RESEARCH ARTICLE

# Confirmation of Parentage of the Pear Cultivar 'Niitaka' (*Pyrus pyrifolia*) Based on Self-incompatibility Haplotypes and Genotyping with SSR Markers

Hoy-Taek Kim and Ill-Sup Nou*

Department of Horticulture, Sunchon National University, Suncheon 57922, Korea

*Corresponding author: nis@sunchon.ac.kr

## Abstract

The parentage of the horticulturally important pear cultivar 'Niitaka' was confirmed by determining its *S*-genotypes based on the *S-RNase* and *PpSFBB*$^{-\gamma}$ genes, and genotyping using simple sequence repeat (SSR) markers. Previous reports suggested that the cultivars 'Amanogawa' and 'Imamuraaki' were the parents of 'Niitaka', although the cultivars 'Chojuro' and 'Shinchu' were also examined as candidate parents, along with two other cultivars. In the present study, the *S*-genotype of 'Niitaka' was determined to be $S^3S^9$. The $S^9$-*RNase* of 'Niitaka' was found to be likely inherited from the parent 'Amanogawa' ($S^1S^9$) and the $S^3$-*RNase* from 'Chojuro' ($S^3S^5$) or 'Shinchu' ($S^3S^5$). Based on the S-genotypes, the cultivar 'Imamuraaki' ($S^1S^6$) had no contribution to the parentage of 'Niitaka' ($S^3S^9$). A total of 67 polymorphic SSR markers were used to further confirm the parentage of 'Niitaka'. Discrepancies were found at several SSR loci between 'Niitaka' and the cultivars 'Imamuraaki' and 'Shinchu', whereas 'Niitaka' inherited alleles from 'Amanogawa' and 'Chojuro' at all SSR loci. Therefore, our findings established that 'Amanogawa' and 'Chojuro' are the parents of pear cultivar 'Niitaka', and not 'Imamuraaki' as previously reported.

**Additional key words:** parent-offspring relationship, PCR-CAPS, SFBB, *S-RNase*



Korean J. Hortic. Sci. Technol. 34(3):453-460, 2016
http://dx.doi.org/10.12972/kjhst.20160046

pISSN : 1226-8763
eISSN : 2465-8588

**Received:** March 4, 2016

**Revised:** April 19, 2016

**Accepted:** April 25, 2016



## Introduction

Pear (*Pyrus* spp.) is an important fruit tree worldwide and has been cultivated in more than 50 countries. In Korea, pear breeding began in the late 1920s by the National Institute of Horticultural and Herbal Science (NIHHS) of the Rural Development Administration (RDA). The goals of this breeding program include improving fruit quality in terms of fruit size, acidity, sugar content, flesh firmness and core ratio; developing disease and pest resistance for black spot, scab and mites; and developing labor-saving varieties by inducing self-compatibility (Shin et al., 2002). Korean pear cultivars 'Chuwhangbae', 'Hanareum', 'Josengwhangkeum', 'Shincheon' and 'Sooyoung' were bred using 'Niitaka' as a parent because 'Niitaka' ripens in mid-October at Suwon and has a high yield potential, producing large fruit with an attractive appearance and good storability (Kim et al., 1986; Hwang et al., 2002, 2005a, 2005b;

Shin et al., 2007). The 'Niitaka' cultivar was developed in 1915 by a breeding program in Japan (Kikuchi, 1927). Identifying the parent-offspring relationships of pear cultivars is important for current efforts to improve breeding efficiencies; however, some Japanese pear cultivars, including 'Housui' (Ishimizu et al., 1998), 'Kisui' (Hiratsuka et al., 1998), 'Tanzawa' (Castillo et al., 2001), 'Oharabeni' (Kim et al., 2007) and 'Niitaka' (Takasaki et al., 2004), exhibit discrepancies from their reported pedigrees in terms of skin type and self-incompatibility traits.

'Housui' (syn. 'Hosui') is a hybrid cultivar that was reported to be developed through a cross between 'Ri-14' and 'Yakumo' in 1954 (Kajiura et al., 1974). However, 'Housui' has russet skin and an $S^3S^5$ genotype for self-incompatibility. The reported parents, 'Ri-14' ($S^1S^2$) and 'Yakumo' ($S^1S^4$ and/or $S^2S^4$), have smooth skin and a different genetic makeup for self-incompatibility (Ishimizu et al., 1998). Kimura et al. (2003) used 20 SSR markers to confirm that 'Ri-14' and 'Yakumo' were not the parents of 'Housui'. The parents of 'Housui' were found to be 'Kousui' (female parent) and 'Hiratsuka 1 gou' (syn. 'I-33') (male parent) by Sawamura et al. (2004) using 61 SSR markers.

