Parentage Confirmation of Korean Bred Pear Cultivars by Simple Sequence Repeat SSR Genotyping and S-Genotypes Analysis

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ABSTRACT Identification and authentication of parentage are important for effective pear breeding. Within Korean pear cultivars discrepancies are often reported between parents and offspring in skin color of fruits and also in S-genotypes suggesting that reported parentage was often inappropriate. In Korea, the parentage of the most of pear cultivars was never confirmed at the molecular level. Simple sequence repeat (SSR) genotyping and S-genotype analysis are considered effective in identifying parents. In this study, parentage of nine Korean bred cultivars was confirmed using SSR genotyping and S-genotype analysis. A total of 53 SSR markers were used. Six different haplotype-specific endonucleases were used for restriction cleavage of S-genotypes. Most of the Korean bred cultivars had six comparatively shorter S-RNase, S^1 , S^3 , S^4 , S^5 , S^6 , or S^7 of 450 bp in length whereas the Japanese control cultivars had four other comparatively longer S-RNase. Out of nine pear cultivars only 'Chuwangbae' and 'Whangkeumbae' had identical SSR genotypes and S-genotype with previously reported parents. For another cultivar, 'Sujeonbae', the parents were the mutants of reported parent, 'Niitaka'. For four other cultivars, SSR and S-genotypes of offspring matched with only one reported parent 'Niitaka' but those of another parent did not match. For the two other pear cultivars 'Soowhangbae' and 'Sooyoung' none of reported parents were confirmed by SSR genotyping and S-genotype analysis. Historically, the parent 'Niitaka' was predominant in the Korean pear breeding programs because of its high yield potential and quality. The methods have been used in this study could be used to identify pear cultivars with diverse S-genotypes to eliminate any existing obscure parent-offspring relations.

Keywords Pears, SSR genotype, S-genotype, S-RNase

INTRODUCTION

Pear (*Pyrus* spp.) is one of the important fruit in the world that is cultivated in more than 50 countries. In Korea, some local pear cultivars such as, 'Whangsilri', 'Cheongsilri' (*Pyrus ussuriensis* var. *ovoidea* Rehder), 'Cheongdangrori', 'Habsilri' (*P. ussuriensis* var. *viridis* T.B. Lee) were being cultivated until the beginning of 19th century. Breeding of Korean pear cultivars (*P. pyrifolia* Nakai) was started in the late 1920s at the National Institute of Horticultural and Herbal Science (NIHHS) of Rural Development Administration (RDA) in South Korea. The breeding goals at that time were increasing fruit size and improving fruit quality that includes desirable pH, sugar content, flesh firmness and core ratio and also development of disease and pest resistance such as, black spot, scab and mite resistance

(Shin *et al.* 2002). Besides those, utilization of self-compatibility phenomenon was an additional breeding objective aimed to develop labor-saving varieties (Shin *et al.* 2002). As a part of that extensive pear breeding efforts throughout the 19th century a total of 27 pear cultivars were released by NIHHS between 1969 and 2009 (Cho *et al.* 2012). A notable number of varieties including 'Chuwangbae', 'Whangkeumbae', 'Hanareum', 'Shincheon', 'Soowhangbae', 'Josengwhangkeum', 'Sunhwang' and 'Sooyoung' were bred by using 'Niitaka' as one of the parent in those breeding programs. But the same parent 'Niitaka' was not used for some other cultivars such as 'Chuwangbae' and 'Soowhangbae' (Kim *et al.* 1986; Hwang *et al.* 2002; 2005a; 2005b; Shin *et al.* 2007). Simple sequence repeat (SSR) genotyping in a few notable studies also confirmed that 'Niitaka' was not the parent of some pear cultivars as

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previously reported (Sawamura *et al.* 2008; Kim and Nou 2016). Other than variation in SSR genotyping of some pear cultivars, such as, ‘Housui’ (Machida *et al.* 1982; Ishimizu *et al.* 1998), ‘Kisui’ (Hiratsuka *et al.* 1998), ‘Tanzawa’ (Castillo *et al.* 2001), ‘Niitaka’ (Takasaki *et al.* 2004) and ‘Oharabeni’ (Kim *et al.* 2007) some of them had discrepancies from their reported parents in terms of skin type and *S*-genotypes. The registrant parents ‘Ri-14’ (S^1S^2) and ‘Yakumo’ (S^1S^4 and/or S^2S^4) of ‘Housui’ had smooth skin and a different genetic makeup for self-incompatibility compared to their offspring ‘Housui’ (Machida *et al.* 1982; Ishimizu *et al.* 1998). Kimura *et al.* (2003) analyzed the parentage of 14 pear cultivars by using 20 SSR markers and confirmed that ‘Ri-14’ and ‘Yakumo’ were not the parents of ‘Housui’. The parents of ‘Housui’ were ‘Kousui’ (female parent) and ‘Hiratsuka 1 gou’ (synonym ‘I-33’, male parent) as reported by Sawamura *et al.* (2004) after SSR genotyping. Furthermore, in a separate study parentage of 55 Japanese pear cultivars was identified by using *S*-RNase allele and 18 SSR markers (Sawamura *et al.* 2008). The study by Sawamura *et al.* (2008) thus identified discrepancy of SSR-genotypes between 10 hybrid cultivars (‘Kisui’, ‘Tanzawa’, ‘Niitaka’, ‘Seiryu’, ‘Akemizu’, ‘Atago’, ‘Echigonishiki’, ‘Ishiiwase’, ‘Shusui’, and ‘Yachiyo’) and their previously reported parents and suggested that these hybrid cultivars have new candidate parents. However, identifying the parent-offspring relationships of pear cultivars is very important to improve breeding efficiencies.

