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DEVELOPMENT OF SSR MARKERS AND GENETIC IDENTIFICATION OF PEAR VARIETIES

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Abstract

We developed 22 SSRs by using 3 approaches, RAHM (random amplified hybridization microsatellites), 5' anchored PCR methods and an enriched genomic library. Seventy of 22 SSRs could be successfully amplified for *Pyrus* spp., which showed highly polymorphic.

Sixty Asian pear accessions from 6 *Pyrus* species were genetically identified by 9 SSR markers with a total of 133 putative alleles. The SSR markers were highly polymorphic and could be utilized as a reliable tool for cultivar identification in Asian pears. In 10 out of 14 cultivars, the parent-offspring relationships were reconfirmed by parentage analysis using 20 SSR markers because the hybrids inherited SSR alleles from their parents without any discrepancy. In maternity analysis of 8 pear varieties derived from interspecific crosses by using the *trnL-trnF* sequences in chloroplast DNA, there was no contradiction between 2 hybrid varieties and their female parent. However, 4 varieties showed different nucleotide sequences from their described female parents, suggesting the possibility of reverse combinations of female and male parents. Therefore, we showed that the combination of nuclear and cytoplasmic molecular markers, e.g., SSR loci and cpDNA sequences, could be applicable to parentage and maternity analysis.

Development of SSR Markers

1. The development of highly informative DNA markers, such as SSRs (simple sequence repeats, also called as microsatellites), is essential for genetic studies and marker-assisted selection of agronomically important traits in pear (*Pyrus* spp.) [1)]. We used 3 approaches, RAHM (random amplified hybridization microsatellites), 5' anchored PCR methods and an enriched genomic library for isolation of SSRs [2•5)].

2. Nine SSRs were isolated, including 6 obtained from RAHM and 3 from 5' anchored PCR approaches (Fig. 1) [3)]. Two different methods were successfully applied for the isolation of pear SSRs. Segregation analysis of the 7 SSRs, 5 from RAHM and 2 from 5' anchored PCR, revealed that amplified fragments were derived from the single loci, using 3 sets of progenies from crosses between pear varieties. Genetic diversity was characterized using 32 varieties, including 10 from Japanese pear, 9 from Chinese pear, 10 from European pear as well as 3 wild relatives.

3. Thirteen polymorphic SSR loci were developed in Japanese pear by using an enriched genomic library [4]. The obtained SSR loci showed a high degree of polymorphism in Japanese pear with 3 to 6 alleles per locus. The average values of observed and expected heterozygosities among these 13 loci were 0.69 and 0.71, respectively. Ten SSRs could be successfully amplified for European pear, which were highly polymorphic as well.

Genetic identification of pear varieties

Identification of Asian pear varieties by SSR analysis

4. Sixty Asian pear accessions from 6 *Pyrus* species were genetically identified by 9 SSR markers with a total of 133 putative alleles (Fig. 2) [6]. Among them, 58 varieties could be successfully differentiated except for 2 pairs of synonymous or clonal varieties. All the SSR markers produced 1 or 2 discrete amplified fragments for all the diploid accessions, whereas a triploid variety showed 3 fragments with some SSRs. The number of putative alleles ranged from 7 to 20, with an average value of 14.8. The observed heterozygosity and the power of discrimination were 0.63 and 0.91, respectively. A phenogram based on the SSR genotypes was obtained, showing 3 major groups corresponding to the Japanese, Chinese and European groups. The SSR markers were highly polymorphic and could be utilized as a reliable tool for cultivar identification in Asian pears.

Parentage analysis in pear cultivars characterized by SSR markers

5. Parentage of 14 pear cultivars, including 8 cultivars derived from intraspecific crosses and 6 from interspecific crosses, was analyzed using 20 SSR markers [7•9]. In 10 out of 14 cultivars, the parent-offspring relationships were reconfirmed because the hybrids inherited SSR alleles from their parents without any discrepancy (Fig. 3). There were 4 questionable parent-offspring relationships with respect to 7 or more SSR loci. 'Housui' is not an offspring from a cross of 'Ri-14' x 'Yakumo' because of discrepancies at 7 loci [8, 9]. Likewise, 'Chojuro' and 'Nijisseiki' could not be confirmed as parents of 'Tanzawa' because of discrepancies at 9 loci. 'Max Red Bartlett' was reconfirmed as a parent of 'Oharabeni' because all SSR loci matched. 'Okusankichi' was not its parent because of discrepancies at 7 loci. In this study, 20 SSR markers were effectively utilized to determine parentage of Japanese pear, and 15 SSR markers could be utilized for interspecific *Pyrus* hybrids.