Similarly, 'Niitaka' was reportedly derived from a cross between the pear cultivars 'Amanogawa' and 'Imamuraaki' (Kikuchi, 1927). The *S*-genotypes of 'Amanogawa' and 'Imamuraaki' are $S^1S^9$ and $S^1S^6$, respectively (Kim et al., 2002; Takasaki et al., 2004), and the *S*-genotypes of any offspring of a cross between these cultivars would have to be $S^1S^6$ or $S^6S^9$. However, the *S*-genotype of 'Niitaka' is $S^3S^9$, leading Takasaki et al. (2004) to suggest that 'Amanogawa' and 'Imamuraaki' may not be the parents of 'Niitaka'. Two other cultivars, 'Chojuro' and 'Shinchu', have been suggested as candidate parents of 'Niitaka', because they were used as genetic resources during the breeding of 'Niitaka' in 1915 and have $S^3$-*RNase* (Kajiura and Sato, 1990; Washio et al., 2006). Here, to determine the parents of 'Niitaka', we analyze the *S*-genotypes of seven pear cultivars based on markers specific for *S-RNase* and *SFBB*$^{\gamma}$ and determine their SSR genotypes using 65 apple (*Malus* spp.) SSR markers and 42 pear SSR markers.

## Materials and Methods

### Plant Materials and DNA Isolation

Young leaves of six pear cultivars, 'Amanogawa', 'Imamuraaki', 'Chojuro', 'Shinchu', 'Niitaka' and 'Housui', and the pear line '126-29' (an offspring from a cross between 'Niitaka' and 'Housui'), were collected at the National Institute of Fruit Tree Science (Ibaraki, Japan). The leaves were frozen in liquid nitrogen and stored at -80ºC. Genomic DNA was extracted from the leaves by the method of Yamamoto et al. (2006) and used for genotyping with SSR markers and PCR-CAPS analysis of *S*-genes.

### *S-RNase* PCR-CAPS Analysis

PCR was performed using the *S-RNase*-specific primers FTQQYQ and PSprI, as described by Kim et al. (2007). The amplified *S-RNase* fragments were digested with the haplotype-specific restriction enzymes *Sfc*I ($S^1$), *Xba*I ($S^2$), *Ppu*MI ($S^3$ and $S^5$), *Alw*NI ($S^5$), *Mlu*I ($S^6$ and $S^7$) and *Bst*BI ($S^9$) and analyzed by agarose gel electrophoresis as described by Kim et al. (2002) and Takasaki et al. (2004).

### *PpSFBB*$^{\alpha}$ PCR-CAPS Analysis

*PpSFBB*$^{\gamma}$ fragments were amplified using the primers PpFBXf7 and PpFBXr3 and subjected to CAPS (cleaved amplified polymorphic sequence) analysis (Kakui et al., 2007). Derived CAPS (dCAPS) analysis was performed to detect *PpSFBB*$^{\gamma}$(Neff et al. 1998). *PpSFBB*$^{\gamma}$ fragments were amplified with the 1 bp-mutated primer GdCAPSS2g1-Rsa and reverse primer PpFBXr11. PCR products were digested with *Taq*I ($S^1$), *Ras*I ($S^2$), *Hpy*CH4IV ($S^3$), *Afl*II ($S^5$), *Dde*I ($S^6$) and *Hae*III ($S^9$). *Taq*I and *Sma*I digestions were incubated for 3 h at 65°C and 30°C, respectively, and the other endonucleases were incubated for 3 h at 37°C. The digested fragments were separated on 2% agarose gels in TAE buffer, stained with ethidium bromide and visualized under UV illumination.

### SSR Analysis

Genotyping was conducted using 107 SSR markers, including 65 SSR markers derived from apple and 42 SSR markers from pear (Yamamoto et al., 2004). Genomic DNA was isolated using the DNeasy Plant Mini Kit (QIAGEN, Germany). PCR amplification was performed according to the method of Yamamoto et al. (2002) with one modification; the forward primers were labeled with a FAM fluorescent dye. PCR products were separated by capillary electrophoresis on a POP-4 polymer using a Genetic Analyzer 3100 (PE Applied Biosystems, USA). The size of the amplified bands was calculated using an internal DNA standard (GeneScan-400HD ROX, PE Applied Biosystems) and GeneScan software (PE Applied Biosystems).

## Results and Discussion

### Analysis of *S*-genotypes using *S-RNase* from the Style and *SFBB*$^{\alpha}$ from Pollen

We identified the *S*-genotypes of the seven pear cultivars (Fig. 1) using a previously developed PCR-RFLP system (Fig. 1) (Kim et al., 2007). *S-RNase* PCR fragments (around 450 bp corresponding to $S^3$ or $S^5$ *RNase* and 1,300 bp corresponding to $S^2$ or $S^9$ *RNase*) were obtained from 'Niitaka' and the other pear cultivars. 'Niitaka' was digested with the $S^3$ and $S^5$ allele-specific restriction endonuclease, *Ppu*MI, producing a 450-bp PCR fragment, but this was not sufficient to determine whether its corresponding *S* allele was $S^3$ or $S^5$. However, when the $S^5$ allele-specific restriction endonuclease, *Alw*NI, was used, it was unable to digest the target allele, indicating that only the $S^3$ allele was present in that locus in 'Niitaka' (lane 5; Fig. 1C, 1D, and 1F). The presence of the $S^3$ allele was confirmed in 'Chojuro' (lane 3), 'Shinchu' (lane 4), 'Housui' (lane 6) and '162-29' (lane 7) when digested with the $S^3$ and $S^5$ allele-specific restriction endonuclease, *Ppu*MI (Castillo et al., 2001; Kim et al., 2002).