Polymorphism exists in *S*-genotypes at the *S*-RNase locus that controls self-incompatibility was previously utilized for the identification of Japanese and Korean bred pear cultivars (Ishimizu *et al.* 1999; Kim *et al.* 2002; 2007). In addition, DNA marker based profiling techniques could be also utilized for identifying the parents of pear cultivars. Teng *et al.* (2002) used random amplified polymorphic DNA markers to classify 118 Asian pear cultivars. Monte-Corvo *et al.* (2001) reported that inter-simple sequence repeat was effective for identifying parents of *P. communis* L. cultivars of European origin. Kim *et al.* (2012) analyzed 80 pear cultivars among them 64 were Japanese (*P. pyrifolia*), 10 were Chinese (*P. ussuriensis*) and six were European in origin based on their BRIP markers associated DNA profiling. The authors were able

to differentiate cultivar ‘Housui’ from the other Japanese pear cultivars. A large number of SSR markers have been developed in the last two decades from genomic DNAs and ESTs in Japanese and European pears (Yamamoto *et al.* 2002; 2004; Fernandez-Fernandez *et al.* 2006; Nishitani *et al.* 2009). The use of these SSR markers has allowed DNA profiling, parentage analysis, and evaluation of genetic diversity in pear cultivars. To discern the real parent-offspring relationship, some apple cultivars were investigated with *S*-RNase analysis and SSR markers and revealed their true parentage (Kitahara *et al.* 2005; Moriya *et al.* 2011).

In Korea, Cho *et al.* (2012) used 19 sequence-characterized amplified region (SCAR) markers and nine SCAR markers to distinguish 39 pear cultivars (25 Korean pear cultivars and 14 Japanese pear cultivars). Recently, the *SNPs*, *InDels*, and *SSRs* detected between two cultivars ‘Whangkeumbae’ and ‘Minibae’ by using NGS data have been mapped to the reference genome of the Chinese pear ‘Suli’ (*P. bretschneideri*) (Oh *et al.* 2015). The number of total *SSRs* for ‘Whangkeumbae’ and ‘Minibae’ was 75,000 among them 1,200 were found polymorphic. Despite availability of sufficient techniques and markers, to date the parent-offspring relationship of Korean bred pear has not been analyzed. In this study, the nine Korean bred pear cultivars were analyzed using the *S*-genotypes with *S*-RNase analysis and *SSR* genotypes with 53 *SSR* markers to confirm their parent-offspring relationship.

MATERIALS AND METHODS

Plant materials, candidate parent selection and isolation of DNA

The nine Korean bred pear cultivars were collected at the NIHHS (Cho *et al.* 2012; Table 1). *SSR* genotyping data of 98 pear cultivars were obtained from the literature (Yamamoto *et al.* 2002; 2004; 2006; 2007; Kimura *et al.* 2003; Sawamura *et al.* 2004; Sawamura *et al.* 2008; submitted manuscript). Candidate parents were selected using MARCO software with 53 *SSR* markers information. All candidate parents were of Japanese origin. Twelve candidate parent-pear cultivars were collected at the National Institute of Fruit Tree Science (Ibaraki, Japan)

Table 1. The 9 Korean bred pear cultivars and 12 Japanese candidate pear parent cultivars used in this experiment.

Cultivar (synonym)	Registrant parents	Origin
Chuwangbae	Imamuraaki (S^1S^6)×Nijisseiki (S^2S^4)	Korea
Whangkeumbae	Niitaka (S^3S^9)×Nijisseiki (S^2S^4)	Korea
Hanareum	Niitaka (S^3S^9)×Chuwangbae (S^3S^4)	Korea
Shincheon	Niitaka (S^3S^9)×Chuwangbae (S^3S^4)	Korea
Soohwangbae	Chojuro (S^2S^3)×Kimitsukawase (S^1S^5)	Korea
Josaenghwangkeum	Niitaka (S^3S^9)×Shinko (S^4S^9)	Korea
Sunhwang	Niitaka (S^3S^9)×Okusankichi (S^5S^7)	Korea
Sooyoung	Niitaka (S^3S^9)×Soohwangbae (S^3S^4)	Korea
Sujeongbae	Mutant of Niitaka (S^3S^9)	Korea
Imamuraaki	Breeding process is unknown	Japan
Nijisseiki	Discovered in 1888, named in 1904	Japan
Niitaka	Amanogawa (S^1S^9)×Chojuro (S^2S^3)	Japan
Chojuro	Sown in 1889, fruited in 1893	Japan
Kimitsukawase	Shinkozo×Doitsu (S^1S^2)	Japan
Okusankichi	Sown in 1840-1850	Japan
Tama	Gion (S^2S^4)×Kosui (S^4S^5)	Japan
Chouju	Asahi (S^4S^5)×Kimitsukawase (S^1S^5)	Japan
Shinsui	Kikusui (S^4S^4)×Kimitsukawase (S^1S^5)	Japan
Shinko	Nijisseiki (S^2S^4)×Amanokawa (S^1S^9)	Japan
Ichiharawase	Discovered in 1892 or 1893	Japan
Kinchaku	Breeding process is unknown, local cultivar	Japan

(Sawamura *et al.* 2008; Table 1). Genomic DNA was extracted from young leaves by using a Genomic tip 20/G (Qiagen, Germany) using the method previously followed by Yamamoto *et al.* (2006). The extracted genomic DNA was used for SSR analysis and polymerase chain reaction (PCR)-cleaved amplified polymorphic sequences analysis of *S*-RNases.

Data analysis and SSR genotyping by SSR markers

A total of 53 SSR markers were selected from 96 SSR markers developed for apple and pear (Yamamoto *et al.* 2002; 2004; 2006; 2007). PCR amplification was performed according Yamamoto *et al.* (2002) except that the forward primers labeled with a fluorescent dye. The PCR products were separated by electrophoresis on a POP-4 polymer and detected using a Genetic Analyzer 3100 (PE Applied Biosystems, DriveFoster City, CA, USA). The size of amplified bands was scored using an internal DNA standard (GeneScan-400HD ROX; PE Applied Biosystems) with GeneScan software (PE Applied Biosystems). The parent-offspring relationships of Korean bred pear cultivars were analyzed by comparing the SSR alleles in each

cultivars with registrant parents using the MARCO software (Fujii *et al.* unpublished as cited by Shoda *et al.* 2012). Furthermore, to identify minimal number of marker subsets, MinimalMarker software (Fujii *et al.* 2007) was used because that was necessary to distinguish all of the cultivars to find out identical genotypes of the 21 cultivars generated by the 53 SSR markers.

S-RNase genotyping by PCR-restriction fragment length polymorphism (RFLP) analysis

PCR amplification was performed using the *S*-RNase-specific primers, FTQQYQ (TTTACGCAGCAATAT-CAG) and PSprI (DKWCSCRCAGGWCCATGTTT), which were designed from *S*-RNase high homology region of pear and amplified S^1 to S^9 -RNase and S^k -RNase, as described by Kim *et al.* (2007). The amplified *S*-RNase fragments were checked and digested with the haplotype-specific restriction enzymes *SfcI* (S^1), *PpuMI* (S^3 and S^5), *NdeI* (S^4), *AhaNI* (S^5), and *BstBI* (S^9) to determine their size after agarose gel electrophoresis as described by Kim *et al.* (2002).

