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IDENTIFICATION OF QUINCE VARIETIES USING SSR MARKERS DEVELOPED FROM PEAR AND APPLE

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IDENTIFICATION OF QUINCE VARIETIES USING SSR MARKERS DEVELOPED FROM PEAR AND APPLE

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Summary

1. Cross-genus application of SSR markers developed in pear and apple was examined for quince (*Cydonia oblonga*) in order to conduct its genetic characterization. It was revealed that 77 out of 118 SSR markers producing 1 or more reproducible amplified bands could be used in quince, including 20 SSRs from pear and 57 SSRs from apple. Twenty quince varieties were analyzed by using 39 polymorphic SSRs. A total of 122 polymorphic amplified fragments were obtained by using 39 SSR markers, which could divide 20 varieties into 12 genotypes. Parentage of 'Kaori' was confirmed because all the putative alleles of 'Kaori' were transmitted from its parents 'Smyrna' and 'Zairaishu' without any discrepancy at tested SSR markers. The SSR markers could be utilized as a reliable tool for the identification of quince varieties.

Key Words: *Cydonia oblonga*, cultivar identification, rootstock, SSR.

Introduction

2. Quince (*Cydonia oblonga* Mill.) belongs to the family Rosaceae, sub-family Maloideae along with pears (*Pyrus* spp.) and apples (*Malus* spp.). A large number of quince varieties have been used as dwarfing rootstocks for pear, while some varieties were used for preserves, jam and jellies (Hedrick 1925). Morphological characteristics of the fruits and trees of quince varieties are so similar that it is very difficult to obtain a reliable classification. There are no reports on genetic studies by DNA markers although Sanchez *et al*. (1988) analyzed isoenzyme patterns in *Cydonia* germplasms and divided them into several types. Therefore, no systematic classification and genetic studies have been conducted based on morphological characteristics as well as molecular markers in quince.

3. In the previous studies, we reported that SSRs from apple produced discrete amplified fragments in pear accessions and could be utilized for evaluation of genetic diversity in *Pyrus* spp (Yamamoto *et al*. 2001). The SSRs derived from pear were highly polymorphic and could be utilized as a reliable tool for cultivar identification in pears (Kimura *et al*. 2002).

4. In the present study, SSRs derived from pear and apple were examined for cross-genus amplification in quince. SSRs transferable to quince were used for variety identification in quince varieties.

Materials and Methods

Plant materials and DNA isolation

5. Twenty quince varieties, including 3 clones of 'Smyrna' and 2 clones of 'Kaori', were used in this study. About half of the quince varieties were cultivated for fruit production, whereas the others were used as rootstocks for pear. Genomic DNA was isolated from fresh

young leaves using the modified CTAB protocol described by Yamamoto *et al*. (2001).

Cross-genus amplification of SSR markers developed from pear and apple

6. One hundred and eighteen SSR markers, including 40 SSRs derived from pear and 78 SSRs from apple (Yamamoto *et al.* 2002a, 2002b, 2002c, Gianfranceschi *et al.* 1998, Guilford *et al.* 1997, Liebhard *et al.* 2002), were tested for cross-genus amplification. SSR markers transferable to quince were used for the identification of 20 quince varieties.

7. PCR amplification was conducted under the following conditions; initial denaturation at 94 °C for 2 min, followed by 35 cycles at 94 °C for 1 min, 55 °C for 1 min and 72 °C for 2 min, for denaturation, annealing and primer extension, respectively. The PCR products were separated and detected using a PRISM 377 DNA sequencer (Applied Biosystems, USA). The size of the amplified bands was calculated based on an internal standard DNA (GeneScan-350TAMRA, Applied Biosystems, USA) using GeneScan software (Applied Biosystems, USA).

Results and Discussion

Amplification of SSR markers in quince

8. Twenty out of 40 SSR markers derived from Japanese and European pears, could successfully produce amplified bands in quince. Fourteen SSRs generated 1 or 2 discrete reproducible bands in quince, whereas 6 other SSRs produced more than 2 amplified bands, presumably derived from multi loci.