To determine the other *S*-allele, the 1,300-bp PCR fragments of 'Niitaka' were digested with the $S^2$ and $S^9$ allele-specific restriction endonucleases, *Xba*I and *Bst*BI, respectively (Fig. 1B and 1F). The PCR fragments of 'Niitaka' (lane 5) were cleaved with the $S^9$ allele-specific restriction endonuclease, but not with the $S^2$ allele-specific enzyme, indicating that 'Niitaka' contained the $S^9$ allele at that locus. The cultivar 'Amanogawa' (lane 1) was also confirmed to have the $S^9$-*RNase* using the $S^9$ allele-specific restriction endonuclease *Bst*BI (Takasaki et al., 2004). Therefore, we concluded that the *S*-genotype of 'Niitaka' must be $S^3S^9$. The *S*-genotypes of the cultivars were determined as follows: 'Amanogawa' ($S^1S^9$), 'Imamuraaki' ($S^1S^6$), 'Chojuro' ($S^2S^3$), 'Shinchu' ($S^3S^5$), 'Niitaka' ($S^3S^9$), 'Housui' ($S^3S^5$) and '162-29' ($S^3S^5$).

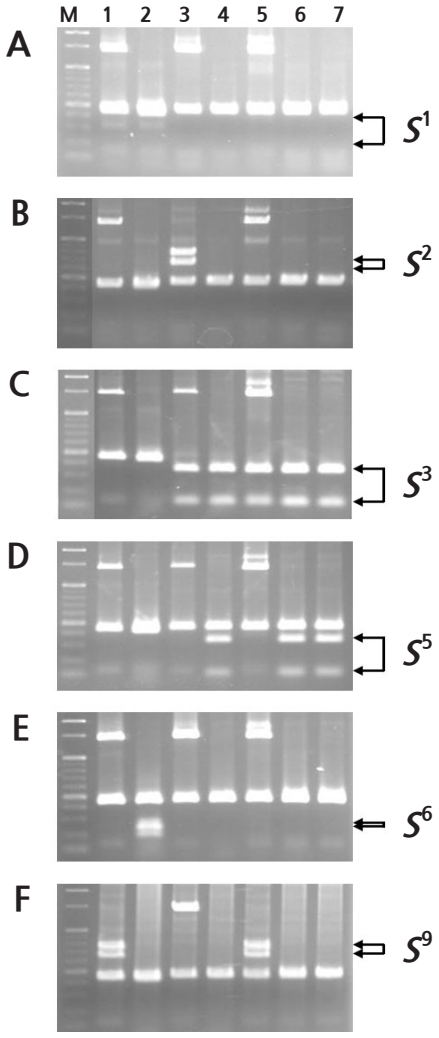


**Fig. 1.** PCR-RFLP analysis for identification of *S*-genotypes of seven Japanese pear cultivars using *S-RNase* genes. The *S-RNase* fragments were amplified by PCR using FTQQYQ and PSprI primers and digested with S allele-specific restriction enzymes; (A) *Sfc*I ($S^1$), (B) *Xba*I ($S^2$ and $S^k$), (C) *Ppu*MI ($S^3$ and $S^5$), (D) *Alw*NI ($S^5$), (E) *Mlu*I ($S^6$ and $S^7$) and (F) *Bst*BI ($S^9$). The pear cultivars examined were lane 1: Amanogawa ($S^1S^9$), lane 2: Imamuraaki ($S^1S^6$), lane 3: Chojuro ($S^2S^3$), lane 4: Shinchu ($S^3S^5$), lane 5: Niitaka ($S^3S^9$), lane 6: Housui ($S^3S^5$) and lane 7: 162-29 ($S^3S^5$). The digested fragments are indicated by arrows.

The $S^9$ allele specific to the 1,300-bp fragment was detected in 'Niitaka' and 'Amanogawa', suggesting that the $S^9$ allele in 'Niitaka' was derived from 'Amanogawa' ($S^1S^9$). The $S^3$ allele was also detected in 'Niitaka'; however, neither the $S^1$ nor $S^6$ allele of the previously reported male parent 'Imamuraaki' was found in 'Niitaka' ($S^3S^9$). Therefore, we concluded that 'Imamuraaki' is not a parent of 'Niitaka'. Furthermore, our results suggest 'Chojuro' ($S^2S^3$) and 'Shinchu' ($S^3S^5$) as possible candidates for the parent that contributed the $S^3$ allele of 'Niitaka'.

The recent identification of the *S* locus F-box brothers (*SFBB*) in Japanese pear and apple suggested that these multiple F-box genes are pollen-specific candidate genes for the *S* haplotypes. These *S* haplotypes exhibit pollen-specific expression, polymorphisms and linkage to the *S* locus (Sassa et al., 2007). In Japanese pear, three *SFBB*s were identified from a single *S* haplotype and were found to be homologous to other haplotype genes of the same group (i.e., α-, β- and γ-groups; Kakui et al., 2007).