RESULTS

Determination of candidate parent of Korean pear cultivars by using SSR genotyping

SSR genotyping based on 53 SSR markers revealed the parentage of nine Korean bred pear cultivars (Table 2). The SSR genotypes of ‘Chuwangbae’ (‘Imamuraaki’ × ‘Nijiiseiki’) and ‘Whangkeumbae’ (‘Nijiiseiki’ × ‘Niitaka’) were confirmed and matched with the registrant parents SSR genotypes by all SSR markers (Table 2). We confirmed that one SSR allele each from ‘Hannareum’, ‘Shincheon’, ‘Josaengwhangkum’ and ‘Sunhwang’ was inherited from registrant parent ‘Niitaka’, i.e., those 4 pear cultivars had one identical SSR allele of ‘Niitaka’ (Table 2). But they did not have identical SSR alleles with another registrant parents. In addition, two cultivars ‘Soowhangbae’ and ‘Sooyoung’ had different SSR alleles that were not matched with parents (Table 2). Therefore, we concluded that two pear cultivars ‘Chuwangbae’ and ‘Whangkeumbae’ were bred from a breeding program between registrant parent, but other six pear cultivars were bred from a cross between other non-reported parents. Also, the SSR genotype of ‘Sujeongbae’ being a mutant of ‘Niitaka’ was identical with ‘Niitaka’ (data not shown).

The 53 SSR alleles of five pear cultivars were inherited from SSR alleles of each parent. The candidate parents of ‘Hannareum’ were ‘Niitaka’ and ‘Tama’; those of ‘Shincheon’ were ‘Niitaka’ and ‘Chouju’; those of ‘Sunhwang’ were ‘Niitaka’ and ‘Shinsui’, and those of ‘Soowhangbae’ were ‘Niitaka’ and ‘Nijisseiki’ (Table 3). The cultivar ‘Josaengwhangkum’ was the offspring of ‘Niitaka’ and ‘Nijisseiki’ similar to ‘Whangkeumbae’ although ‘Shinko’ was the reported parent instead of ‘Nijisseiki’ (Table 3). Therefore we suggested that the pear cultivars developed between 1966 and 1986, ‘Soowhangbae’, ‘Josaengwhangkum’ and ‘Whangkeumbae’ were the selected offspring of a cross between ‘Niitaka’ and ‘Nijisseiki’. The cultivar ‘Okusankichi’ was identified as one candidate parent of ‘Sooyoung’ (Table 3). The cultivar ‘Niitaka’ was another reported parent of ‘Sooyoung’ (Table 3). However out 53 SSR alleles not even one allele of ‘Sooyoung’ matched to ‘Niitaka’. Thus the second parent of cultivar ‘Sooyoung’ remain obscure.

S-genotyping by S-RNase PCR-RFLP analysis

The S-genotypes of following five Korean pear cultivars: ‘Sujeongbae’, ‘Hannareum’, ‘Shincheon’, ‘Sooyoung’ and ‘Josaengwhangkum’ were identified by S-RNase PCR-RFLP analysis in this study. The S-genotypes of remaining four cultivars in Table 1 were studied by Kim *et al.* (2002). The S-RNase PCR analysis amplified S-locus of 11 pear cultivars including six control pear cultivars of Japanese origin and five Korean cultivars. S-genotyping identified nine different types of S-locus from S^1 to S^9 and S^k -RNase (Fig. 1). S^1 , S^3 , S^4 , S^5 , S^6 or S^7 -RNase were about 450 bp in length, S^8 -RNase was 500 bp in length, S^k -RNase was 1,065 bp length and S^2 and S^9 -RNase were 1,414 bp and 1,374 bp in length respectively. The Japanese control pear cultivars had all S-RNase whereas four Korean cultivars (except ‘Sujeongbae’) had only the shortest S^1 , S^3 , S^4 , S^5 , S^6 , or S^7 -RNase of 450 bp length. The cultivar ‘Sujeongbae’ had S^9 -RNase in addition to other six types of S-RNase present in four other Korean cultivars (Fig. 1). For the determination of S-genotype, the S-RNase PCR fragments of 5 Korean pear cultivars were digested with S-allele specific enzymes (*PpuMI*; S^3 - and S^5 -allele specific, *AlwNI*; S^5 -allele specific, *BstBI*; S^9 -allele specific, *NdeI*; S^4 -allele specific and *ScfI*; S^1 -allele specific) (Fig. 2). The S-RNase PCR fragments of ‘Sujeongbae’ were digested with *PpuMI* (S^3 - and S^5 -allele specific) and *BstBI* (S^9 -allele specific), but was not digested with *AlwNI* (S^5 -allele specific) (Fig. 2A). Thus, the S-genotype of ‘Sujeongbae’ was determined as S^3S^9 . The S-genotype of ‘Sujeongbae’ corresponded with the S-genotype of ‘Niitaka’ (S^3S^9) because the ‘Sujeongbae’ is a mutant of ‘Niitaka’ (Fig. 2). The S-RNase PCR fragments of ‘Hannareum’ and ‘Josaengwhangkum’ were digested with S^3 - and S^4 -allele specific-enzymes (Fig. 2B, C). The S-genotypes of ‘Hannareum’ and ‘Josaengwhangkum’ were identical, S^3S^4 (Fig. 2). The S-RNase PCR fragments of ‘Shincheon’ were digested with S^1 - and S^3 -allele specific endonucleases. The S^5 -allele specific endonuclease was unable to digest the S-RNase PCR fragments of ‘Shincheon’ (Fig. 2D). Thus, the S-genotype of ‘Shincheon’ was determined as S^1S^3 that was not corresponded with the S-genotype of registrant parents ‘Niitaka’ (S^3S^9) and ‘Chuwangbae’ (S^4S^5). The

Table 2. SSR genotyping analysis of registrant parent and nine Korean bred pear cultivars by the 53 SSR markers and the MARCO software.

