HortScience 40(2):314–317. 2005.

Parent Identification of Eight Apple Cultivars by S-RNase Analysis and Simple Sequence Repeat Markers

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Additional index words. *Malus* ×*domestica*, microsatellites, parentage analysis, simple sequence repeat, *S*-RNase allele

***Abstract*. As the parents of the some of the apple cultivars were unknown and others were uncertain, we investigated the parent–offspring relationships of eight apple cultivars by S-RNase analysis and SSR markers. The paternal parent of 'Hida' was identified as 'Golden Delicious', not the previously mentioned 'Orin'. It was indicated that 'Ryoka No Kisetsu' and 'Korin' showing identical SSR genotype are likely sports of 'Fuji'. 'Fuji', rather than 'Toko', seemed to be a maternal parent of 'Kotoku', but was not a paternal parent of 'Orei', 'Starking Delicious', 'Nero 26', 'Empire', or 'Aori 3'. Previously mentioned 'Mutsu', 'Indo', and 'Shin Indo' were excluded as paternal parents of 'Hokuto'. 'Tsugaru' and 'Jonathan' and were identified as the respective paternal parents of three cultivars described as having unknown paternal parents, i.e., 'Aika No Kaori', 'Yoko', and 'Tsugaru'.**

Apples (*Malus* ×*domestica* Borkh.) are produced commercially throughout most temperate-climate zones. In Japan, modern commercial apple production averages 1 million tons each year. 'Fuji', grown in 50% of the total cultivated area, is the leading cultivar, followed by 'Tsugaru' (14%) and 'Orin' (10%) (Soejima et al., 2000).

Apples exhibit gametophytic self-incompatibility (GSI), causing self pollen tube growth to be arrested in the style. GSI enforces outbreeding and results in heterozygosity. The *S*-RNase gene located within the *S*-locus encodes ribonucleases (Franklin-Tong and Franklin, 2003; McClure et al., 1989). From the nucleotide sequences of the S-RNases, the PCR-based S-RNase allele genotype analysis method was developed (Broothaerts, 2003; Kitahara and Matsumoto, 2002a; Matsumoto and Kitahara, 2000). To date, we investigated the *S*-RNase content of more than 300 apple cultivars, lineages and species, and found that the pedigrees of some cultivars were uncertain due to discrepancies in the inheritance of *S*-genes (Matsumoto et al., 2003a, 2003b).

Simple sequence repeats (SSRs, also called microsatellites) have become the accepted markers of plant species, and SSR markers have been used for parentage analyses in grapes (Bowers and Meredith, 1997; Bowers et al., 1999; Sefc et al., 1997), peaches (Testolin et al., 2000; Yamamoto et al., 2003), and pears (Kimura et al., 2003).

Determining accurate parent-offspring relationships is important for the development of efficient apple-breeding programs. We selected 'Tsugaru', one of the major cultivars in Japan, and seven important cultivars having 'Fuji' or 'Golden Delicious' as a maternal parent, and then investigated their parentages using 19 SSR markers.

Materials and Methods

Plant material. T. Kobayashi and Y. Miyamoto supplied 'Hida B6' and 'Hida B7'. Other *Malus* plants used in this study were from collections at the Apple Research Center of the National Institute of Fruit Tree Science, Japan, or the Nagano Fruit Tree Experiment Station, Japan. Young leaves were collected and stored at –80 °C until use.

S-RNase allele specific PCR-digestion analysis. Total DNA from the leaves of individual plants was isolated as described by Thomas et al. (1993). The primers and conditions used for the *S*-RNase allele-specific PCR amplification and digestion were essentially those described by Broothaerts (2003) (*S2*-, *S3*-, *S4*-, *S7*-, *S9*-, and *S16*-RNase allele), Kitahara et al. (2000) (*S24*-RNase allele), Kitahara and Matsumoto (2002b) (*S10*-RNase allele), Kitahara and Matsumoto (2002a) (*S25*-RNase allele), Matsumoto et al. (1999a) (*S5*- and *S7*-RNase allele), Matsumoto et al. (1999b) (*S1*- and *S20*-RNase allele), and Matsumoto and Kitahara (2000) (*S28*-RNase allele).

