J. Japan. Soc. Hort. Sci. 77 (4): 388–394. 2008.
Available online at www.jstage.jst.go.jp/browse/jjshs1

Cultivar Differentiation Identified by SSR markers and the Application for Polyploid Loquat Plants

Manabu Watanabe[1]*, Toshiya Yamamoto[2], Mari Ohara[1], Chikako Nishitani[2] and Shigeki Yahata[3]

[1]*Chiba Prefectural Agriculture and Forestry Research Center, 808 Daizenno, Midori-ku, Chiba 266-0006, Japan*
[2]*National Institute of Fruit Tree Science, National Agriculture and Food Research Organization, 2-1 Fujimoto, Tsukuba 305-8605, Japan*
[3]*Southern Prefectural Horticulture Institute, Chiba Prefectural Agriculture and Forestry Research Center, 1762 Yamamoto, Tateyama 294-0014, Japan*

Here we established a DNA marker-based method to identify cultivars of loquat (*Eriobotrya japonica* Lindl.) using simple sequence repeat (SSR) markers derived from pears and apples. A total of 24 loquat cultivars commercially grown in Japan were used for genetic identification, including 15 diploid-, 6 triploid-, and 3 tetraploid cultivars. Analysis using the 88 SSR markers derived from pears and apples, which belong to the same subfamily (family Rosaceae, subfamily Maloideae) as the loquat, indicated that 26 SSR markers were applicable to the identification of loquat cultivars. A total of 82 putative SSR alleles were obtained by the 26 SSR markers. SSR analysis enabled the identification of all loquat varieties tested, except for 'Tomihusa' and '4N-Tomihusa'. In addition, the parentages of several cultivars, including 'Oohusa' and 'Mizuho', were confirmed from the SSR genotype data obtained. Triploid and tetraploid varieties could be identified from the SSR genotypes because some SSR loci generated 2 or more alleles for polyploids. The relationship between 'Kibou' and its parents was confirmed since SSR alleles of the seedless triploid offspring 'Kibou' were clearly inherited from the female parent '4N-Tanaka 1' and the male parent 'Nagasakiwase'. The phenogram obtained by cluster analysis showed no distinctive separation of Japanese commercial cultivars from Chinese cultivars. This result was consistent with the hypothesis that Japanese commercial cultivars were derived from introduced Chinese loquat.

Key Words: DNA marker, *Eriobotrya japonica*, simple sequence repeat, triploid, tetraploid.

Introduction

The loquat (*Eriobotrya japonica* Lindl.) is an evergreen fruit tree, belonging to the family Rosaceae, subfamily Maloideae, and is widely cultivated in Asia, including China and Japan, and in Europe, especially in coastal areas of the Mediterranean. The genus *Eriobotrya* has about 20 species with wide ranges in the temperate and subtropical regions of China and southeastern Asia, centered in the upper Yangtze River area in Szechwan (Sichuan) Province. Among the *Eriobotrya* species, only one, *E. japonica*, is edible. In China, where *E. japonica* originated, many cultivars were developed in the 7th century and the loquat was established as one of the major fruit tree species (Ichinose, 2002; Yu, 1979). A temperate climate, with mild temperatures and no extreme temperature changes, is considered to be suitable for the cultivation of loquats. International data show that loquats are primarily grown in Asia (China 42,000 ha, Japan 2,420 ha, Pakistan 11,000 ha) followed by the Mediterranean coastal regions (Spain 2,914 ha, Turkey 1,470 ha, Italy 663 ha, Israel 330 ha) and South America (Brazil 300 ha) (Caballero et al., 2002).

In Japan, 'Mogi', 'Tanaka', and 'Kusunoki', which are the current major commercial cultivars, were developed from seedlings of Chinese varieties in the early 19th century. This led to full-scale cultivation of the loquat in Japan. Many of the loquat cultivars that are commercially cultivated in Japan were developed by hybridization using 'Mogi' or 'Tanaka'. Early-maturing cultivars 'Mogi' and 'Nagasakiwase' are cultivated in Kyushu (e.g., Nagasaki Prefecture and Kagoshima Prefecture) and the middle- to late-maturing cultivars

Received; January 10, 2008. Accepted; April 15, 2008.
* Corresponding author (E-mail: m.wtnb38@ma.pref.chiba.lg.jp).

'Oohusa' and 'Tanaka' are cultured in Honshu and Shikoku (e. g., Chiba Prefecture, Kagawa Prefecture, and Ehime Prefecture).

In recent years, superior loquat cultivars with big fruit, high fruit quality, and high production have been developed; however, as the loquat can be clonally propagated by grafting, the unlawful use or violation of rights concerning these new cultivars is feared. As the confirmation of illegal activities by cultivation testing is time-consuming in fruit trees, including the loquat, the identification of cultivars is generally carried out by analyzing phenotypes. However, phenotypes can be affected by environmental factors and the exact identification of cultivars is difficult even for experienced experts involved in the production and distribution of loquats; therefore, a scientific method for identifying cultivars has been strongly hoped for. In addition, in the case of fresh fruit, a method of rapid identification is necessary to prevent the loss of profits that occurs during standard identification procedures. Techniques using DNA markers are promising, as they can be rapid, reliable, and objective.