Errors ²⁾	Parent/ Offspring	Cultivar	SSR markers ³⁾												
			TsuENH003	CN444636	TsuENH045	CH02b10	NH023a	NBI13a	TsuENH074	BGA35	NH011b	CH02h11a	CH02c02b	CH03a09	NBI03a
0	Parent	Imamuraaki	FF	BG	BD	DF	BC	DH	AB	EE	FG	AC	BI	AC	DD
0	Parent	Nijiseiki	GG	AE	DD	DF	CJ	CC	CC	EE	DE	BE	BF	AC	BB
0	Offspring	Chuwangbae	FG	BE	BD	DF	CC	CD	AC	EE	DF	AE	BB	AA	BD
0	Parent	Nijiseiki	GG	AE	DD	DF	CJ	CC	CC	EE	DE	BE	BF	AC	BB
0	Parent	Niitaka	FG	AB	BE	AD	CG	DE	AD	EE	EF	AB	BG	AB	BD
0	Offspring	Whangkeumbae	GG	AE	DE	AD	GJ	CD	AC	EE	EE	BB	BB	BC	BB
0	Parent	Niitaka	FG	AB	BE	AD	CG	DE	AD	EE	EF	AB	BG	AB	BD
10	Parent	Chuwangbae	FG	BE	BD	DF	CC	CD	AC	EE	DF	AE	BB	AA	BD
11	Offspring	Hanareum	GG	BE	BD	DD	CC	CD	AC	EE	EF	AD	BG	BC	BD
0	Parent	Niitaka	FG	AB	BE	AD	CG	DE	AD	EE	EF	AB	BG	AB	BD
12	Parent	Chuwangbae	FG	BE	BD	DF	CC	CD	AC	EE	DF	AE	BB	AA	BD
22	Offspring	Shincheon	FG	AE	EE	AF	CG	CE	CD	EE	EF	BB	BB	AA	BF
0	Parent	Niitaka	FG	AB	BE	AD	CG	DE	AD	EE	EF	AB	BG	AB	BD
17	Parent	Shinko	FG	BE	DD	DF	CC	CD	AC	EE	DF	AE	BG	AC	BD
20	Offspring	Josaengwhangkum	GG	AE	DE	DD	CG	CE	CD	EE	EE	BB	FG	AA	BB
0	Parent	Niitaka	FG	AB	BE	AD	CG	DE	AD	EE	EF	AB	BG	AB	BD
20	Parent	Okusankichi	EF	BE	DD	DF	CC	DE	AE	AE	FF	AC	BF	CC	DE
28	Offspring	Sunhwang	GG	BC	BE	DF	GG	CE	CD	AE	EE	BB	BB	AA	DF
10	Parent	Chojuro	BC	AD	BD	AA	AD	CC	CD	AB	BC	BE	AA	AB	AC
18	Parent	Kimitsukawase	CC	CD	BD	AD	AC	CD	CD	BB	CC	AB	AA	AC	BE
34	Offspring	Soowhangbae	BC	AB	BC	CD	BB	AC	CD	BB	BD	AE	AA	AC	AC
12	Parent	Niitaka	FG	AB	BE	AD	CG	DE	AD	EE	EF	AB	BG	AB	BD
13	Parent	Soowhangbae	FG	AB	BD	DF	CC	CE	CD	EE	DF	AE	BB	AC	BD
29	Offspring	Sooyoung	EF	BB	AD	CF	BC	DE	AD	EE	AF	AA	BB	AC	DD

²⁾Numbers of the not matched simple sequence repeat (SSR) alleles among the parent and offspring by the MARCO software. Letters A to J was generated by the MARCO software correspond to each SSR fragment.

³⁾Different SSR alleles among the parent and offspring were indicated by shaded box.

Table 2. Continued.

Parent/ Offspring	Cultivar	SSR markers ^{y)}												
		CH01b11	TsuENH046	TsuENH006	CH04e05	NB114a	IPPN13	NH029a	TsuENH008	CH05a03	NH039a	NH017a	EMPe105	CH01f07a
Parent	Imamuraaki	AA	BB	BC	CG	CD	AF	BB	BG	BC	CE	DE	CH	FG
Parent	Nijiiseiki	AC	AB	BC	GH	AC	AE	BB	JJ	BD	BB	EF	AB	BF
Offspring	Chuwangbae	AC	AB	BC	GH	CD	AA	BB	BJ	CD	BE	DF	AC	FF
Parent	Nijiiseiki	AC	AB	BC	GH	AC	AE	BB	JJ	BD	BB	EF	AB	BF
Parent	Nitaka	AA	BB	AC	CE	CD	CG	BC	CJ	BF	BE	DH	EH	EF
Offspring	Whangkeumbae	AA	BB	CC	CH	CD	AC	BC	CJ	BB	BE	DE	BH	EF
Parent	Nitaka	AA	BB	AC	CE	CD	CG	BC	CJ	BF	BE	DH	EH	EF
Parent	Chuwangbae	AC	AB	BC	GH	CD	AA	BB	BJ	CD	BE	DF	AC	FF
Offspring	Hanareum	AA	BB	BC	CG	DD	EG	BB	JJ	BD	BE	DF	EH	EF
Parent	Nitaka	AA	BB	AC	CE	CD	CG	BC	CJ	BF	BE	DH	EH	EF
Parent	Chuwangbae	AC	AB	BC	GH	CD	AA	BB	BJ	CD	BE	DF	AC	FF
Offspring	Shincheon	AA	BB	AB	EG	CD	CG	BC	CJ	FG	BE	DE	EG	FH
Parent	Nitaka	AA	BB	AC	CE	CD	CG	BC	CJ	BF	BE	DH	EH	EF
Parent	Shinko	AC	AB	BC	CG	AC	CE	BC	CJ	DD	BE	DE	BH	EF
Offspring	Josaengwhangkum	AA	AB	AB	CG	CC	EG	BB	JJ	DF	BB	FH	BE	FF
Parent	Nitaka	AA	BB	AC	CE	CD	CG	BC	CJ	BF	BE	DH	EH	EF
Parent	Okusankichi	AA	BB	CC	DG	CE	AG	AC	CJ	GI	BB	CF	AI	BE
Offspring	Sunhwang	AA	BB	AC	CE	DD	CG	BC	CJ	AB	BE	DF	GH	EH
Parent	Chojuro	AA	BB	AA	DH	BC	AE	BB	AE	EF	AA	DE	DE	DF
Parent	Kimitsukawase	AA	BB	AC	BH	BC	EE	AA	DE	EF	AA	CD	BE	BG
Offspring	Soowhangbae	AB	BB	AC	DF	AB	AB	BC	BE	BE	AC	BD	AF	AC
Parent	Nitaka	AA	BB	AC	CE	CD	CG	BC	CJ	BF	BE	DH	EH	EF
Parent	Soowhangbae	AC	BB	AC	EG	AC	AC	BC	CJ	BF	BE	DF	AH	BE
Offspring	Sooyoung	AA	AB	AC	DF	CE	AG	AB	CJ	FG	BB	FH	AE	BF

Table 2. Continued.