We also determined the *S*-genotypes of the seven pear cultivars using the PCR-CAPS/dCAPS method based on the *PpSFBB*$^{\gamma}$ genes (Fig. 2A-2F). The 1,245-bp $S^3$-*PpSFBB*$^{\gamma}$ PCR fragments of 'Niitaka' ($S^3S^9$) (lane 5), 'Shinchu' ($S^3S^5$) (lane 4), 'Housui' ($S^3S^5$) (lane 6) and '162-29' ($S^3S^5$) (lane 7) were not cleaved with *Hpy*CH4IV (Fig. 2C). The *PpSFBB*$^{\gamma}$ fragments of 'Shinchu' (lane 4), 'Housui' (lane 6) and '162-29' (lane 7) were cleaved by the $S^5$-specific restriction endonuclease *Afl*II, but 'Niitaka' (lane 5) was not cleaved by this enzyme (Fig. 2D). The $S^9$-*PpSFBB*$^{\gamma}$ PCR fragments of 'Niitaka' (lane 5) and Amanogawa ($S^1S^9$) were cleaved by *Hae*III (Fig. 2F). Based on these polymorphisms of the *PpSFBB*$^{\gamma}$ genes, the *S*-genotype of 'Niitaka' was concluded to be $S^3S^9$ and the *S*-genotypes of 'Shinchu', 'Housui' and '162-29' were concluded to be $S^3S^5$, which correlates to the results of the *S-RNase* analysis. Therefore, we suggest that PCR-CAPS/dCAPS analysis of both *PpSFBB*$^{\gamma}$ and *S-RNase* genes can be used to determine the *S*-genotypes of Japanese pear, identify cultivars with highly diverse *S*-genotypes and reveal obscure relationships between parents and progeny. However, the *S*-genotype alone cannot perfectly describe the parent-offspring relationships or be used to identify new potential parents.

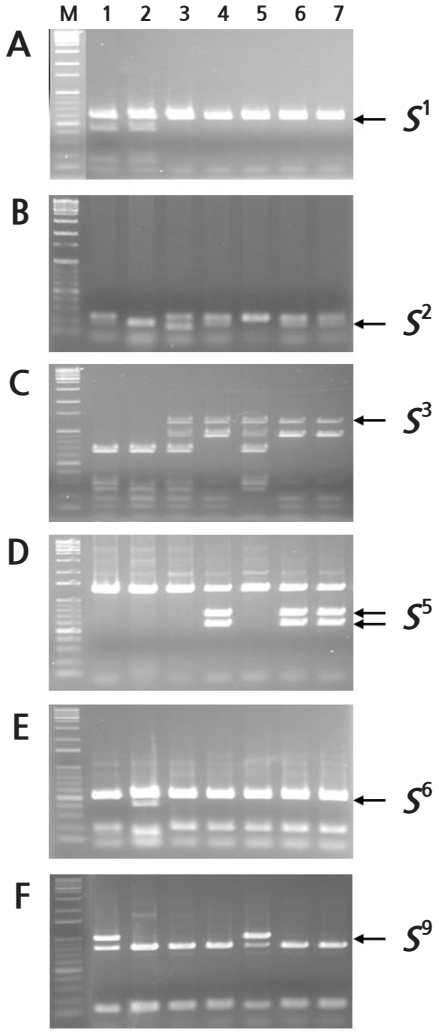


**Fig. 2.** PCR-CAPS and dCAPS analysis of *PpSFBB*$^{\gamma}$ genes. Amplified *PpSFBB*$^{\gamma}$ genes were digested with six restriction endonucleases; *Taq*I (A), *Rsa*I (B), *Hpy*CH4IV (C), *Afl*II (D), *Dde*I (E) and *Hae*III (F). Arrows show the representative *S* haplotype-specific fragments. The pear cultivars examined were lane 1: Amanogawa ($S^1S^9$), lane 2: Imamuraaki ($S^1S^6$), lane 3: Chojuro ($S^2S^3$), lane 4: Shinchu ($S^3S^5$), lane 5: Niitaka ($S^3S^9$), lane 6: Housui ($S^3S^5$) and lane 7: 162-29 ($S^3S^5$).

## SSR Analysis

To determine the parentage of the pear cultivar 'Niitaka', 107 SSR markers were tested. Among them, 67 SSR markers contained one or two alleles represented by discrete fragments and 14 SSRs contained three or more alleles, whereas 26 SSRs did not amplify. The 67 polymorphic SSR markers were used to determine the parentage of 'Niitaka' (Table 1). Sixteen alleles from ten SSR markers, NZ28f4, CH01h01, CH01f07a, CH04h02, CH03d02, CN444542, NH004a, NH014a, NH015a and TsuENH008, amplified in the previously reported parent 'Imamuraaki' but were not found in 'Niitaka', indicating that 'Imamauraaki' is not the male parent of 'Niitaka'. Based on similar discrepancies at seven SSR markers, the cultivar 'Shinchu' was also ruled out as a parent of 'Niitaka'.