SSR amplification and parentage analysis. We used the following 19 SSR markers: CH01c06, CH01d08, CH01d09, CH01f03b, CH01f07a, CH01g05, CH02b07, CH02c02b, CH02c09, CH02d08, CH02g04, CH03a04, CH03a09, CH03d07, CH03d12, CH03e03, CH05a04, CH05c04, and CH05g08 (Liebhard et al., 2002). PCR was conducted in a 20-μL mixture comprised of 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin, 200 μM deoxynucleotides, 0.5 μM of each of three forward primers labeled with a fluorescent chemical (FAM, VIC, or NED) in addition to an unlabelled reverse primer, 10 ng of genomic DNA, and a 1 unit of *Taq* polymerase. We did not combine the three primer pairs in the same PCR reaction. The analysis was programmed in a thermal cycler (GeneAmp 2700 apparatus; PE Applied Biosystems) and conducted under the following conditions: 5 min preheating at 94 °C, 1 min at 94 °C, 1 min at 55 °C, and 2 min at 72 °C for 35 cycles, followed by an extension for 7 min at 72 °C. The PCR products were basically diluted about 10 to 20 times with distilled water, then separated and detected using a PRISM 310 DNA sequencer (PE Applied Biosystems). The size of the amplified bands was calculated based on an internal standard DNA (GeneScan 400HD Rox, PE Applied Biosystems) with GeneScan software (PE Applied Biosystems).

Results and Discussion

Parentage analysis of 'Hida'. 'Hida' (sowed in 1972, selected in 1978, registered in 1985) was produced from an orchard of 'Fuji' ('Ralls Janet' was crossed with 'Delicious' in 1939, selected in 1958, registered in 1962) and 'Orin' ('Golden Delicious' was crossed with 'Indo', named in 1952). However, the *S*-RNase content of 'Hida' (S_3S_9) did not match any of the expected *S*-RNase contents from its supposed parents 'Fuji' (S_1S_9) and 'Orin' (S_2S_7) (Matsumoto et al., 2003a). We confirmed the *S*-RNase content of 'Hida' as S_3S_9 using the plants 'Hida B6' and 'Hida B7' from the Hida region of Japan (data not shown). From the *S*-RNase content, S_9 seemed to be inherited from its maternal parent 'Fuji' (S_1S_9), and S_3

Received for publication 26 May 2004. Accepted for publication 30 Aug. 2004. We wish to thank T. Kobayashi and Y. Miyamoto for supplying the plant samples and offering useful suggestions. We are also indebted to T. Fukazawa-Akada for her useful suggestions and R. Horiguchi for her technical assistance. This research was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (Nos. 14360019 and 15208004).

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not from its paternal parent 'Orin' (S_2S_7), but from an unknown paternal parent having an S_3. 'Golden Delicious' (introduced into Japan in 1923) and 'Orei' ('Golden Delicious' was crossed with 'Delicious' in 1932, selected in 1947, registered in 1951) had been planted with 'Orin' within or nearby the orchard that produced 'Hida'. 'Golden Delicious' (S_2S_3), not 'Orei' (S_2S_{28}) seemed to be the paternal parent of 'Hida' based on their *S*-RNase contents. We investigated 19 SSR loci to confirm the paternal parent of 'Hida'. All 38 alleles in 'Hida' had been inherited from 'Fuji' and 'Golden Delicious' without discrepancy (Table 1), confirming that 'Hida' is a hybrid of those two cultivars (Table 2).

Parentage analysis of 'Ryoka No Kisetsu' and 'Hokuto'. A chance seedling, 'Ryoka No Kisetsu', was discovered at 1981 from an orchard of 'Fuji' and 'Starking Delicious' (introduced into Japan in 1929), and has been considered to be a hybrid of 'Starking Delicious' (S_9S_{28}) × 'Fuji' (S_1S_9) or a sport of 'Fuji' (S_1S_9) based on its *S*-RNase content S_1S_9 (Matsumoto et al., 2003a). All 38 alleles from the 19 SSR loci in 'Ryoka No Kisetsu' matched completely those in 'Fuji' (Table 1), strongly suggesting that 'Ryoka No Kisetsu' was a sport of 'Fuji' (Table 2). However, we could not deny the possibility of a 'Starking Delicious' × 'Fuji' parentage since all 38 alleles in 'Ryoka No Kisetsu' could have been inherited from those two cultivars (Table 1).