Parent/ Offspring	Cultivar	SSR markers ^{y)}													
		CH04h02	TsuENH083	NB105a	CH04d08	NH207a	CH04g04	CH05d11	NB104a	CH04d02	NH009b	NB120a	NH004a	CH04c07	TsuENH032
Parent	Imamuraaki	AB	AA	DH	AA	AB	BG	DE	CE	DI	AC	GK	AJ	BD	AG
Parent	Nijiseiki	CD	BC	BB	AC	BB	BB	DD	EE	DD	EE	AJ	EI	AB	AG
Offspring	Chuwangbae	BD	AC	BD	AA	AB	BG	DE	CE	DI	AE	JK	EJ	BB	GG
Parent	Nijiseiki	CD	BC	BB	AC	BB	BB	DD	EE	DD	EE	AJ	EI	AB	AG
Parent	Nitaka	CD	AC	DF	AD	BD	BC	CD	CD	FI	CE	BK	EI	DE	AH
Offspring	Whangkeumbae	DD	CC	BD	AC	BB	BB	DD	CE	DI	EE	JK	EI	AD	AA
Parent	Nitaka	CD	AC	DF	AD	BD	BC	CD	CD	FI	CE	BK	EI	DE	AH
Parent	Chuwangbae	BD	AC	BD	AA	AB	BG	DE	CE	DI	AE	JK	EJ	BB	GG
Offspring	Hanareum	DD	AC	DF	AD	BD	BC	CD	CD	FI	CE	AB	EI	DG	AG
Parent	Nitaka	CD	AC	DF	AD	BD	BC	CD	CD	FI	CE	BK	EI	DE	AH
Parent	Chuwangbae	BD	AC	BD	AA	AB	BG	DE	CE	DI	AE	JK	EJ	BB	GG
Offspring	Shincheon	CD	AC	BD	AC	CD	BC	CD	CD	FI	AE	AB	AI	DF	AE
Parent	Nitaka	CD	AC	DF	AD	BD	BC	CD	CD	FI	CE	BK	EI	DE	AH
Parent	Shinko	AD	AC	BD	AA	AB	BG	DE	CE	DI	DE	AK	II	BE	GH
Offspring	Josaengwhangkum	CD	CC	BF	AD	BD	BC	DD	DE	DF	EE	BJ	EE	AD	AA
Parent	Nitaka	CD	AC	DF	AD	BD	BC	CD	CD	FI	CE	BK	EI	DE	AH
Parent	Okusankichi	BC	AA	BE	AC	BD	BC	DI	AE	DF	AD	AB	GI	AB	FG
Offspring	Sunhwang	CD	AC	BF	CD	BC	BB	DD	CD	FI	CE	BJ	AE	DD	AB
Parent	Chojuro	DE	AC	BE	BC	AB	AE	BC	BB	FF	DD	AG	BC	AC	AB
Parent	Kimitsukawase	DE	AA	BB	BB	AC	AE	BC	BB	FF	AD	GG	AA	BC	BE
Offspring	Soowhangbae	CD	AC	BC	AC	BD	AB	AB	CD	CD	BD	BF	DD	BC	AE
Parent	Nitaka	CD	AC	DF	AD	BD	BC	CD	CD	FI	CE	BK	EI	DE	AH
Parent	Soowhangbae	CD	AC	BD	AD	BD	BC	CD	DE	DF	CE	BJ	II	BD	AG
Offspring	Sooyoung	BD	AA	AE	AA	CD	CE	DD	AA	BG	AE	BC	GG	AA	AG

Table 2. Continued.

Parent/ Offspring	Cultivar	SSR markers ^{y)}												
		CH05d03	TsuENH093	CH02e12	NH204a	IPP17	NH025a	CH02c09	TsuENH035	NH007b	CH05a04	TsuENH033	NB125a	NH008b
Parent	Inamuraaki	CC	BC	DE	CD	BE	GG	BE	DG	BB	CD	BC	BC	BD
Parent	Nijiseiki	CE	AA	FF	CE	CF	BI	DE	DE	BG	DD	BG	BH	CG
Offspring	Chuwangbae	CC	AC	DF	CC	EF	GI	DE	EG	BG	CD	BC	BC	CD
Parent	Nijiseiki	CE	AA	FF	CE	CF	BI	DE	DE	BG	DD	BG	BH	CG
Parent	Nitaka	CG	AB	EF	CE	BE	EG	AB	DE	BF	AC	BE	BF	BE
Offspring	Whangkeumbae	CE	AB	EF	CE	BF	EI	AD	DE	FG	AD	BB	BB	EG
Parent	Nitaka	CG	AB	EF	CE	BE	EG	AB	DE	BF	AC	BE	BF	BE
Parent	Chuwangbae	CC	AC	DF	CC	EF	GI	DE	EG	BG	CD	BC	BC	CD
Offspring	Hanareum	CC	AA	FF	DE	BD	BE	AA	EE	BB	AD	BB	BB	EE
Parent	Nitaka	CG	AB	EF	CE	BE	EG	AB	DE	BF	AC	BE	BF	BE
Parent	Chuwangbae	CC	AC	DF	CC	EF	GI	DE	EG	BG	CD	BC	BC	CD
Offspring	Shincheon	CD	AB	EF	CD	DE	GH	BE	DE	EF	AC	AB	AB	EG
Parent	Nitaka	CG	AB	EF	CE	BE	EG	AB	DE	BF	AC	BE	BF	BE
Parent	Shinko	CG	AB	EF	CC	EF	GI	EE	EE	BG	CD	BG	BH	BG
Offspring	Josaengwhangkum	CE	AB	EF	CE	BC	BE	AD	DE	BF	CD	BG	BH	EG
Parent	Nitaka	CG	AB	EF	CE	BE	EG	AB	DE	BF	AC	BE	BF	BE
Parent	Okusankichi	BC	AC	CF	CD	DE	GH	DE	DE	FG	CD	AD	AD	BC
Offspring	Sunhwang	CE	AA	FF	DE	BD	EI	BD	DD	EF	AC	BE	BF	EE
Parent	Chojuro	CE	AA	EE	BC	AC	BD	AB	CD	AD	AC	EF	FG	EE
Parent	Kimitsukawase	CE	AA	EE	BB	CC	AA	BC	CC	BD	AC	AB	AB	CF
Offspring	Soowangbae	CC	AA	EE	CC	AB	AB	AD	CC	AD	AC	BB	BG	BF
Parent	Nitaka	CG	AB	EF	CE	BE	EG	AB	DE	BF	AC	BE	BF	BE
Parent	Soowangbae	CC	AA	FF	EE	BC	BE	AE	EE	BG	AD	BB	BH	BG
Offspring	Sooyoung	AC	BC	AC	CC	BE	GH	AE	CE	BF	AC	AD	AD	AC