**Table 1.** Parentage analysis of 'Niitaka' using 39 apple SSR markers and 28 pear SSR markers.

| Cultivar | *S*-genotype | SSR genotype (bp) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | NZ02b1 | NZ05g8 | NZ28f4 | CH01h01 | CH02b03b | CH02b10 | CH02d11 | CH01f07a | CH01g05 | CH02c09 | CH02c11 | CH02d08 |
| Amanogawa | $S^1S^9$ | 247/252 | 101/101 | 97/99 | 108/117 | 88/90 | 119/130 | 123/139 | 195/200 | 134/134 | 240/254 | 226/226 | 201/201 |
| Imamuraaki | $S^1S^6$ | 247/252 | 101/106 | 97/107 | 108/108 | 88/90 | 119/130 | 115/123 | 200/200 | 130/134 | 240/254 | 226/234 | 201/201 |
| Chojuro | $S^2S^3$ | 252/252 | 106/117 | 97/99 | 70/117 | 90/92 | 113/113 | 98/115 | 199/204 | 130/134 | 229/240 | 214/218 | 201/201 |
| Shinchu | $S^3S^5$ | 252/252 | 117/117 | 97/99 | 70/110 | 90/92 | 113/119 | 98/115 | 199/204 | 132/134 | 229/247 | 218/221 | 201/201 |
| Niitaka | $S^3S^9$ | 252/252 | 101/117 | 99/99 | 70/117 | 90/90 | 113/119 | 115/123 | 195/199 | 130/134 | 229/240 | 214/226 | 201/201 |
| Housui | $S^3S^5$ | 252/252 | 101/101 | 99/107 | 108/108 | 83/90 | 119/130 | 98/115 | 176/199 | 132/134 | 229/254 | 224/234 | 201/215 |
| 162-29 | $S^3S^5$ | 252/252 | 101/117 | 99/99 | 70/108 | 90/90 | 119/119 | 115/123 | 199/199 | 132/134 | 229/254 | 214/224 | 201/201 |

| Cultivar | *S*-genotype | CH02h11a | CH03a09 | CH03d11 | CH03g06 | CH04a12 | CH04d02 | CH04e05 | CH04g04 | CH04h02 | CH05c07 | MS06c09 | CH03a08 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Amanogawa | $S^1S^9$ | 105/105 | 111/116 | 95/105 | 135/147 | 165/165 | 131/155 | 184/192 | 163/175 | 155/172 | 132/134 | 107/127 | 152/152 |
| Imamuraaki | $S^1S^6$ | 105/111 | 111/116 | 95/95 | 135/135 | 157/165 | 125/155 | 184/204 | 159/175 | 155/170 | 132/134 | 107/111 | 152/153 |
| Chojuro | $S^2S^3$ | 109/136 | 111/113 | 97/101 | 135/135 | 137/165 | 155/155 | 194/214 | 159/175 | 182/184 | 116/134 | 111/111 | 153/153 |
| Shinchu | $S^3S^5$ | 109/132 | 111/113 | 97/105 | 135/147 | 144/165 | 121/155 | 182/214 | 165/175 | 170/182 | 116/128 | 111/111 | 153/153 |
| Niitaka | $S^3S^9$ | 105/109 | 111/113 | 95/101 | 135/143 | 165/165 | 131/155 | 184/194 | 159/163 | 172/182 | 116/134 | 111/127 | 152/152 |
| Housui | $S^3S^5$ | 109/109 | 116/116 | 113/115 | 135/147 | 165/165 | 125/125 | 204/204 | 159/159 | 172/182 | 116/128 | 111/111 | 152/152 |
| 162-29 | $S^3S^5$ | 109/109 | 111/116 | 101/113 | 135/147 | 165/165 | 125/131 | 184/204 | 159/159 | 172/172 | 116/116 | 111/111 | 152/152 |

| Cultivar | *S*-genotype | CH03d02 | CH04g07 | CH05d04 | CH01f12 | CH04c07 | CH04f06 | CH05d03 | CH05d11 | CH05a03 | CN493139 | AU223657 | Hi04a08 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Amanogawa | $S^1S^9$ | 184/204 | 171/189 | 183/183 | 151/169 | 106/121 | 176/176 | 169/182 | 175/183 | 197/202 | 140/140 | 236/257 | 204/204 |
| Imamuraaki | $S^1S^6$ | 184/188 | 189/208 | 183/189 | 161/169 | 106/119 | 176/176 | 169/169 | 178/183 | 197/202 | 140/149 | 245/257 | 204/204 |
| Chojuro | $S^2S^3$ | 175/184 | 171/171 | 183/183 | 152/156 | 100/119 | 159/176 | 169/173 | 178/183 | 205/205 | 140/140 | 236/253 | 204/216 |
| Shinchu | $S^3S^5$ | 173/175 | 171/171 | 183/183 | 151/152 | 100/100 | 159/178 | 165/173 | 183/188 | 207/220 | 140/147 | 253/255 | 204/216 |
| Niitaka | $S^3S^9$ | 175/204 | 171/189 | 183/183 | 156/169 | 119/121 | 176/176 | 169/182 | 175/178 | 197/205 | 140/140 | 253/257 | 204/204 |
| Housui | $S^3S^5$ | 173/178 | 171/208 | 183/183 | 149/157 | 106/106 | 159/178 | 169/169 | 178/178 | 197/202 | 140/147 | 236/253 | 204/204 |
| 162-29 | $S^3S^5$ | 178/204 | 171/171 | 183/183 | 156/157 | 106/119 | 176/178 | 169/169 | 178/178 | 197/202 | 140/140 | 253/253 | 204/204 |