The identification of loquat cultivars using DNA markers such as random amplified polymorphic DNA (RAPD) has been reported. Vilanova et al. (2001) analyzed 33 cultivars, including those cultivated in Spain by the RAPD method, and observed 29 polymorphic bands. They reported that 22 cultivars could be identified among 33 cultivars. Fukuda et al. (2002) showed that 69 varieties, including cultivars introduced from Mexico and China, could be identified by 108 polymorphic bands observed in RAPD analysis. Badenes et al. (2003) showed rather narrow genetic diversity in the loquat varieties cultivated in Europe by using RAPD and simple sequence repeat (SSR) markers. They also reported the usefulness of SSR markers. Soriano et al. (2005), in a study to identify 40 cultivars using SSR markers, reported that the cultivars tested could be classified into 34 genotypes by 39 alleles. In spite of these findings, the identification of cultivars by DNA markers remains in the preliminary stage, and further developments using reliable DNA markers are necessary.

Many major fruit tree species, such as the Japanese pear (*Pyrus pyrifolia* Nakai) and the apple (*Malus domestica* Borkh.), as well as the loquat, belong to family Rosaceae, subfamily Maloideae. The applicability of SSR markers developed for the apple and the Japanese pear has been reported (Liebhard et al., 2002; Yamamoto et al., 2001, 2002c, 2004). Yamamoto et al. (2001, 2002c) also indicated that apple-derived SSR markers could be used for linkage map construction and genetic analysis. Liebhard et al. (2002) further reported that apple-derived SSR markers could be applied widely to plants belonging to subfamily Maloideae, such as the loquat. In addition, SSR markers derived from the apple and the Japanese pear could be used effectively to identify quince cultivars and determine parentage relationships (Yamamoto et al., 2004).

To develop a reliable DNA technique for the identification of loquat cultivars, the applicability of SSR markers developed in apples and the Japanese pear was tested. Identification of polymorphisms with respect to SSR markers, confirmation of the parent-offspring relationship, analyses of triploid and tetraploid strains, and genotyping of the triploid seedless cultivar 'Kibou' are reported.

Materials and Methods

Plant materials

The plants used in this study were: 15 major diploid cultivars commercially grown in Japan, which had been maintained in the Southern Prefectural Horticulture Institute, Chiba Prefectural Agriculture and Forestry Research Center; 6 triploid varieties, including 'Kibou' and its siblings; and 3 tetraploid varieties, including a parent of 'Kibou' (Table 1). '4N-Tanaka 1' and '3N-Tanaka seedling' were kindly supplied by Mr. Muranishi (South Kyushu University) as scions. The other polyploids were bred and obtained from the Southern Prefectural Horticulture Institute, Chiba Prefectural Agriculture and Forestry Research Center. The tetraploid strains either spontaneously formed or were induced by colchicines treatment of the apical buds of trees or seedlings (Yahata et al., 2004). The triploid varieties were bred by hybridizing tetraploid seed parents with diploid cultivars. The ploidy of these varieties was confirmed by flow cytometry (Ploidy Analyzer FAS Type, Ikeda Scientific Co., Tokyo, Japan).

Extraction of DNA

DNA was extracted from the leaves immediately after their unfolding. Samples for DNA extraction were 0.05 g of leaves taken from each variety. Genomic DNA was extracted using the Nucleon Phytopure DNA extraction kit (GE Healthcare UK Ltd., Buckinghamshire, England). To avoid high viscosity and coloring, which were probably due to contaminants, Reagent 1 in the kit was modified by adding mercaptoethanol (1% (v/v)), pectinase (0.5% (w/v)), and α-amylase (135 U/mL) just before application. Concentrations of DNA were determined, after 0.8% (w/v) agarose gel electrophoresis and ethidium bromide staining, by comparison with λ DNA of known concentrations under UV irradiation.

SSR analysis

A total of 88 SSR markers were used: 43 markers derived from Japanese pears (Sawamura et al., 2004; Yamamoto et al., 2002a, b, c; Table 3) and 45 markers derived from apples (Liebhard et al., 2002) (Table 2). PCR was carried out in a reaction volume of 20 μL, with 5 ng of DNA extracted from the loquat varieties being used as a template, 5'-fluorescence-labeled (Fam/Vic/Ned) primer pair (250 nM, 0.2 mM dNTP (Takara Co., Ohtsu, Japan), 1.5 mM $MgCl_2$ (Takara Co.), and 1 U of Taq polymerase (Takara Co.). The reaction mixture was

Table 1. Plant materials used in this study.