Table 3. SSR genotyping analysis of candidate parents and 7 Korean bred pear cultivars by the 53 SSR markers and the MARCO software.

Errors ²⁾	Parent/ Offspring	Cultivar	SSR markers ³⁾													
			TsuENH003	CN444636	TsuENH045	CH02b10	NH023a	NB113a	TsuENH074	BGA35	NH011b	CH02h11a	CH02c02b	CH03a09	NB103a	
0	Parent	Tama	GG	EE	DD	DD	CJ	CC	CC	EE	DE	BD	BF	AC	BB	
0	Parent	Niitaka	FG	AB	BE	AD	CG	DE	AD	EE	EF	AB	BG	AB	BD	
0	Offspring	Hanareum	GG	BE	BD	DD	CC	CD	AC	EE	EF	AD	BG	BC	BD	
0	Parent	Chouju	GG	EE	BE	AF	BG	CE	CD	EE	DE	BD	BB	AC	BF	
0	Parent	Niitaka	FG	AB	BE	AD	CG	DE	AD	EE	EF	AB	BG	AB	BD	
0	Offspring	Shincheon	FG	AE	EE	AF	CG	CE	CD	EE	EF	BB	BB	AA	BF	
0	Parent	Nijisseiki	GG	AE	DD	DF	CJ	CC	CC	EE	DE	BE	BF	AC	BB	
0	Parent	Niitaka	FG	AB	BE	AD	CG	DE	AD	EE	EF	AB	BG	AB	BD	
0	Offspring	Soowhangbae	FG	AB	BD	DF	CC	CE	CD	EE	DF	AE	BB	AC	BD	
0	Parent	Nijisseiki	GG	AE	DD	DF	CJ	CC	CC	EE	DE	BE	BF	AC	BB	
0	Parent	Niitaka	FG	AB	BE	AD	CG	DE	AD	EE	EF	AB	BG	AB	BD	
0	Offspring	Josaengwhangkum	GG	AE	DE	DD	CG	CE	CD	EE	EE	BB	FG	AA	BB	
0	Parent	Shinsui	GG	CE	DE	DF	GG	CH	CC	AE	EE	BB	BF	AA	BF	
0	Parent	Niitaka	FG	AB	BE	AD	CG	DE	AD	EE	EF	AB	BG	AB	BD	
0	Offspring	Sunhwang	GG	BC	BE	DF	GG	CE	CD	AE	EE	BB	BB	AA	DF	
1	Parent	Okusankichi	EF	BE	DD	DF	CC	DE	AE	AE	FF	AC	BF	CC	DE	
12	Parent	Niitaka	FG	AB	BE	AD	CG	DE	AD	EE	EF	AB	BG	AB	BD	
20	Offspring	Sooyoung	EF	BB	AD	CF	BC	DE	AD	EE	AF	AA	BB	AC	DD	

Parent/ Offspring	Cultivar	SSR Markers													
		CH01b11	TsuENH046	TsuENH006	CH04c05	NB114a	IPP13	NH029a	TsuENH008	CH05a03	NH039a	NH017a	EMPc105	CH01f07a	
Parent	Tama	AC	AB	AB	EG	DD	AE	BC	JJ	DG	BB	FF	AE	BF	
Parent	Niitaka	AA	BB	AC	CE	CD	CG	BC	CJ	BF	BE	DH	EH	EF	
Offspring	Hanareum	AA	BB	BC	CG	DD	EG	BB	JJ	BD	BE	DF	EH	EF	
Parent	Chouju	AA	BB	BC	GI	CC	GG	AB	HJ	GG	BB	EH	EG	FH	
Parent	Niitaka	AA	BB	AC	CE	CD	CG	BC	CJ	BF	BE	DH	EH	EF	
Offspring	Shincheon	AA	BB	AB	EG	CD	CG	BC	CJ	FG	BE	DE	EG	FH	
Parent	Nijisseiki	AC	AB	BC	GH	AC	AE	BB	JJ	BD	BB	EF	AB	BF	
Parent	Niitaka	AA	BB	AC	CE	CD	CG	BC	CJ	BF	BE	DH	EH	EF	
Offspring	Soowhangbae	AC	BB	AC	EG	AC	AC	BC	CJ	BF	BE	DF	AB	BE	
Parent	Nijisseiki	AC	AB	BC	GH	AC	AE	BB	JJ	BD	BB	EF	AB	BF	
Parent	Niitaka	AA	BB	AC	CE	CD	CG	BC	CJ	BF	BE	DH	EH	EF	
Offspring	Josaengwhangkum	AA	AB	AB	CG	CC	EG	BB	JJ	DF	BB	FH	BE	FF	
Parent	Shinsui	AA	BB	BC	CG	AD	EG	AB	HJ	AG	BB	FF	AG	BH	
Parent	Niitaka	AA	BB	AC	CE	CD	CG	BC	CJ	BF	BE	DH	EH	EF	
Offspring	Sunhwang	AA	BB	AC	CE	DD	CG	BC	CJ	AB	BE	DF	GH	EH	
Parent	Okusankichi	AA	BB	CC	DG	CE	AG	AC	CJ	GI	BB	CF	AI	BE	
Parent	Niitaka	AA	BB	AC	CE	CD	CG	BC	CJ	BF	BE	DH	EH	EF	
Offspring	Sooyoung	AA	AB	AC	DF	CE	AG	AB	CJ	FG	BB	FH	AE	BF	

^{z)}Numbers of the not matched simple sequence repeat (SSR) alleles among the parent and offspring by the MARCO software. Letters A to J was generated by the MARCO software correspond to each SSR fragment.