9. Fifty-seven out of 78 SSR markers developed in apple could amplify bands in quince. Forty-one of the 57 SSRs produced 1 or 2 discrete reproducible bands in quince, whereas 16 other SSRs produced more than 2 amplified bands presumably derived from multi loci. A total of 77 SSR markers, including 20 SSRs from pear and 57 SSRs from apple, could be transferred from pear and apple to quince across genus.

10. Since no SSR markers have been developed in quince, it is considered that the use of transferable SSRs from pear and apple could be suitable for the genetic identification of quince varieties.

Variety identification

11. Twenty quince varieties were differentiated into 12 genotypes by using 122 polymorphic amplified fragments from 39 SSR markers. These 12 genotypes were differentiated based on differences of 1 to 55 SSR fragments (Table 1). Four sets of cultivars, i.e., ‘Angers’ vs. ‘Champion’, ‘Acucar’ vs. ‘Portugal’ vs. ‘Zairaishu’, ‘C-98-4’ vs. ‘Quince C’, ‘Quince B’ vs. ‘Sydo’, showed identical genotypes. ‘Zairaishu’, which was introduced and selected in Japan, showed the same genotype as ‘Portugal’ and ‘Acucar’. SSR analysis suggested that these 3 cultivars showed an identical (or very close) genetic relationship. ‘Quince A’ was differentiated from ‘Quince B’ by a difference of 1 band. It was reported that ‘Quince B’ showed similar plant characteristics to those of ‘Quince A’ (Tukey 1964). SSR analysis revealed that ‘Quince A’ and ‘Quince B’ were genetically very close but could be differentiated from each other.

12. Three ‘Smyrna’ clones maintained in Akita, Aomori and Nagano showed the same SSR genotypes, suggesting that they were vegetatively propagated clones. Similarly, it was considered that ‘Kaori’ in Aomori and Nagano had been propagated from the same original clone because of the identical SSR band pattern. ‘Kaori’ was bred from a cross of ‘Smyrna’ x ‘Zairaishu’ (Tsuchiya 1989). In a parentage analysis of ‘Kaori’ based on the use of 22 SSR markers presumably derived from a single locus, all the putative alleles of ‘Kaori’ were transmitted from its parents ‘Smyrna’ and ‘Zairaishu’ without any discrepancy, confirming that ‘Kaori’ was a hybrid between ‘Smyrna’ x ‘Zairaishu’.

13. In the present study, we showed that SSR markers were very effective for the identification of closely related quince varieties.

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Table 1. Differences of SSR fragments between each pair of quince varieties

No.	Cultivar name	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	Acucar																			
2	Angers	50																		
3	BA-29	32	52																	
4	Champion	50	0	52																
5	Cheldow	2	50	32	50															
6	C-26-L-1	32	52	2	52	32														
7	C-98-4	31	51	1	51	31	1													
8	Doue	32	48	4	48	32	4	3												
9	Kaori (Aomori)	38	40	48	40	40	48	47	46											
10	Kaori (Nagano)	38	40	48	40	40	48	47	46	0										
11	Portugal	0	50	32	50	2	32	31	32	38	38									
12	Quince A	34	50	4	50	34	4	3	4	46	46	34								
13	Quince B	33	51	3	51	33	3	2	3	47	47	33	1							
14	Quince C	31	51	1	51	31	1	0	3	47	47	31	3	2						
15	Smyrna (Akita)	55	37	55	37	55	55	54	53	25	25	55	53	54	54					
16	Smyrna (Aomori)	55	37	55	37	55	55	54	53	25	25	55	53	54	54	0				
17	Smyrna (Nagano)	55	37	55	37	55	55	54	53	25	25	55	53	54	54	0	0			
18	Sydo	33	51	3	51	33	3	2	3	47	47	33	1	0	2	54	54	54		
19	Vitory	32	52	2	52	32	2	1	4	48	48	32	4	3	1	55	55	55	3	
20	Zairaishu	0	50	32	50	2	32	31	32	38	38	0	34	33	31	55	55	55	33	32

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