Variety name	Origin	Ploidy
Tanaka	Chance seedlings of Chinese loquat	diploid
Oohusa	Tanaka × Kusunoki	diploid
Mizuho	Tanaka × Kusunoki	diploid
Kusunoki	Chance seedlings of Chinese loquat	diploid
Nagasakiwase	Mogi × Hondawase	diploid
Toi	Chance seedlings of Chinese loquat	diploid
Satomi	Seedlings of Kusunoki	diploid
Husahikari	Mizuho × Tanaka	diploid
Tomihusa	Tsukumo × Mizuho	diploid
Husahime	Kusunoki × Tsukumo	diploid
Mogi	Chance seedlings of Chinese loquat	diploid
Shiromogi	Gamma-ray irradiation of seedlings of Mogi	diploid
Hakugyoku	Introduced from China	diploid
Kahou 2 gou	Introduced from China	diploid
Daishouhou	Introduced from China	diploid
Kibou	4N-Tanaka 1 × Nagasakiwase	triploid
3N-Nagasaki 17	4N-Tanaka 1 × Nagasakiwase	triploid
3N-Nagasaki 30	4N-Tanaka 1 × Nagasakiwase	triploid
3N-Tanaka seedling	4N-Tanaka × Tanaka	triploid
3N-Kusunoki 4	4N-Tanaka 1 × Kusunoki	triploid
3N-Dohi 22	4N-Tanaka 1 × Toi	triploid
4N-Tanaka 1	Colchicine treatment of Tanaka seedlings	tetraploid
4N-So 75	Chance seedlings of Husahikari × Daishouhou	tetraploid
4N-Tomihusa	Colchicine treatment of shoot buds of Tomihusa	tetraploid

put through 35 cycles of 1 min at 94°C, 1 min at 52–55°C and 2 min at 72°C in the GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, USA). The amplification products obtained were separated and detected by a DNA sequencer, ABI PRISM 377 (Applied Biosystems). The fragment lengths of the amplification products were analyzed by the fragment analysis program GeneScan (Applied Biosystems) using fluorescence-labeled DNA molecular-weight markers (400HD-ROX, Applied Biosystems) as internal standards.

Analysis of genotype data

The presence (1) and absence (0) of the amplification products were scored for all alleles obtained from the 28 SSR loci in each cultivar or strain. A similarity matrix was prepared from the scored data. Nei's degrees of genetic identity (Nei, 1972) between two cultivars were calculated. A phenogram was constructed from cluster analysis by UPGMA (unweighted pair-group method using arithmetic averages) using the NTSYS-pc program (Rohlf, 2005).

Results and Discussion

SSR amplification in loquat

In the experimental application of 43 SSR markers derived from Japanese pears, 20 markers produced stable amplified bands. Among them, 12 markers showed polymorphism among varieties. Of 45 SSR markers derived from apples, amplified fragments were observed in 25 markers, among which 14 markers showed polymorphism (Table 2). Of the 88 SSR markers derived from pears and apples, 45 SSRs, about half, were applicable to the loquat, of which 26, about 30% of the total, showed polymorphism. The ratios of SSR markers applicable to the loquat were not much different between pear-derived and apple-derived markers.

When compared to the observations that all 12 SSR markers derived from apples were applicable to pears (Yamamoto et al., 2001) and that 77 of 118 SSR markers (65% of the total) derived from apples and pears could be used for quince analysis (Yamamoto et al., 2004), the percentage of SSRs applicable to loquat was somewhat lower. Soriano et al. (2005) reported that 13 of 30 SSR markers originated from apple could be applicable to the loquat. In this study, 14 additional SSR markers derived from apple and 12 SSRs from pear were found to be applicable to the loquat.

Genetic identification of diploid loquat by SSR markers

In genotype analysis of diploid loquat cultivars using 26 SSR markers, 1 or 2 stable amplification bands were obtained in diploid materials. This suggested that each of the SSR markers used amplified putative alleles derived from a single locus in the genome, as in the case of SSR analysis in the Japanese pear (Kimura et al., 2004). Two to 6 alleles were detected for each SSR marker, totaling 82 alleles. An average of 3.2 alleles was

detected for each SSR marker. Diploid loquat cultivars could be identified by differences in the responses to plural SSR markers (Table 3).

The parent-offspring relationship was examined in cultivars where the parentages were known. The parent-offspring relationship of 'Oohusa' (offspring) and 'Tanaka' and 'Kusunoki' (parents) was confirmed, with no discrepancy in the alleles found. The parent-offspring relationship between 'Mizuho' and its parents 'Tanaka' and 'Kusunoki' was also confirmed by SSR markers. On the other hand, the parent-offspring relationship between 'Husahikari' (putatively 'Mizuno' × 'Tanaka'), 'Mizuno' and 'Tanaka' was not supported, as the genetic inheritance of alleles was contradictory. In our attempt to determine the parentage of 'Husahikari', the allele results of 'Kusunoki' × 'Tanaka' showed no discrepancies; therefore, the possibility that the parents of 'Husahikari' are 'Kusunoki' and 'Tanaka' is high. For each of 'Nagasakiwase', 'Tomihusa', 'Husahime', and 'Kusunoki', one of their parents was included in the varieties tested. As the genetic inheritance of alleles had no inconsistencies, the parent-offspring relationships were confirmed. It was shown that SSR markers could be widely applied across the genus and that they were useful in diagnosing loquat parentage, as has been reported for the Japanese pear (Kimura et al., 2003). Soriano et al. (2005) presented the grouping and relationships among loquat cultivars by SSR markers; however, the parent-offspring relationships were not analyzed. In this study, we showed that SSR markers could be successfully utilized for confirmation of the parent-offspring relationship and origin.