^{y)}Different SSR alleles among the parent and offspring were indicated by shaded box.

Table 3. Continued.

Parent/ Offspring	Cultivar	SSR markers ^{y)}													
		CH04h02	TsuENH083	NB105a	CH04d08	NH207a	CH04g04	CH05d11	NB104a	CH04d02	NH009b	NB120a	NH004a	CH04c07	TsuENH033
Parent	Tama	DD	AC	BF	CD	BB	BB	DD	CE	DI	EE	AF	EE	BG	AG
Parent	Nitaka	CD	AC	DF	AD	BD	BC	CD	CD	FI	CE	BK	EI	DE	AH
Offspring	Hanareum	DD	AC	DF	AD	BD	BC	CD	CD	FI	CE	AB	EI	DG	AG
Parent	Chouju	DD	AC	BB	AC	BC	BB	DD	CE	DI	AE	AK	AE	BF	EG
Parent	Nitaka	CD	AC	DF	AD	BD	BC	CD	CD	FI	CE	BK	EI	DE	AH
Offspring	Shincheon	CD	AC	BD	AC	CD	BC	CD	CD	FI	AE	AB	AI	DF	AE
Parent	Nijisseiki	CD	BC	BB	AC	BB	BB	DD	EE	DD	EE	AJ	EI	AB	AG
Parent	Nitaka	CD	AC	DF	AD	BD	BC	CD	CD	FI	CE	BK	EI	DE	AH
Offspring	Soowhangbae	CD	AC	BD	AD	BD	BC	CD	DE	DF	CE	BJ	II	BD	AG
Parent	Nijisseiki	CD	BC	BB	AC	BB	BB	DD	EE	DD	EE	AJ	EI	AB	AG
Parent	Nitaka	CD	AC	DF	AD	BD	BC	CD	CD	FI	CE	BK	EI	DE	AH
Offspring	Josaengwhangkum	CD	CC	BF	AD	BD	BC	DD	DE	DF	EE	BJ	EE	AD	AA
Parent	Shinsui	DE	AC	BB	CC	BC	BB	DD	CE	DI	AE	JK	AA	BD	BG
Parent	Nitaka	CD	AC	DF	AD	BD	BC	CD	CD	FI	CE	BK	EI	DE	AH
Offspring	Sunhwang	CD	AC	BF	CD	BC	BB	DD	CD	FI	CE	BJ	AE	DD	AB
Parent	Okusankichi	BC	AA	BE	AC	BD	BC	DI	AE	DF	AD	AB	GI	AB	FG
Parent	Nitaka	CD	AC	DF	AD	BD	BC	CD	CD	FI	CE	BK	EI	DE	AH
Offspring	Sooyoung	BD	AA	AE	AA	CD	CE	DD	AA	BG	AE	BC	GG	AA	AG

Parent/ Offspring	Cultivar	SSR Markers													
		CH05d03	TsuENH093	CH02e12	NH204a	IPPNI7	NH025a	CH02c09	TsuENH035	NH007b	CH05a04	TsuENH033	NB125a	NH008b	
Parent	Tama	CH	AA	FF	DE	CD	BB	AA	EE	BG	DD	BB	BB	EG	
Parent	Nitaka	CG	AB	EF	CE	BE	EG	AB	DE	BF	AC	BE	BF	BE	
Offspring	Hanareum	CC	AA	FF	DE	BD	BE	AA	EE	BB	AD	BB	BB	EE	
Parent	Chouju	CD	AA	FF	DD	DD	BH	BE	EE	BE	AA	AE	AF	EG	
Parent	Nitaka	CG	AB	EF	CE	BE	EG	AB	DE	BF	AC	BE	BF	BE	
Offspring	Shincheon	CD	AB	EF	CD	DE	GH	BE	DE	EF	AC	AB	AB	EG	
Parent	Nijisseiki	CE	AA	FF	CE	CF	BI	DE	DE	BG	DD	BG	BH	CG	
Parent	Nitaka	CG	AB	EF	CE	BE	EG	AB	DE	BF	AC	BE	BF	BE	
Offspring	Soowhangbae	CC	AA	FF	EE	BC	BE	AE	EE	BG	AD	BB	BH	BG	
Parent	Nijisseiki	CE	AA	FF	CE	CF	BI	DE	DE	BG	DD	BG	BH	CG	
Parent	Nitaka	CG	AB	EF	CE	BE	EG	AB	DE	BF	AC	BE	BF	BE	
Offspring	Josaengwhangkum	CE	AB	EF	CE	BC	BE	AD	DE	BF	CD	BG	BH	EG	
Parent	Shinsui	CE	AA	FF	CD	DF	BI	BD	DE	BE	AD	BB	BB	CE	
Parent	Nitaka	CG	AB	EF	CE	BE	EG	AB	DE	BF	AC	BE	BF	BE	
Offspring	Sunhwang	CE	AA	FF	DE	BD	EI	BD	DD	EF	CD	BE	BF	EE	
Parent	Okusankichi	BC	AC	CF	CD	DE	GH	DE	DE	FG	AD	AD	AD	BC	
Parent	Nitaka	CG	AB	EF	CE	BE	EG	AB	DE	BF	AC	BE	BF	BE	
Offspring	Sooyoung	AC	BC	AC	CC	BE	GH	AE	CE	BF	AC	AD	AD	AC

Fig. 1. *S*-RNase polymerase chain reaction analysis of 6 Japanese pear cultivars of known *S*-genotypes and 5 Korean bred pear cultivars by using the FTQQYQ-Fam and PspRI primers. Lane 1: Ichiharawase (S^1S^8), lane 2: Nijisseiki (S^2S^4), lane 3: Niitaka (S^1S^9), lane 4: Okusankichi (S^5S^7), lane 5: Imamuraaki (S^1S^6), lane 6: Kinchaku (S^4S^k), lane 7: Sujeongbae, lane 8: Hannareum, lane 9: Shincheon, lane 10: Sooyoung, lane 11: Josaengwhangkum, and M: 100 bp DNA Ladder.

