that are commercially important and/or used in breeding. This information is critical to the breeding program for predicting the compatibility of S genotypes in hybridization and for selection of parents in modified back crossing to avoid inbreeding. Such results also will provide information on the pollination requirements of new cultivars to ensure successful pollination in the orchard.

Materials and Methods

Plant material included 15 apple cultivars developed by Japanese breeders and the parents of these cultivars. This material was obtained from collections at the New York State Agriculture Experiment Station and U.S. Dept. of Agriculture Germplasm Repository in Geneva, N.Y. DNA was extracted from ≈0.1 g of unexpanded or partially expanded leaflets using a procedure based on Torres et al. (1993). Extracted DNA was diluted in autoclaved distilled water and RNA was eliminated by the addition of RNase A. Allele-specific PCR and restriction enzyme digestion were carried out under conditions based on the method of Janssens et al. (1995) (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, Watertown, Mass.) programmed for 30 cycles of denaturation (1 min at 94 °C), annealing (1 min at an optimal temperature for each primer combination), and extension (1 min at 72 °C). The annealing temperatures for the S5- and S7-allele were reduced to 54 °C and 58 °C, respectively. After PCR, the appropriate buffer and restriction enzyme were added directly to one half of the PCR reaction volume (Table 1). Digestion was done for 1 h at 37 °C. 'Gala', 'Golden Delicious', and 'Jonathan', determined to be S2S5, S2S3, and S7S9, respectively (Janssens et al., 1995), were used as

positive or negative controls. PCR products and digests were separated on 2% agarose gels. After staining with ethidium bromide, gels were observed and photographed under ultraviolet light.

Results and Discussion

Both S-alleles were determined for 'Himekami', 'Kinsei', 'Kogetsu', 'Megumi', 'Orin', 'Sansa', 'Sayaka', and 'Sekai-ichi' (Table 2). In most cases, the S-genotypes corresponded to the presumed parents. However, 'Hokuto' and 'Kinsei' had S-alleles different from that predicted by their parentage. Although sports of a cultivar are known to be cross-incompatible with the parent cultivar (Ishiyama et al., 1995; Way, 1995; Yamada et al., 1971), in our study one accession of 'Ralls Janet' was designated S3S* and a presumed sport of 'Ralls Janet' was S2S*. This difference in S genotypes, coupled with distinct fruit phenotypes, suggested that one of these samples was mislabeled. The late-ripening phenotype and S genotype (S2S*) of the sport of 'Ralls Janet' corresponds most closely to the true 'Ralls Janet'. The S2S* genotype of 'Ralls Janet' was confirmed using DNA samples obtained from another clone of 'Ralls Janet'.

'Himekami' was reported to be a result of the cross 'Fuji' (S9S*) by 'Jonathan' (S7S9) (Yoshida et al., 1985). Therefore, its S genotype should be S7S9 or S7S* and a S7S9 designation was confirmed (Table 2). Komori et al. (1996) and Yoshida et al. (1985) found that 'Himekami' and 'Jonathan' were cross-incompatible in pollination tests.

'Hokuto', a triploid cultivar, was determined to be S7S9S*, but its parents are 'Fuji' (S9S*) and 'Mutsu' (S2S3S*) (Yamada et al., 1987). On the basis of the 2D-PAGE profiles of apple S-glycoproteins (named S^a, S^b, S^c, S^d, S^e, and S^f), 'Hokuto' was designated S^cS^eS^f (Sassa et al., 1994). Comparison of the proposed S-genotypes based on S-glycoprotein with those deduced by S-allele-specific PCR analysis revealed that the S^c- and S^e-glycoproteins could be related to the S9- and S7-allele, respectively (Janssens et al., 1995). Sassa et al. (1994) reported that 'Fuji' contributed the unreduced gamete based on the S-glycoprotein profiles, and the S-genotype of 'Hokuto' in our study supports this. Furthermore, 'Hokuto' (S7S9S*) was found to be cross-incompatible with 'Fuji' (S9S*) and 'Jonathan' (S7S9) (Ishiyama et al., 1995), which supports the S7S9S* genotype. Therefore, these studies suggest that 'Mutsu' is not the true pollen parent of 'Hokuto'. 'Mutsu' is triploid, does not produce viable pollen, and thus may not be a competitive pollen parent (Sato, 1990; Yamada et al., 1971).

'Kinsei' was genotyped as S2S9, but its parents, 'Golden Delicious' (S2S3) and 'Ralls Janet' (S2S*), do not possess the S9 allele. Pollination tests by Ishiyama et al. (1995) between 'Kinsei' and 'Redgold' (both determined to be S2S9 in this study) support our results, as only 8% fruit set was obtained. The S-genotype of 'Megumi', a hybrid of 'Ralls Janet' (S2S*) and 'Jonathan' (S7S9), was also

Received for publication 24 Feb. 1997. Accepted for publication 9 June 1997. This research was supported in part by Hatch funds and by a grant from the USDA NRICRG 93-37300-8776. We gratefully acknowledge Dr. W. Broothaerts, Centre for Fruit Culture, Katholieke Universiteit Leuven, Belgium, for helpful discussions during the course of this work. We also thank David Terry for his assistance. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

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Table 1. Oligonucleotides sequences and conditions for allele-specific PCR and restriction enzyme digestion.²