Analysis of triploid and tetraploid loquats

As in the case of diploid cultivars, by analyzing triploid and tetraploid varieties using 26 SSR markers, SSR genotypes were characterized and all varieties could be identified (Table 3). In the analysis, the parentage relationship between 'Kibou' and its parents '4N-Tanaka 1' (♀) and 'Nagasakiwase' (♂) was confirmed (Yahata et al., 2005). '4N-Tanaka 1' is a colchicine-induced tetraploid variety that showed 1 or 2 alleles in all SSR analyses. 'Kibou' is a triploid cultivar that showed 3 alleles in the analysis with SSR markers NH007b and NH014a. Thus, the triploidy of 'Kibou' was also confirmed by molecular markers. The analysis of genotypes showed that SSR alleles of '4N-Tanaka 1' and 'Nagasakiwase' were transferred to 'Kibou' (Table 4). From these data, 'Nagasakiwase' was confirmed to be the pollen parent of 'Kibou', as has been assumed. That parentage of '4N-So75' is described as 'Husahikari' (♀) and 'Daishouhou' (♂). In this study, a parent-offspring relationship between '4N-So75' and 'Daishouhou' was rejected because of the discrepancy at the 2 SSRs NH014a and CH05g08, whereas 'Husahikari' shared the same alleles as '4N-So75' in all tested SSRs.

Table 2. SSR markers used for cross-genus amplification in the loquat.

SSR name[z]	Origin	References[y]
NH032a, **NH035a**, NH037a, NH201a, NH203a, **NH204a**, NH206a, **NH207a**, NH208a, **NH209a**, NH210a, NH212a, **NB104a**	pear	d
NB102a, NB103a, **NB105a**, NB106a, NB109a, **NB110a**, NB111a, NB113a, NH019b, **NH020a**, NH021a	pear	c
NH004a, NH005a, **NH007b**, **NH009b**, **NH011b**, **NH014a**, **NH015a**, NH017a	pear	b
BGT23b, BGA35, **KA14**	pear	a
NH033b, NH202a, NH205a, NH211a, **NB101a**, **NB114a**, NB135a, **NB141b**	pear	Table 3
CH02g04, CH02g09, **CH02h11a**, CH03a04, **CH03a09**, **CH03d01**, **CH03d07**, CH03d11, **CH03d12**, CH03e03, **CH03g06**, **CH03g07**, **CH03g12**, **CH03h03**, CH04a12, **CH04d02**, CH04e02, **CH04e03**, **CH04e05**, **CH04g04**, CH04h02, **CH05a02**, **CH05a04**, CH05a05, **CH05c06**, **CH05c07**, CH05d02, CH05d08, CH05e06, CH05f06, **CH05g03**, **CH05g08**, MS01a05, **MS06c09**, **MS14b04**, **CH02a03**, **CH02d10a**, CH03a08, CH03d02, **CH04f10**, CH04g07, CH05c04, **CH05d04**, CH05e04, CH05f04	apple	e

[z] SSR markers showing stable amplification are in bold and polymorphic SSR markers are underlined.

[y] References for SSR are as follows: a, Yamamoto et al., 2002a; b, Yamamoto et al., 2002b; c, Yamamoto et al., 2002c; d, Sawamura et al., 2004; e, Liedhard et al., 2002.

Table 3. Characteristics of SSR markers developed in pear.

SSR name	Forward primer sequences (5'-3')	Reverse primer sequences (5'-3')[z]	Origin	Motif	Accession No.
NH033b	GTCTGAAACAAAAAGCATCGCAA	CTGCCTCGTCTTCCTCCTTATCTCC	Housui	$(AG)_{22.5}$	AB302413
NH202a	ATCGACCAATTCAAAGTCTGAT	gtttcttTAGTCTTCCACAACAAACCCTA	Housui	$(AC)_7$	AB302415
NH205a	ATGTCTCGATATGCGTGCTAGG	gtttcttCTTCAAAGCATTCACGGTAATCA	Housui	$(TA)_6(CA)_8$	AB302416
NH211a	GGGAAATTCACACAACTCTTAGGG	gtttcttTTCGAATATGCAAAACAACAAGTG	Housui	$(AC)_6$	AB302417
NB101a	GAAAGAGAAGGATAGCTGGTTA	TTTGCTGCTTGCTTCTGCTT	Bartlett	$(AG)_{27.5}$	AB302411
NB114a	AAGAAATAAAACCCACAAAGCC	gtttcttTGTCTTCTCTCTCCGCTTATTC	Bartlett	$(GA)_{15}$	AB302423
NB135a	TGAGAGAAGAACAGCCAATGAT	gtttcttCTCCCACTCAGATCGCTCCT	Bartlett	$(GA)_{22.5}$	AB302441
NB141b	GGATTGATCGCCTTATGGTTGT	gtttcttCAGAGAAAGACAGAGGTAGAGAGAA	Bartlett	$(AT)_8(AG)_{15.5}$	AB302443