Although the maternal and paternal parents of 'Hokuto' (selected in 1980, registered in 1983) were thought to be 'Fuji' and 'Mutsu' ('Golden Delicious' was crossed with 'Indo' in 1930, selected in 1939, registered in 1949), respectively, Sakurai et al. (1997) pointed out that 'Mutsu' ($S_2S_3S_{20}$) was not the true paternal parent of 'Hokuto' ($S_1S_7S_9$) based on their *S*-RNase contents, whereas 'Indo' (sowed in 1875, the first cultivar produced in Japan) (S_2S_{20}) seemed to be its true paternal parent based on its *S*-RNase content and genetic markers relating to *Alternaria* blotch disease, etc. (Fukazawa-Akada, personal communication). In the case of triploid, such as 'Hokuto' and 'Mutsu', 2n and n gamete are contributed from the maternal and paternal parent, respectively. The allele of one locus (222, 224, and 228 bp at CH02d08) in 'Mutsu' was not inherited by 'Hokuto' (Table 1). Assuming that 'Fuji' is the maternal parent of 'Hokuto', the alleles of one locus in 'Mutsu' (146 bp at CH01g05) and six loci in 'Indo' (200 bp at CH03e03, 139 bp at CH03a09, 218 bp at CH03d07, 188 bp at CH01c06, 186 bp at CH05c04 and 192 bp at CH02g04) were not inherited by 'Hokuto' (Table 1). Based on those results, both 'Mutsu' and 'Indo' would have to be excluded as paternal parents of 'Hokuto'. We examined another candidate for 'Hokuto' paternal parentage 'Shin Indo' ('Indo' was crossed with 'Golden Delicious' in 1930, selected in 1947, named in 1948), but one locus in 'Shin Indo' (206 bp and 218 bp at CH03d07) was not inherited by 'Hokuto' (Table 1). Moreover, neither 186 bp at CH05c04 nor 192 bp at CH02g04 were inherited by it on the basis of its maternal parent 'Fuji' (Table 1), indicating that 'Shin Indo' must also be excluded as the paternal parent of 'Hokuto' (Table 2). One of the clones that vanished during the breeding program and thus was not registered as a new cultivar might also have been used as a paternal parent of 'Hokuto'.

Parentage analysis of 'Aika No Kaori', 'Korin', 'Kotoku', 'Tsugaru', and 'Yoko'. The paternal parents of 'Aika No Kaori' (sowed in 1972, registered in 2001), 'Korin' (breeding process are largely unknown), 'Kotoku' (sowed in 1971, selected in 1979, registered in 1985), 'Tsugaru' (crossed in 1930, selected in 1943, registered in 1975) and 'Yoko' (sowed in 1962, selected in 1969, registered in 1981) are unknown. 'Yoko' (S_3S_9) or 'Tsugaru' (S_3S_7) is likely to have been used as a paternal parent of 'Aika No Kaori' (S_3S_9) based on its *S*-RNase content analysis, and fruit and branch characteristics (Matsumoto et al., 2003a; Komatsu, unpublished results). Within the 19 SSR loci, four loci in 'Yoko' (135 bp and 143 bp at CH03a09, 156 bp and 188 bp at CH01c06, 224 bp and 228 bp at CH02d08, and 134 bp at CH01d09) were not inherited by 'Aika No Kaori' (Table 1). An additional two loci (144 bp at CH01g05 and 191 bp at CH02g04) were also not inherited by 'Aika No Kaori', assuming its maternal parent to be 'Fuji' (Table 1). In contrast, 37 within 38 alleles in 'Aika No Kaori' had been inherited from 'Fuji' and 'Tsugaru' without discrepancy (Table 1), indicating that 'Aika No Kaori' is a hybrid of those two cultivars (Table 2). One allele (228 bp at CH03d07) in 'Aika No Kaori', not inherited from 'Fuji', was detected only in 'Aika No Kaori'. The 228 bp band might be generated from the corresponding 226 bp in 'Fuji' by an error at DNA replication.

'Korin' is a chance seedling of 'Fuji' × unknown paternal parent. However, the *S*-RNase content of 'Korin' (S_1S_9) was identical to its reported maternal parent 'Fuji' (S_1S_9), suggesting that 'Korin' might be a sport of 'Fuji' (Matsumoto et al., 2003b). The fact that all 38 alleles from the 19 SSR loci in 'Korin' completely matched those in 'Fuji' (Table 1) indicated that 'Korin' was the sport of 'Fuji' (Table 2).