| Cultivar | *S*-genotype | CN444542 | Hi04e04 | AT000174 | BGT23b | BGA35 | HGA8b | KA4b | KU10 | NH001c | NH002b | NH004a | NH005b |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Amanogawa | $S^1S^9$ | 115/121 | 228/228 | 150/150 | 196/206 | 136/136 | 147/164 | 90/90 | 249/249 | 120/124 | 173/183 | 81/118 | 330/350 |
| Imamuraaki | $S^1S^6$ | 112/112 | 228/228 | 150/150 | 196/206 | 136/136 | 147/164 | 90/90 | 249/287 | 120/122 | 173/183 | 81/126 | 326/351 |
| Chojuro | $S^2S^3$ | 109/112 | 228/228 | 150/161 | 196/206 | 128/136 | 147/164 | 90/102 | 249/249 | 120/122 | 175/175 | 104/112 | 330/352 |
| Shinchu | $S^3S^5$ | 109/114 | 228/228 | 150/150 | 196/226 | 128/136 | 147/164 | 90/102 | 236/249 | 107/120 | 175/175 | 104/112 | 330/353 |
| Niitaka | $S^3S^9$ | 109/121 | 228/228 | 150/150 | 196/206 | 136/136 | 147/164 | 90/102 | 249/249 | 122/124 | 175/183 | 104/118 | 330/354 |
| Housui | $S^3S^5$ | 109/114 | 228/228 | 150/161 | 196/226 | 136/136 | 147/164 | 90/102 | 249/249 | 124/124 | 177/177 | 81/118 | 346/350 |
| 162-29 | $S^3S^5$ | 109/114 | 228/228 | 150/161 | 196/226 | 136/136 | 147/164 | 90/102 | 249/249 | 122/124 | 177/183 | 118/118 | 350/350 |

**Table 1** (Continued)

| Cultivar | *S*-genotype | NH007b | NH008b | NH009b | NH011b | NH013a | NH014a | NH015a | NH017a | NB105a | NB109a | NB113a | NH023a |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Amanogawa | $S^1S^9$ | 126/144 | 176/176 | 151/159 | 188/188 | 205/205 | 71/78 | 99/135 | 85/87 | 150/156 | 146/146 | 143/143 | 137/137 |
| Imamuraaki | $S^1S^6$ | 126/126 | 176/183 | 145/151 | 188/244 | 205/215 | 78/92 | 99/99 | 87/89 | 150/172 | 146/146 | 143/149 | 133/137 |
| Chojuro | $S^2S^3$ | 126/154 | 176/196 | 163/163 | 178/186 | 201/205 | 71/88 | 98/135 | 95/108 | 146/160 | 182/182 | 145/149 | 133/176 |
| Shinchu | $S^3S^5$ | 154/154 | 180/196 | 163/163 | 186/244 | 201/217 | 79/88 | 104/135 | 85/95 | 146/160 | 182/182 | 145/149 | 137/176 |
| Niitaka | $S^3S^9$ | 126/144 | 176/196 | 151/163 | 186/188 | 201/205 | 71/88 | 135/135 | 87/108 | 150/160 | 146/182 | 143/145 | 137/176 |
| Housui | $S^3S^5$ | 154/154 | 196/200 | 163/163 | 186/186 | 201/217 | 71/88 | 98/135 | 85/95 | 146/146 | 146/182 | 143/149 | 137/196 |
| 162-29 | $S^3S^5$ | 144/154 | 196/196 | 163/163 | 186/186 | 201/205 | 71/88 | 99/135 | 95/108 | 146/160 | 146/182 | 143/149 | 137/137 |

| Cultivar | *S*-genotype | NH025a | NH027a | NH029a | NH036b | NB125a | NB141b | TsuENH008 |
|---|---|---|---|---|---|---|---|---|
| Amanogawa | $S^1S^9$ | 82/82 | 115/135 | 80/88 | 164/185 | 235/235 | 128/130 | 155/158 |
| Imamuraaki | $S^1S^6$ | 82/82 | 115/135 | 80/80 | 181/185 | 235/237 | 130/142 | 155/162 |
| Chojuro | $S^2S^3$ | 76/94 | 143/143 | 80/80 | 180/185 | 264/277 | 130/134 | 155/165 |
| Shinchu | $S^3S^5$ | 82/94 | 143/143 | 77/80 | 179/185 | 260/260 | 128/130 | 163/165 |
| Niitaka | $S^3S^9$ | 76/82 | 135/143 | 80/88 | 185/185 | 235/264 | 130/130 | 158/165 |
| Housui | $S^3S^5$ | 82/98 | 143/143 | 80/80 | 179/185 | 233/235 | 130/130 | 163/165 |
| 162-29 | $S^3S^5$ | 82/98 | 143/143 | 80/80 | 179/185 | 235/264 | 130/130 | 165/165 |