[z] gtttctt: pig-tail

Table 4. Genotypes for 24 loquat varieties identified by 26 SSR markers.

Variety name	CH03a09	CH03d07	CH03d12	CH03g06	CH04h03	CH04e03	CH05a04	NH033b	NH035a	NH204a	NH207a	KA14	NH007b
Tanaka	140/150	306/308	115/140	176/178	102/102	178/182	185/185	196/198	144/144	110/110	156/156	175/175	129/147
Oohusa	140/140	308/308	95/140	176/178	102/102	178/182	185/185	184/198	144/144	110/110	156/156	175/175	141/147
Mizuho	140/140	306/306	95/115	176/178	102/102	178/182	185/185	184/198	144/144	110/110	156/156	175/175	129/141
Kusunoki	140/148	306/308	95/95	178/178	102/102	182/182	185/185	184/198	144/144	110/110	156/156	173/175	141/147
Nagasakiwase	134/140	308/314	115/138	176/180	94/94	178/182	185/187	184/196	144/144	110/114	156/156	175/175	129/141
Toi	134/152	308/314	95/115	180/180	94/94	182/182	185/185	184/196	144/144	110/110	156/158	173/173	147/147
Satomi	140/148	306/306	95/115	178/178	102/102	182/182	185/185	184/198	144/144	110/110	156/156	173/175	147/147
Husahikari	140/140	306/306	95/115	176/178	102/102	182/182	185/185	198/198	144/144	110/110	156/156	173/175	147/147
Tomihusa	140/140	306/308	115/115	176/180	102/102	178/182	185/185	184/196	144/144	110/110	156/156	175/175	129/129
Husahime	140/148	306/308	95/115	178/178	102/102	178/182	185/185	184/198	144/144	110/110	156/156	175/175	141/147
Mogi	140/140	308/310	115/140	176/180	94/102	182/184	185/185	196/196	144/144	110/114	156/156	175/175	129/129
Shiromogi	140/140	308/310	140/140	180/180	94/102	182/184	185/185	196/196	144/144	110/110	156/156	175/175	129/129
Hakugyoku	136/140	308/316	115/140	176/180	94/102	182/182	185/185	184/184	152/152	110/114	156/158	175/175	129/141
Kahou 2 gou	148/150	306/308	95/121	176/180	94/102	182/182	185/185	184/196	144/144	110/114	156/158	175/175	129/129
Daishouhou	140/148	306/306	95/140	176/178	102/102	178/182	185/185	184/198	144/144	110/110	156/156	173/175	147/147
Kibou	140/150	306/308	115/140	178/180	94/102	178/182	185/187	184/196	144/144	110/110	156/156	175/175	129/141/147
3N-Nagasaki 17	140/150	306/308	138/140	178/180	94/102	178/182	185/185	196/196	144/144	110/114	156/156	175/175	129/147
3N-Nagasaki 30	134/150	306/314	115/140	178/180	94/102	178/178	185/185	184/196	144/144	110/114	156/156	175/175	129/147
3N-Tanaka seedling	150/150	306/308	115/140	176/178	102/102	178/182	185/185	196/198	144/144	110/110	156/156	175/175	129/129
3N-Kusunoki 4	148/150	306/308	95/115/140	178/178	102/102	182/182	185/185	196/198	144/144	110/110	156/156	175/175	129/141/147
3N-Dohi 22	140/150/152	306/308	95/140	178/180	94/102	178/182	185/185	196/196	144/144	110/110	156/156	173/175	129/147
4N-Tanaka 1	140/150	306/308	115/140	178/178	102/102	178/182	185/185	196/196	144/144	110/110	156/156	175/175	129/147
4N-So 75	134/140	306/314	95/140	170/176	94/102	182/182	179/185	198/198	144/152	110/114	156/156	173/175	147/147
4N-Tomihusa	140/140	306/308	115/115	176/180	102/102	178/182	185/185	184/196	144/144	110/110	156/156	175/175	129/129