Fig. 2. *S*-genotypes analysis of five Korean bred pear cultivars by polymerase chain reaction (PCR)-restriction fragment length polymorphism analysis. The *S*-RNase PCR fragments were digested with *S*-allele-specific restriction enzymes; a: *Ppu*MI (S^3 and S^5), b: *Alw*NI (S^5), c: *Bst*BI (S^9), d: *Nde*I (S^4), e: *Sfc*I (S^1). The digested fragments are indicated by arrows.

digested *S*-RNase fragments with *Ppu*MI and *Alw*NI of ‘Sooyoung’ were identical and that was only S^5 -RNase (Fig. 2). Thus, the only one *S*-RNase PCR product of ‘Sooyoung’ was identified; the other *S*-RNase PCR product was not amplified. Therefore the *S*-genotype of ‘Sooyoung’ was $S^5(S^5S^?)$. The predicted *S*-genotype of ‘Sooyoung’ matched neither with registrant parent

‘Niitaka’ (S^3S^9) nor with ‘Soowhangbae’ (S^3S^4).

DISCUSSION

In this study, we confirmed the parents of nine Korean pear cultivars by using the 53 SSR markers and *S*-RNase

based PCR-RFLP analysis. The SSR and *S*-genotypes of ‘Chuwangbae’, ‘Whangkeumbae’ and ‘Sujeongbae’ matched with registrant parents; ‘Imamuraaki’×‘Nijisseiki’, ‘Nijisseiki’×‘Niitaka’ and mutant of ‘Niitaka’, respectively (Table 4). However, the SSR genotypes of ‘Hannareum’ (S^3S^4) and ‘Josaengwhangkum’ (S^3S^4) did not matched with the SSR genotypes of registrant parents, but those matched with SSR genotypes of the new candidate parents (‘Niitaka’(S^3S^9)×‘Tama’(S^4S^5) for ‘Hannareum’ and ‘Niitaka’ (S^3S^9)×‘Nijisseiki’ (S^2S^4) for ‘Josaengwhangkum’. Thus our results confirmed that at least one parent of those two pear cultivars was wrongly reported earlier (Table 4).

The *S*-genotypes of ‘Soowhangbae’ and ‘Sunhwang’ were determined as S^3S^4 and S^3S^5 respectively by Kim *et al.* (2002). However, the authors were unable to confirm the parentage of these two Korean pear cultivars at that time. In this study, the SSR genotypes of two pear cultivars and their registrant parents were found different. Our results suggested that ‘Nijisseiki’×‘Niitaka’ and ‘Niitaka’×‘Shinsui’ were the candidate parents of ‘Soowhangbae’ and ‘Sunhwang’ respectively (Table 4). The S^3S^4 genotype of ‘Soowhangbae’ could not be obtained from registrant parent ‘Chojuro’ (S^2S^3)×‘Kimitsukawase’ (S^1S^5), but that could be obtained from the new candidate parents ‘Nijisseiki’ (S^2S^4)×‘Niitaka’ (S^3S^9) (Table 4). ‘Shincheon’ and ‘Sooyoung’ were also different from their registrant parents by their SSR genotyping and *S*-genotyping. ‘Shincheon’ (S^1S^3) might be originated from the following candidate parent combination: ‘Niitaka’ (S^3S^9)×‘Chouju’

(S^1S^5) but the candidate parent of ‘Sooyoung’ was not possible to determine by comparing SSR genotypes of 96 Japanese pear cultivars. Therefore, it could be suggested that ‘Sooyoung’ was possibly a bred of a Korean local cultivars and was not probably a bred of any Japanese pear cultivars (Table 4).

The three Korean pear cultivars namely, ‘Whangkeumbae’, ‘Soowhangbae’ and ‘Josaengwhangkum’ were bred by crossing between ‘Niitaka’×‘Nijisseiki’ as these three cultivars had identical (S^3S^4) *S*-genotypes even though fruit characters of those three were different (<http://www.nihhs.go.kr/search/search.asp>). Seven out of nine Korean bred pear cultivars namely ‘Whangkeumbae’, ‘Hanareum’, ‘Shincheon’, ‘Soowhangbae’, ‘Josaengwhangkum’ and ‘Sunhwang’ were bred using ‘Niitaka’ as a parent probably because ‘Niitaka’ had very high yield potential, producing large fruit with an attractive appearance and good storability (Saito 2016).

In conclusion, we have used 53 reliable SSR markers this study, which were found substantial to determine pear parentage, and the use of *S-RNase* genes associated PCR-RFLP systems led us towards a reliable determination of *S*-genotypes. This methods and approaches have been used in this study could be successfully utilized in identifying cultivars with highly diverse *S*-genotypes and also in identifying obscure relationships between parents and progenies of pear and apple.

Table 4. *S*-genotypes relationship among the 9 Korean pear cultivars and their parents.

Cultivars	Registrant parents	SSR genotyping ²⁾	Candidate parents	<i>S</i> -genotype
Sujeongbae	Mutant of Niitaka (S^3S^9)	O		S^3S^9
Chuwangbae ³⁾	Imamuraki (S^1S^6)×Nijisseiki (S^2S^4)	O		S^4S^6
Whangkeumbae ³⁾	Niitaka (S^3S^9)×Nijisseiki (S^2S^4)	O		S^3S^4
Hanareum	Niitaka (S^3S^9)×Chuwangbae (S^4S^6)	X	Niitaka (S^3S^9)×Tama (S^4S^5)	S^3S^4
Shincheon	Niitaka (S^3S^9)×Chuwangbae (S^4S^6)	X	Niitaka (S^3S^9)×Chouju (S^1S^5)	S^1S^3
Soowhangbae ³⁾	Chojuro (S^2S^3)×Kimitsukawase (S^1S^5)	X	Niitaka (S^3S^9)×Nijisseiki (S^2S^4)	S^3S^4
Josaengwhangkum	Niitaka (S^3S^9)×Shinko (S^4S^9)	X	Niitaka (S^3S^9)×Nijisseiki (S^2S^4)	S^3S^4
Sunhwang ³⁾	Niitaka (S^3S^9)×Okusankichi (S^5S^7)	X	Niitaka (S^3S^9)×Shinsui (S^4S^5)	S^3S^5
Sooyoung	Niitaka (S^3S^9)×Soowhangbae (S^3S^4)	X	?×Okusankichi (S^5S^7)	S^5S^7

²⁾O is matched the simple sequence repeat (SSR) genotypes among the registrant parents and cultivar, X is not matched the SSR genotypes among the registrant parents and cultivar.

³⁾*S*-genotypes were previously determined by Kim *et al.* (2002).

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