Underlined values indicate that specific alleles of 'Niitaka' may have been transmitted from 'Amanogawa' or 'Chojuro'

In humans, the probability of parentage is calculated based on the allele frequency (Hashiyada et al., 1997; Katsumata et al., 2001). According to the criteria for human parentage, an allele frequency of 0.9911 indicates an extremely high probability that an individual is a parent, whereas an allele frequency of 0.9999 shows a definitive parent-offspring relationship. In cultivated pears, it is very difficult to evaluate allele frequency, because it varies according to cultivar and species; however, the parent-offspring relationship of eight pear cultivars was determined with 15 to 20 SSR loci and the probability of parentage ranged from 0.9911 to 0.9999 (Kimura et al., 2003). Recently, Sawamura et al. (2008) analyzed the parent-offspring relationship of 55 Japanese pear cultivars using 18 SSR markers and determined that the reported parents of ten hybrid cultivars, including 'Niitaka', were incorrect due to discrepancies at three or more SSR loci.

In this study, we used 67 SSR markers, which should give a high level of confidence in parentage determination. Seven specific alleles of 'Amanogawa' were identified in 'Niitaka' by the SSR markers CH01f07a, CH04h02, MS06c09, CH03d02, CN444542, NH004a and TsuENH008. Eight specific alleles of 'Chojuro' were also found in 'Niitaka' using the SSR markers CH02c11, CH03d11, CH04e05, CH01f12, CH05a03, NH017a, NH025a and NB125a. Therefore, we conclude that 'Niitaka' was produced from a cross between 'Amanogawa' and 'Chojuro'. Based on the SSR analysis, we also confirmed that 'Niitaka' and 'Housui' are the parents of '162-29'.

**Acknowledgments:** We thank Prof. Toshiya Yamamoto of NARO Institute of Fruit Tree Science, Japan for providing pear plant materials. This research was supported by the Golden Seed Project (Center for Horticultural Seed Development, No. 213003-04-4-SB110), Ministry of Agriculture, Food and Rural Affairs (MAFRA), Ministry of Oceans and Fisheries (MOF), Rural Development Administration (RDA) and Korea Forest Service (KFS).

## Literature Cited


**Castillo C, Takasaki T, Saito T, Yoshimura Y, Norioka S, Nakanishi T** (2001) Reconsideration of *S*-genotype assignments and discovery of a new allele based on *S-RNase* PCRR-FLP in Japanese pear cultivars. Breed Sci 51:5-11. doi:10.1270/jsbbs.51.5

**Hashiyada M, Nata M, Adachi N, Kanetake J, Ji G, Sagisaka K, Kimura T, Ujiie K, Takaki T, Tsuchiya M** (1997) Allele frequency and application to paternity test of STR loci in Japanese population utilizing multiplex fluorescent PCR kit. DNA Polymorphism 6:152-155. (In Japanese with English title)

**Hiratsuka S, Kubo T, Okada Y** (1998) Estimation of self-incompatibility genotype in Japanese pear cultivars by stylar protein analysis. J Jpn Soc Hortic Sci 67:491-496. doi:10.2503/jjshs.67.491

**Hwang HS, Shin IS, Kim WC, Cho HM, Shin YU, Hwang JH** (2002) Breeding of the new early season pear cultivar 'Shincheon'. Acta Hortic 587: 299-320. doi:10.17660/ActaHortic.2002.587.39

**Hwang HS, Shin IS, Kim WC, Shin YU, Hwang JH, Hong SS** (2005a) Breeding of pear (*Pyrus pyrifolia* Nakai) cv. Hanareum characterized by early maturity and superior fruit quality. Korean J Hortic Sci Technol 23:60-63

**Hwang HS, Kim WC, Shin IS, Cho H M, Shin YU, Lee DK, Hong SS** (2005b) Breeding of a new yellow green pear cultivar 'Josengwhangkeum' (*Pyrus pyrifolia* Nakai) with high quality for summer season. Korean J Hortic Sci Technol 23:56-59

**Ishimizu T, Norioka S, Nakanishi T, Sakiyama F** (1998) *S*-genotype of Japanese pear 'Housui'. J Jpn Soc Hortic Sci 67:35-38. doi:10.2503/jjshs.67.35

**Kakui H, Tsuzuki T, Koba T, Sassa H** (2007) Polymorphisms of *SFBB*$^{\alpha}$ and its use for *S* genotyping in Japanese pear (*Pyrus pyrifolia*). Plant Cell Rep 26:1619-1625. doi:10.1007/s00299-007-0386-8