Genetic characterization of pear varieties revealed by chloroplast DNA sequences

6. Nucleotide sequences at 6 noncoding regions of cpDNA (chloroplast DNA), *atpB-rbcL* spacer, *trnL-trnF* spacer, *accD-psaI* spacer, *ndhA* intron, *rpl16* intron and *rpoC1* intron, were identified for 8 pear varieties from 5 different species in order to apply for maternity analysis. A total of 38 mutations such as nucleotide substitution, deletion and insertion were found in more than 5.7 kbp of nucleotide sequences [10]. A cladogram based on the mutations showed 4 types including 1 European pear group and 3 Asian pear groups. Nucleotide sequences at the *trnL-trnF* were revealed for 33 pear varieties and 8 mutations were identified. A cladogram obtained showed that Asian pear varieties were divided into 6 groups and that diversity existed within as well as between species in Asian pears. In contrast, European pear varieties were identical with respect to the *trnL-trnF* region. Maternity analysis of 8 pear varieties derived from interspecific crosses was conducted using the *trnL-trnF* sequences (Table 1). There was no contradiction between 2 hybrid varieties and their female parent. However, 4 varieties showed different nucleotide sequences from their described female parents, suggesting the possibility of reverse combinations of female and male parents. Therefore, we showed that the combination of nuclear and cytoplasmic molecular markers, e.g., SSR loci and cpDNA sequences, could be applicable to parentage and maternity analysis.

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Forward primer

1: AATACTAATCCTTTTTGCTAATAAT : 25

26: ATCTTGACAC**GAGAGAGAGAGAGAG** : 50

51: **AGAGAGAGAGAGAGAGAGAGAGAGA** :100

101: **GA**AGGGAATAAGGGATGATAATACA :120

121: GAAGGTGACCGAGACAGATTGAATG :125

126: GA Reverse primer :127

Fig. 1. Sequence of SSR marker NH001c
(modified from Kimura *et al* 2003a)
Bold letters indicate the SSR region of GA repeats