'Kotoku' resulted from a hybrid of 'Toko' ('Golden Delicious' was crossed with 'Indo' in 1930, fruited in 1938, named in 1962) with an unknown paternal parent. However, 'Toko' (S_2S_7) seemed not to be the maternal parent of 'Kotoku' (S_1S_{28}) based on the *S*-RNase content analysis (Matsumoto et al., 2003b). From its *S*-RNase content and fruit characteristics, 'Kotoku' (S_1S_{28}) may possibly be a hybrid of 'Fuji' (S_1S_9) × 'Orei' (S_2S_{28}). Based on our results, one allele in each locus of 'Fuji' was inherited by 'Kotoku' without discrepancy (Table 1), suggesting that 'Fuji' was used as a maternal parent of 'Kotoku' (Table 2). We also examined whether or not 'Orei' was a paternal parent of 'Kotoku'. Six alleles at three loci in 'Orei' (178 bp and 198 bp at CH01f07a, 103 bp and 111 bp at CH02b07, and 252 bp and 270 bp at CH01d08) were not inherited by 'Kotoku' (Table 1). Assuming that 'Fuji' is the maternal parent of 'Kotoku', an additional 10 alleles at five loci in 'Orei' (204 bp at CH03e03, 124 bp at CH03a04, 179 bp at CH01f03b, 222 bp at CH02d08 and 243 bp at CH02c09) were not inherited by 'Kotoku', suggesting that 'Orei' was not the paternal parent of 'Kotoku' (Table 1). Based on the *S*-RNase content of 'Kotoku' (S_1S_{28}) and its maternal parent 'Fuji' (S_1S_9), the paternal parent of 'Kotoku' must have the S_{28}-allele. We chose 'Starking Delicious' (S_9S_{28}), 'Nero 26' ['45 Gou' (' Jonathan' × 'Golden Delicious') was crossed with 'Richared Delicious' in 1954, selected in 1961] (S_7S_{28}), Empire (introduced into Japan in 1965) ($S_{10}S_{28}$) and Aori 3 ('Toko' was crossed with 'Richared Delious' in 1952, selected in 1963, named in 1970) (S_2S_{28}) as candidates for the paternal parent of 'Kotoku'. However, all of them were excluded for the reasons given below. Six alleles at three loci in 'Nero 26' (96 bp and 116 bp at CH03a04, 194 bp and 198 bp at CH01f07a, and 218 bp and 222 bp at CH02d08), eight at four loci in 'Empire' (96 bp and 118 bp at CH03a04, 192 bp and 198 bp at CH01f07a, 172 bp at CH01d09 and 208 bp at CH05c04), and four at two loci in 'Aori 3' (112 bp and 122 bp at CH02c02b, and 218 bp and 224 bp at CH02d08) were not inherited by 'Kotoku' (Table 1). Moreover, assuming that 'Fuji' is the maternal parent of 'Kotoku', 10 alleles at five loci in 'Starking Delicious' (204 bp at CH03e03, 96 bp at CH03a04, 103 bp at CH02b07, 218 bp at CH02d08, and 172 bp at CH01d09), six at three loci in 'Nero 26' (175 bp at CH05g08, 200 bp at CH03e03 and 132 bp at CH01d09), eight at four loci in 'Empire' (180 bp at CH05g08, 186 bp at CH03e03, 152 bp at CH01g05, and 233 bp at CH02c09), and 18 at nine loci in 'Aori 3' (175 bp at CH05g08, 204 bp at CH03e03, 96 bp at CH03a04, 131 bp at CH03a09, 111 bp at CH02b07, 132 bp at CH01d09, 208 bp at CH05c04, 140 bp at CH01g05 and 243 bp at CH02c09) were not inherited by 'Kotoku' (Table 1). A clone possessing the S_{28}-allele that was unregistered during the breeding process might have been used as a paternal parent of 'Kotoku'.

Both 'Tsugaru' and 'Yoko' were produced as hybrids of 'Golden Delicious' with an unknown paternal parent. Either of 'Megumi' ('Ralls Janet' was crossed with 'Jonathan' in 1931, named in 1948, registered in 1950) (S_2S_9), 'Fuji' (S_1S_9) or 'Jonathan' (introduced into Japan in 1871) (S_7S_9) could have been the paternal parent of 'Yoko' (S_3S_9) based on their *S*-RNase contents and fruit characteristics. Six alleles at three loci in 'Megumi' (206 bp and 226 bp at CH03d07, 190 bp and 196 bp at CH01f07a, and 250 bp and 254 bp at CH02d08), and 10 at five loci in 'Fuji' (131 bp at CH03a09, 226 bp at CH03d07, 158 bp and 160 bp at CH01c06, 212 bp at CH02d08, and 233 bp and 245 bp at CH02c09) were not inherited by 'Yoko' (Table 1). Moreover, four alleles at two loci in 'Megumi' (175 bp at CH05g08 and 270 bp at CH01d08), and eight at four loci in 'Fuji' (175 bp at CH05g08, 94 bp at CH03a04, 206 bp at CH01f07a and 192 bp at CH02g04) were not inherited by 'Yoko' assuming the maternal parent to be 'Golden Delicious' (Table 1). In contrast, all 38 alleles in 'Yoko' had been inherited from 'Golden Delicious' and 'Jonathan' without discrepancy

(Table 1), indicating that 'Yoko' is a hybrid of those two cultivars (Table 2).