Variety name	NH011b	NH014a	NB114a	NB141b	CH05c06	CH05g03	CH05g08	MS06c09	MS14b04	CH02d10a	CH05d04	NH015a	NB105a
Tanaka	174/184	164/182	133/137	143/151	107/107	161/180	160/199	141/141	267/267	220/220	192/192	113/113	165/165
Oohusa	174/184	135/182	133/137	143/151	107/107	143/161	199/199	141/141	267/267	212/220	192/192	113/119	165/165
Mizuho	174/184	135/182	133/137	143/151	107/107	143/180	160/199	141/141	267/267	212/220	192/192	113/119	155/165
Kusunoki	174/174	135/164	133/133	143/143	107/109	143/143	164/199	141/141	267/267	212/220	192/194	119/119	155/165
Nagasakiwase	174/174	135/188	133/133	143/161	107/107	143/151	164/199	119/141	267/267	212/212	192/192	113/113	155/165
Toi	174/174	135/164	137/137	143/161	109/109	161/180	160/199	149/149	273/273	216/216	192/194	113/119	155/165
Satomi	174/184	135/164	133/133	143/151	107/109	143/180	164/199	141/141	267/267	212/220	192/194	113/119	165/165
Husahikari	174/174	135/164	133/133	143/143	107/109	143/180	160/199	141/141	267/267	220/220	192/194	113/119	165/165
Tomihusa	174/184	182/182	133/133	151/151	107/107	151/180	164/199	141/141	267/267	216/220	192/192	113/113	165/165
Husahime	174/184	135/182	133/133	143/151	107/107	143/151	160/164	141/141	267/267	212/220	192/192	113/119	155/165
Mogi	174/186	135/188	133/133	143/161	107/107	143/151	164/164	141/141	267/267	212/216	192/192	113/113	155/165
Shiromogi	186/186	135/188	133/133	143/143	107/107	143/151	164/164	145/145	267/267	216/216	192/192	113/113	155/165
Hakugyoku	174/174	135/135	133/137	143/161	107/107	161/161	160/199	149/149	267/273	210/220	192/194	113/113	153/165
Kahou 2 gou	174/174	135/182	133/137	143/143	107/109	143/143	160/160	119/119	267/267	212/216	192/194	113/113	165/165
Daishouhou	174/174	164/164	133/137	143/143	107/107	143/180	164/199	141/141	267/267	212/220	192/194	113/119	155/165
Kibou	174/184	164/182/188	133/137	143/151	107/107	151/161	160/199	141/141	267/267	212/220	192/192	113/113	165/165
3N-Nagasaki 17	174/184	164/182/188	133/137	143/151	107/107	151/161	164/199	141/141	267/267	212/220	192/192	113/113	155/165
3N-Nagasaki 30	174/184	164/182/188	133/137	143/151	107/107	143/161	160/164/199	119/141	267/267	212/220	192/192	113/113	155/165
3N-Tanaka seedling	174/184	164/182	133/137	143/151	107/107	161/180	160/199	141/141	267/267	220/220	192/192	113/113	165/165
3N-Kusunoki 4	174/174	135/164/182	133/137	143/143	107/107	143/161/180	160/199	141/141	267/267	220/220	192/194	113/119	165/165
3N-Dohi 22	174/184	164/182	137/137	143/151	107/109	161/180	160/199	141/149	267/273	216/220	192/194	113/119	155/165
4N-Tanaka 1	174/184	164/182	137/137	143/151	107/107	161/180	160/199	141/141	267/267	220/220	192/192	113/113	165/165
4N-So 75	174/174	135/135	133/137	143/161	107/122	161/180	160/179	141/145	267/273	216/220	194/194	113/113	155/165
4N-Tomihusa	174/184	182/182	133/133	151/151	107/107	151/180	164/199	141/141	267/267	216/220	192/192	113/113	165/165

Genetic relatedness analyzed by SSR markers

A phenogram of the 15 diploid cultivars analyzed in this study was preliminarily constructed (Fig. 1) and cultivars belonging to the 'Tanaka'/'Kusunoki' group, those belonging to the 'Mogi' group and the Chinese cultivar group formed three loose groups, but with no clear distinctive grouping. 'Mogi', 'Tanaka', and 'Kusunoki', which were derived from seedlings of Chinese loquat cultivars, are the current major commercial cultivars as well as the parents of many recent hybrid cultivars. These genetic relationships were reflected in the phenogram. The cultivars bred in Chiba

Table 5. SSR genotypes of 'Kibou' and its parent cultivars.