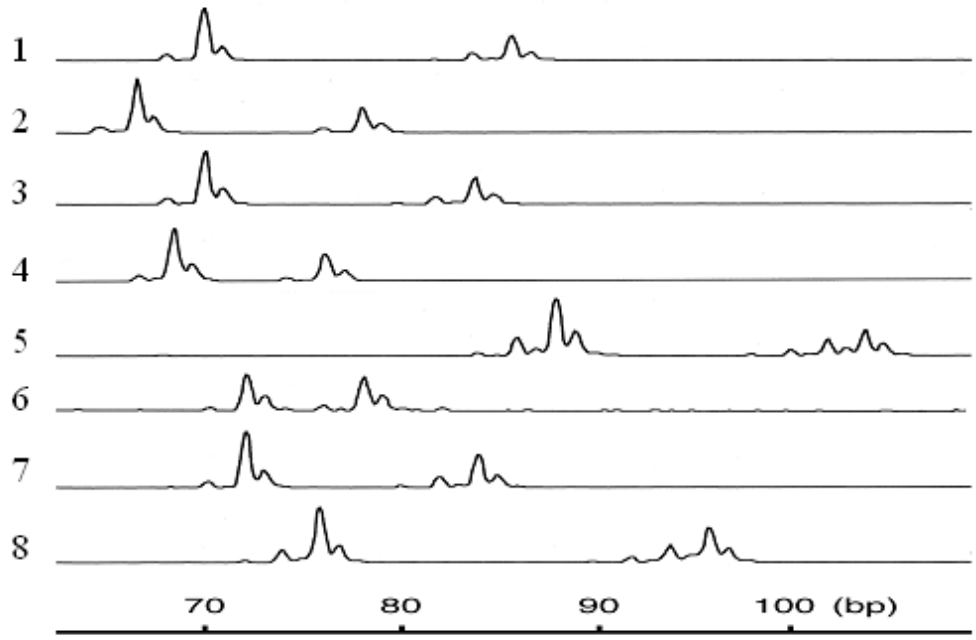

Fig. 2. Amplified fragment patterns of NH 014 a SSR from 8 pear varieties.
Lanes 1 to 8 display amplified products the following varieties.
Lane 1: Housui; 2: Okusankichi; 3: Yali; 4: Laiyangcili; 5: Balixiang; 6: Hongnahe; 7: La France; 8: Mamenashi 6
(Kimura *et al.* 2002a

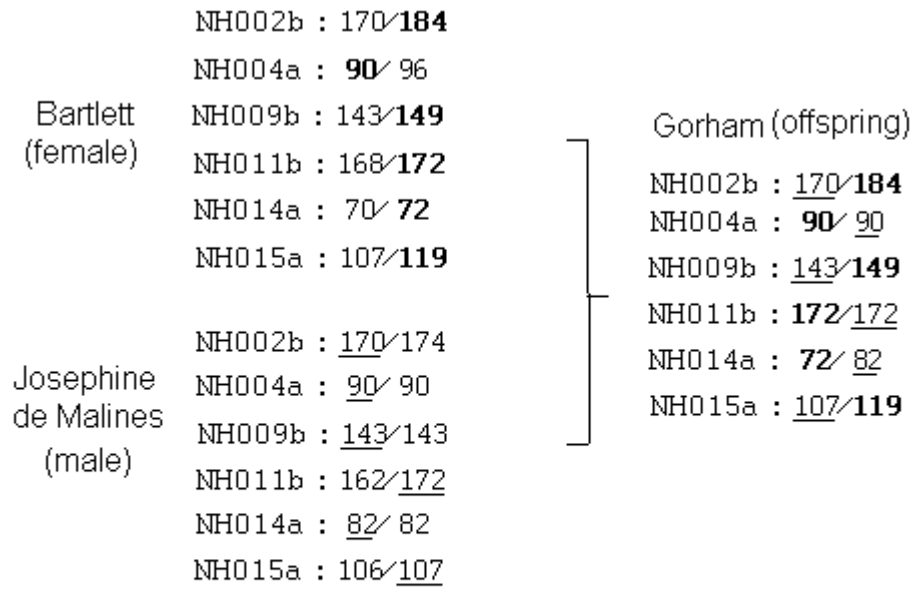

Fig. 3. Parentage analysis of Gorham and its parents Bartlett and Josephine de Malines by 6 SSR loci. Putative alleles transmitted from Bartlett and Josephine de Malines are indicated by bold type and underlines, respectively (Kimura *et al.* 2003b).

Table 1. Maternity analysis of 9 interspecific hybrids by *trnL-F* sequences (Kimura *et al*. 2003c)

Variety name	Described genetic origin	Results in this study
Taiheiyo	Okusankichi x La France	Okusankichi is not confirmed as a female parent. La France is presumably a female parent.
Oharabeni	Ishiiwase x Max Red Bartlett[a]	Ishiiwase is not confirmed as a female parent. Max Red Bartlett is presumably a female parent.
Mishirazu	unknown (*P. pyrifolia* x *P. communis*)	Japanese pear is not a female parent. European pear is presumably a female parent.
Le Conte	unknown (*P. communis* x *P. pyrifolia*)	European pear is not a female parent. Japanese pear is presumably a female parent.
Ninomiya	Chojuro x Le Conte[b]	Not determined because of lack of difference.
Ninomiyabaili	Yali x Shinchu	Not determined because of lack of difference.
Kangyoku	Chojuro x Mishirazu	Chojuro is confirmed as a female parent.
Zaosu	Pingguoli x Mishirazu	Pingguoli is confirmed as a female parent.

a One parent was not Okusankichi but Ishiiwase, as identified by SSR analysis (Kimura *et al.* 2002b; 2003b)

b Parent-offspring relationship between Ninomiya and Le Conte was disproven (Kimura *et al.* 2003b)

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