S-allele	Primers	Sense/antisense	Nucleotide sequence (5' → 3')	Annealing temp (°C)	Restriction enzymes
S2	OWB122	Sense	gttcaaacgtgacttatg	64	<u>Eco</u> RV
	OWB123	Antisense	ggtttggttccttaccatgg		
S3	OWB134	Sense	atggatccgaattcttgaacaayattattcaatg	56	<u>Pst</u> I
	OWB145	Antisense	tagaattcattgctaggacacgc		
S5	OWB139	Sense	atgaattctgaaggtaaacccacg	54 ^y	<u>Eco</u> RV
	OWB138	Antisense	atgaattcatatggataatggtaaccg		
S7	OWB126	Sense	gccttcagactcgaatggaca	58 ^y	<u>Acc</u> I
	OWB127	Antisense	tggcatttacaatatctacc		
S9	OWB154	Sense	cggccggctgtctgccactt	62	<u>Eco</u> RI
	OWB155	Antisense	cgggtcgatcgagtagcttg		
D	OWB128	Sense	atgacggttcttatccatcc	59	<u>Eco</u> RV
	OWB75	Antisense	tgagccattcccgctggggc		

²Developed by Janssens et al. (1995).

^yAnnealing temperatures reduced from 58 °C and 62 °C, respectively.

Table 2. S-allele genotyping and parentage of Japanese varieties.

Cultivar	S-genotype	Seed parent	S-genotype	Pollen parent	S-genotype
Akane	S7S*	Jonathan	<u>S7S9</u> ^x	Worcester Pearmain	<u>S</u> *S*
Beni No Mai	S7S*	Fuji	<u>S9S</u> *	Unknown	
Fuji	S9S*	Ralls Janet	<u>S2S</u> *	Delicious	<u>S9S</u> *
Himekami	S7S9	Fuji	<u>S9S</u> *	Jonathan	<u>S7S9</u> ^y
Hokuto (triploid)	S7S9S*	Fuji	<u>S9S</u> *	Mutsu	<u>S2S3S</u> *
Kinsei	S2S9*	Golden Delicious	<u>S2S3</u> ^y	Ralls Janet	<u>S2S</u> *
Kogetsu	S3S7	Golden Delicious	<u>S2S3</u> ^y	Jonathan	<u>S7S9</u> ^y
Megumi	S2S9	Ralls Janet	<u>S2S</u> *	Jonathan	<u>S7S9</u> ^y
Mutsu (triploid)	S2S3S*	Golden Delicious	<u>S2S3</u> ^y	Indo	no data
Orin	S2S7	Golden Delicious	<u>S2S3</u> ^y	Indo	no data
Sansa	S5S7	Gala	<u>S2S5</u> ^y	Akane	<u>S7S</u> *
Sayaka	S3S9	Jonathan	<u>S7S9</u> ^y	Sekai-ichi	<u>S3S9</u>
Sekai-ichi	S3S9	Delicious	<u>S9S</u> *	Golden Delicious	<u>S2S3</u> ^y
Senshu	S7S*	Toko	No data	Fuji	<u>S9S</u> *
Shizuka (triploid)	S2S3S*	Golden Delicious	<u>S2S3</u> ^y	Indo	No data

S: S allele unknown, S allele is not S2, S3, S5, S7, or S9.

^yDetermined by Janssens et al. (1995) and verified in our study.

^xS allele that is underlined is the allele contributed to the offspring.

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

S2S9. When 'Megumi' was used as pollen parent, little fruit set occurred in crosses with 'Kinsei' and 'Redgold' (Ishiyama et al., 1995; Yamada et al., 1971) except when 'Redgold' was used as seed parent in pollination test by Ishiyama et al. (1995). This supports the S-genotype for both 'Kinsei' and 'Megumi' as S2S9. Komori et al. (1996) also placed 'Kinsei' and 'Redgold' in the same incompatibility group, but designated S alleles that correspond to S2S*.

'Fuji', 'Ralls Janet', and 'Delicious' all had one S allele that was other than S2, S3, S5, S7, or S9. The characterization of additional S alleles by Van Haute et al. (1996) may provide a means to identify these unknown alleles in the future.

Allele-specific PCR analysis supported the cross-incompatibility groups suggested by pollination test results, and it also clearly distinguished between compatible (both S alleles different) and semi-compatible (one S allele in common) reactions. This technique is a useful and rapid method because it does not require pollination tests, S-glycoprotein profiles, or the study of progenies from these cultivars, and avoids environmental effects.

Japanese apple cultivars are of limited genetic diversity. Bessho and Soejima (1992) described 26 apple cultivars developed in Japan. All cultivars were descended from five cultivars: 'Delicious', 'Golden Delicious', 'Indo', 'Jonathan', and 'Fuji'. The eight commercial cultivars that account for more than 90% of apple production in Japan ('Fuji', 'Tsugaru', 'Orin', 'Jonagold', 'Mutsu', 'Red Delicious', 'Hokuto', and 'Senshu') are derived primarily from these five cultivars and will be parents of new cultivars in the future.

Despite common ancestry, many of the Japanese cultivars are in distinct pollination groups and can be intercrossed for the production of new hybrids. The S allele genotyping was also useful for confirming or challenging the pedigree of a cultivar. Knowledge of the S alleles in Japanese apple cultivars of known and unknown parentage will aid in the selection of parents for hybridization and provide important information about pollination requirements in the orchard.

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