SSR marker	Origin	Genotype[z]		
		Kibou	4N-Tanaka 1 (female parent)	Nagasakiwase (male parent)
CH03a09	apple	140/*150*	140/*150*	134/140
CH03d07	apple	*306*/308	*306*/308	308/314
CH03d12	apple	115/*140*	115/*140*	115/138
CH03g06	apple	*178*/180	*178*/178	176/180
CH04h03	apple	94/*102*	*102*/102	94/94
CH05a04	apple	*185*/187	*185*/185	185/187
NH033b	pear	184/*196*	*196*/196	184/196
NH035a	pear	*144*/144	*144*/144	144/144
NH204a	pear	*110*/110	*110*/110	110/114
NH207a	pear	*156*/156	*156*/156	156/156
KA14	pear	*175*/175	*175*/175	175/175
NH007b	pear	*129*/141/*147*	*129*/*147*	129/141
NH011b	pear	174/*184*	174/*184*	174/174
NH014a	pear	*164*/*182*/188	*164*/*182*	135/188
NB114a	pear	133/*137*	*137*/137	133/133
NB141b	pear	143/*151*	143/*151*	143/161
CH05c06	apple	*107*/107	*107*/107	107/107
CH05g03	apple	151/*161*	*161*/180	143/151
CH05g08	apple	*160*/199	*160*/199	164/199
MS06c09	apple	*141*/141	*141*/141	119/141
MS14b04	apple	*267*/267	*267*/267	267/267
CH02d10a	apple	212/*220*	*220*/220	212/212
CH05d04	apple	*192*/192	*192*/192	192/192
NH015a	pear	*113*/113	*113*/113	113/113
NB105a	pear	*165*/165	*165*/165	155/165

[z] Alleles derived from '4N-Tanaka 1' and 'Nagasakiwase' are indicated in italics and underlined, respectively.

Prefecture, such as 'Satomi', 'Husahikari', 'Tomihusa', and 'Husahime', formed a genetically close group in the phenogram. This may have been due to the use of common or related cultivars, such as 'Kusunoki', 'Tanaka', or their offspring, as the parents in hybridization.

More comprehensive and reliable information about the genetic diversity of the loquat will be obtained in the future by increasing the number of varieties analyzed, including indigenous varieties and Chinese cultivars.

Acknowledgements

The research was technically supported by Kazusa DNA Research Institute. The authors are grateful to Dr. Aoki (Kazusa DNA Research Institute) for valuable suggestions.

Literature Cited

Badenes, M. L., T. Canyamas, C. Romero, J. M. Soriano, J. Martinez and G. Llácer. 2003. Genetic diversityIN in European collection of loquat (*Eriobotrya japonica* Lindl.). Acta Hort. 620: 169–174.

Caballero, P. and M. A. Fernandez. 2002. Plenary lecture: Loquat, production and market. First international symposium on loquat (Valenia, Spain). Agenda and Abstract: 11–15.

Fukuda, S., J. Nagato, T. Yamamoto, N. Hiehata and O. Terai. 2002. Cultivar identification in loquat assessed by RAPD analysis. J. Japan. Soc. Hort. Sci. 71: 826–828 (In Japanese with English abstract).

Ichinose, I. 2002. Kajuengeigakudaihyakka 11. p. 7–10 (In Japanese). Nousanryousonbunkakyoukai, Tokyo.

Kimura, T., Y. Sawamura, K. Kotobuki, N. Matsuta, T. Hayashi, Y. Ban and T. Yamamoto. 2003. Parentage analysis in pear