**Kajiura M, Kanato K, Machida Y, Maeda M, Kozaki I, Tashiro T, Kishimoto O, Seike K** (1974) New Japanese pear cultivar 'Hakko' and 'Hosui'. Bull Fruit Tree Res Stn A 1:1-12. (In Japanese)

**Kajiura I, Sato Y** (1990) Recent progress in Japanese pear (*Pyrus pyrifolia*) breeding and descriptions of cultivars based on literature review. Bull Fruit Tree Res Stn Extra No. 1:1-329. (In Japanese with English abstract)

**Katsumata Y, Katsumata R, Yamamoto T, Tamaki K** (2001) Estimating probabilities and dealing with mutations in paternity testing: verification of DNA testing with commercially available STR kits. Jpn J Legal Med 55: 205-216. (In Japanese)

**Kikuchi A** (1927) Some studies on pear and pear breeding. Bull Agric Exp St Kanagawa 53:1-104. (In Japanese)

**Kim H, Kakui H, Koba T, Hirata Y, Sassa H** (2007) Cloning of a new *S-RNase* and development of a PCR-RFLP system for the determination of the *S*-genotypes of Japanese pear. Breed Sci 57:159-164. doi:10.1270/jsbbs.57.159

**Kim H, Hirata Y, Nou I** (2002) Determination of *S*-genotype of pear (*Pyrus pyrifolia*) cultivars by S-RNase sequencing and PCR-RFLP analyses. Mol Cells 13:444-451

**Kim YS, Hong KH, Kim JB, Yiem MS, Lee UJ, Kim WC, Kim JH, Hong SB, Kim SB, Moon JY, Kim KY, Cho MD, Lee DK** (1986) A new late-season pear cultivar 'Chuwhangbae'. Res Rpt RDA (Hortic) 28:57-61

**Kimura T, Sawamura Y, Kotobuki K, Matsuta N, Hayashi T, Ban Y, Yamamoto T** (2003) Parentage analysis in pear cultivars characterized by SSR marker. J Jpn Soc Hortic Sci 72:182-189. doi:10.2503/jjshs.72.182

**Neff MM, Neff JD, Chory J, Pepper AE** (1998) dCAPS, a simple technique for the genetic analysis of single nucleotide polymorphisms: experimental applications in *Arabidopsis thaliana* genetics. Plant J 14:387-392. doi:10.1046/j.1365-313X.1998.00124.x

**Sassa H, Kakui H, Miyamoto M, Suzuki Y, Hanada T, Ushijima K, Kusaba M, Hirano H, Koba T** (2007) *S locus F-box brothers*: multiple and pollen-specific F-box genes with *S* haplotype-specific polymorphisms in apple and pear. Genetics 175:1869-1881. doi:10.1534/genetics.106.068858

**Sawamura Y, Saito T, Takada N, Yamamoto T, Kimura T, Hayashi T, Kotobuki K** (2004) Identification of parentage of Japanese pear 'Housui'. J Jpn Soc Hortic Sci 73:511-518. doi:10.2503/jjshs.73.511

**Sawamura Y, Takada N, Yamamoto T, Saito T, Kimura T, Kotobuki K** (2008) Identification of parent-offspring relationships in 55 Japanese pear cultivars using *S-RNase* allele and SSR markers. J Jpn Soc Hortic Sci 77:364-373. doi:10.2503/jjshs1.77.364

**Shin IS, Kim WC, Hwang HS, Shin YU** (2002) Achievements of pear breeding in Korea. Acta Hortic 596:247-250. doi:10.17660/ActaHortic.2002.596.36

**Shin IS, Hwang HS, Shin YU, Kim WC** (2007) 'Sooyoung': a mid-season pear (*Pyrus pyrifolia* Nakai) cultivar with high soluble solids and medium size. J Am Pomol Soc 61:170-173

**Takasaki T, Okada K, Castillo C, Moriya Y, Saito T, Sawamura Y, Norioka N, Norioka S, Nakanishi T** (2004) Sequence of the $S^9$-RNase cDNA and PCR-RFLP system for discriminating $S^1$- to $S^9$-allele in Japanese pear. Euphytica 135:157-167. doi:10.1023/B:EUPH.0000014907.50575.d0

**Washio K, Takahashi T, Handa M** (2006) A new Japanese pear cultivar, 'Kirari'. Bull Tochigi Agric Exp Stn 58:1-5. (In Japanese)

**Yamamoto T, Kimura T, Hayashi T, Ban Y** (2006) DNA profiling of fresh and processed fruits in pear. Breed Sci 56:165-171. doi:10.1270/jsbbs.56.165

**Yamamoto T, Kimura T, Soejima J, Sanada T, Ban Y,Hayashi T** (2004) Identification of quince varieties using SSR markers developed from pear and apple. Breed Sci 54:239-244. doi:10.1270/jsbbs.54.239

**Yamamoto T, Kimura T, Shoda M, Ban Y, Hayashi T, Matsuta N** (2002) Development of microsatellite markers in Japanese pear (Pyrus *pyrifolia Nakai*). Molec Ecol Notes 2:14-16. doi:10.1046/j.1471-8286.2002.00128.x