'Jonathan' and 'American Summer Pearmain' (discovered in 1817) were thought to be candidates for the paternal parent of 'Tsugaru' from the breeding register. 'Jonathan' (S_7S_9), not 'American Summer Pearmain' (S_1S_{20}) seemed to be the paternal parent of 'Tsugaru' (S_3S_7) based on the *S*-RNase contents and fruit characteristics of both. All 38 alleles in 'Tsugaru' had been inherited from 'Golden Delicious' and 'Jonathan' without discrepancy (Table 1), confirming that 'Tsugaru' is a hybrid of both those cultivars (Table 2).

Table 1. SSR genotypes of 22 apple cultivars

Cultivar	SSR genotype (bp)				
name	CH05g08	CH03e03	CH02c02b	CH03a04	CH03a09
Fuji	175/175	186/198	112/116	94/124	131/131
Hida	175/175	186/204	112/116	94/124	131/131
Orin	175/175	200/200	112/122	124/124	135/139
Orei	175/179	186/204	112/116	124/124	135/135
Golden Delicious	175/175	200/204	116/122	124/124	131/135
Ryoka No Kisetsu	175/175	186/198	112/116	94/124	131/131
Starking Delicious	175/179[z]	186/204[z]	112/116[z]	96/124[z]	131/135[z]
Hokuto	175/175/175	186/198/204	112/116/	94/124/	131/135/
Mutsu	175/179/	186/200/204	112/116/122	120/124/	131/135/139
Indo	175/179	186/200	112/112	120/124	131/139
Shin Indo	175/179	186/204	112/116	120/124	131/135
Aika No Kaori	175/175	186/198	112/112	94/124	131/131
Yoko	175/179	198/204	112/116	116/124	135/143
Megumi	175/175	198/206	112/112	94/116	131/143
Tsugaru	175/175	198/200	112/122	116/124	131/131
Jonathan	175/179	198/206	112/112	100/116	131/143
Korin	175/175	186/198	112/116	94/124	131/131
Kotoku	175/179	186/206	116/116	100/124	131/135
Toko	175/175	186/204	112/122	120/124	131/139
Nero 26	175/175	186/200	112/116	96/116	135/143
Empire	175/180	186/186	116/116	96/118	135/139
Aori 3	175/175	186/204	112/122	96/124	131/131
	CH03d07	CH03d12	CH01c06	CH01f03b	CH01f07a
Fuji	226/226	116/124	158/160	171/179	178/206
Hida	188/226	116/124	156/158	139/171	178/198
Orin	206/226	124/124	162/188	139/177	196/198
Orei	206/206	124/124	160/162	171/179	178/198
Golden Delicious	188/206	124/124	156/162	139/171	178/198
Ryoka No Kisetsu	226/226	116/124	158/160	171/179	178/206
Starking Delicious	206/226[z]	116/124[y]	160/160[x]	139/179[z]	198/206[z]
Hokuto	188/226/	116/124/	158/160/162	171/177/179	178/206/
Mutsu	188/206/226	116/124/	156/162/188	139/171/177	178/198/
Indo	218/226	116/124	160/188	177/179	178/196
Shin Indo	206/218	116/124	162/188	139/177	178/178
Aika No Kaori	202/228	116/124	160/162	139/171	178/178
Yoko	188/202	124/124	156/188	139/171	178/194
Megumi	206/226	124/124	158/188	171/171	190/196
Tsugaru	188/202	124/124	162/188	139/171	178/194
Jonathan	202/206	124/124	188/188	171/171	194/196
Korin	226/226	116/124	158/160	171/179	178/206
Kotoku	226/226	116/124	158/160	139/171	206/206
Toko	188/218	124/124	162/188	139/177	178/178
Nero 26	206/226	124/124	160/162	139/171	194/198
Empire	226/226	122/124	160/162	139/171	192/198
Aori 3	188/226	124/124	160/162	139/139	178/206

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Table 1. SSR genotypes of 22 apple cultivars