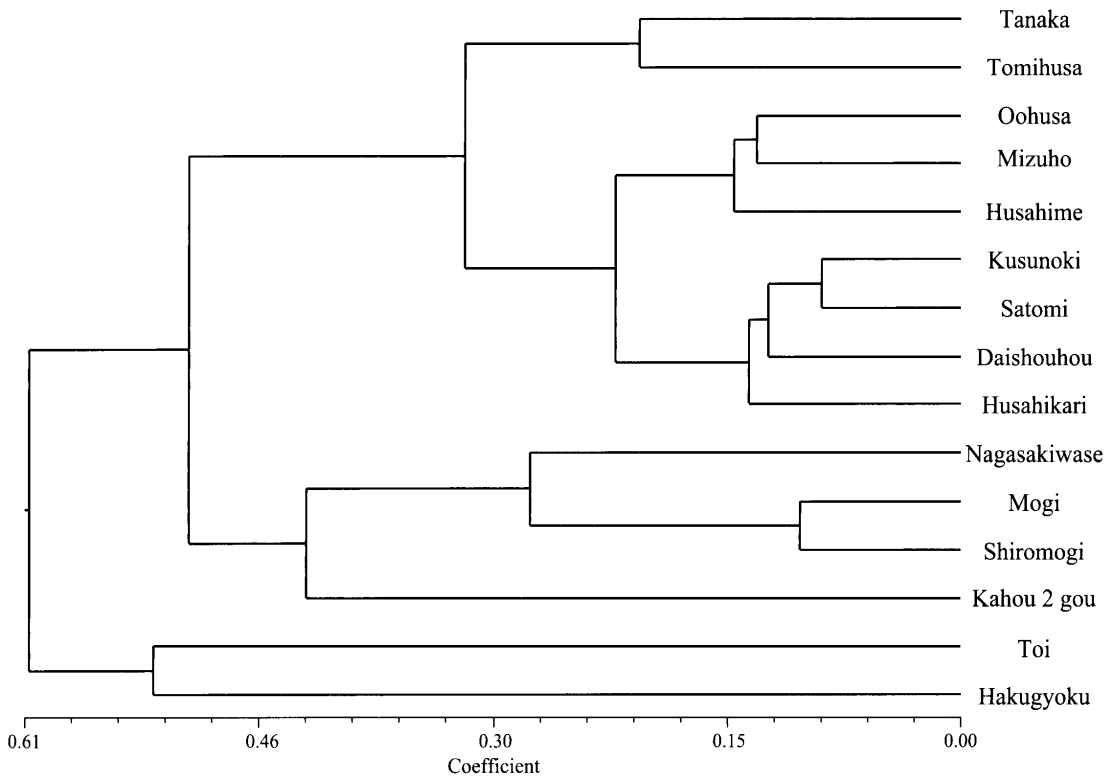

Fig. 1. Phenogram of 15 diploid loquat accessions identified by SSR genotyping generated by the UPGMA method.

cultivars characterized by SSR markers. J. Japan. Soc. Hort. Sci. 72: 182–189.

Kimura, T., Y. Z. Shi, M. Shoda, K. Kotobuki, N. Matsuta, T. Hayashi, Y. Ban and T. Yamamoto. 2002. Identification of Asian pear varieties by SSR analysis. Breed. Sci. 52: 115–121.

Liebhard, R., L. Gianfranceschi, B. Koller, C. D. Ryder, R. Tarchini, E. Van de Weg and C. Gessler. 2002. Development and characterization of 140 new microsatellites in apple (*Malus* × *domestica* Borkh.). Mol. Breed. 10: 217–241.

Nei, M. 1972. Genetic distance between populations. Am. Nat. 106: 283–292.

Rohlf, F. J. 2005. NTSYS-pc, Numerical taxonomy and multivariate analysis system, version 2.2. Exeter Publishing, Ltd., Setauket, New York.

Sawamura, Y., T. Saito, N. Takada, T. Yamamoto, T. Kimura, T. Hayashi and K. Kotobuki. 2004. Identification of parentage of Japanese pear 'Housui'. J. Japan. Soc. Hort. Sci. 73: 511–518.

Soriano, J. M., C. Romero, S. Vilanova, G. Llácer and M. L. Badenes. 2005. Genetic diversity of loquat germplasm (*Eriobotrya japonica* (Thunb) Lindl.) assessed by SSR markers. Genome 48: 108–114.

Vilanova, S., M. L. Badenes, J. Martínez-Calvo and G. Llácer. 2001. Analysis of loquat germplasm (*Eriobotrya japonica* Lindl) by RAPD molecular markers. Euphytica 121: 25–29.

Yahata, S., S. Sato, H. Ohara and H. Matsui. 2004. Induction of tetraploid in loquat with amiprofos-methyl and colchicine. Hort. Res. 3: 339–344 (In Japanese with English abstract).

Yahata, S., S. Sato, H. Ohara and H. Matsui. 2005. Differences of morphological and fruit bearing characteristics among the different ploidy plants and triploid production by crossings between diploid and triploid plants in loquat (Breeding & Germplasm Resources). Hort. Res. 4: 379–384 (In Japanese with English abstract).

Yahata, S., S. Sato, K. Sekiyama and K. Koide. 2005. A new loquat cultivar, 'Kibou'. J. Japan. Soc. Hort. Sci. 74 (Suppl. 1): 248 (In Japanese).

Yamamoto, T., T. Kimura, Y. Sawamura, K. Kotobuki, Y. Ban, T. Hayashi and N. Matsuta. 2001. SSRs isolated from apple can identify polymorphism and genetic diversity in pear. Theor. Appl. Genet. 102: 865–870.

Yamamoto, T., T. Kimura, Y. Sawamura, T. Manabe, K. Kotobuki, T. Hayashi, Y. Ban and N. Matsuta. 2002a. Simple sequence repeats for genetic analysis in pear. Euphytica 124: 129–137.

Yamamoto, T., T. Kimura, M. Shoda, Y. Ban, T. Hayashi and N. Matsuta. 2002b. Development of microsatellite markers in the Japanese pear (*Pyrus pyrifolia* Nakai). Mol. Ecol. Notes 2: 14–16.

Yamamoto, T., T. Kimura, M. Shoda, T. Imai, T. Saito, Y. Sawamura, K. Kotobuki, T. Hayashi and N. Matsuta. 2002c. Genetic linkage maps constructed by using an interspecific cross between Japanese and European pears. Theor. Appl. Genet. 106: 9–18.

Yamamoto, T., T. Kimura, J. Soejima, T. Sanada, Y. Ban and T. Hayashi. 2004. Identification of quince varieties using SSR markers developed from pear and apple. Breed. Sci. 54: 239–244.

Yu, D. J. 1979. Taxonomical classification of Chinese fruits. p. 309–316. In: D. J. Yu (ed.). Tropical subtropics fruit trees. 2. (In Chinese). Agricultural publisher, Chinese Beijing.