Cultivar	SSR genotype (bp)				
name	CH02b07	CH02d08	CH01d09	CH05c04	CH01g05
Fuji	105/105	212/212	148/148	186/208	140/144
Hida	103/105	212/222	132/148	186/186	140/140
Orin	103/103	222/228	130/134	186/186	140/140
Orei	103/111	212/222	134/148	186/200	140/157
Golden Delicious	103/111	222/224	132/134	186/200	140/146
Ryoka No Kisetsu	105/105	212/212	148/148	186/208	140/144
Starking Delicious	103/105[w)]	212/218[z)]	148/172[z)]	200/208[z)]	144/157[z)]
Hokuto	103/105/	212/212/212	130/148/	186/200/208	140/144/157
Mutsu	103/111/	222/224/228	130/132/134	186/200/	140/146/
Indo	103/111	212/228	130/148	186/186	140/157
Shin Indo	103/111	212/222	130/134	186/186	140/157
Aika No Kaori	105/105	212/254	132/148	186/208	140/146
Yoko	103/105	224/228	134/134	186/186	140/144
Megumi	105/105	250/254	148/148	186/210	140/144
Tsugaru	103/105	224/254	132/134	186/200	144/146
Jonathan	105/126	228/254	134/134	186/186	144/144
Korin	105/105	212/212	148/148	186/208	140/144
Kotoku	105/126	212/228	134/148	186/200	144/157
Toko	103/111	224/228	130/132	186/200	140/157
Nero 26	105/126	218/222	132/148	200/208	144/157
Empire	105/126	218/228	172/172	208/208	144/152
Aori 3	105/111	218/224	132/148	186/208	140/144
	CH01d08	CH02c09	CH05a04	CH02g04	
Fuji	238/252	233/245	189/195	192/192	
Hida	238/248	245/257	166/195	192/192	
Orin	248/252	243/243	165/166	192/192	
Orei	252/270	243/245	159/195	187/192	
Golden Delicious	248/270	243/257	166/195	187/192	
Ryoka No Kisetsu	238/252	233/245	189/195	192/192	
Starking Delicious	238/252[z)]	245/255[z)]	159/189[z)]	192/192[z)]	
Hokuto	238/252/	233/243/245	189/195/	187/192/	
Mutsu	248/252/270	243/257/	166/195/	187/192/	
Indo	252/252	243/245	165/195	192/192	
Shin Indo	248/252	243/245	165/195	192/192	
Aika No Kaori	238/252	233/249	189/189	187/192	
Yoko	238/270	249/257	166/189	191/192	
Megumi	270/270	249/257	169/189	191/192	
Tsugaru	238/248	243/249	166/189	187/191	
Jonathan	238/270	249/257	189/189	191/191	
Korin	238/252	233/245	189/195	192/192	
Kotoku	238/238	245/255	189/195	192/192	
Toko	248/252	243/243	166/195	187/192	
Nero 26	238/248	255/257	189/195	191/192	
Empire	252/252	233/245	189/189	192/192	
Aori 3	238/252	243/245	159/195	187/192	

[z]Determined by Liebhard et al. (2002) and this work.
[y]Liebhard et al. (2002) has reported the SSR genotype as 124/126 bp, but we detected 116/124 bp instead.
[x]Liebhard et al. (2002) has reported the SSR genotype as 146/160 bp, but we could not detect a 146bp allele.
[w]Liebhard et al. (2002) has reported the SSR genotype as 180/182 bp, but we detected 103/105 bp instead.

Table 2. Analyses of eight parent-offspring relationships by SSR loci.

Cultivar	Reputed parents	Our results
Hida	Fuji × Orin	Fuji × Golden Delicious
Ryoka No Kisetsu	Starking Delicious × Fuji or Sport of Fuji	Sport of Fuji
Hokuto	Fuji × Mutsu	Fuji × unknown pollen parent (neither Mutsu, Indo, nor Shin Indo were used as pollen parents)
Aika No Kaori	Fuji × unknown pollen parent	Fuji x Tsugaru
Korin	Fuji × unknown pollen parent	Sport of Fuji
Kotoku	Toko × unknown pollen parent	Fuji x unknown pollen parent (neither Orei, Starking Delicious, Nero 26, Empire nor Aori 3 were used as pollen parents)
Tsugaru	Golden Delicious × unknown pollen parent	Golden Delicious × Jonathan
Yoko	Golden Delicious × unknown pollen parent	Golden Delicious × Jonathan