E

BMT/7/11
ORIGINAL: English
DATE: November 6, 2001

UPOV

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

# WORKING GROUP ON BIOCHEMICAL AND MOLECULAR TECHNIQUES AND DNA-PROFILING IN PARTICULAR

## Seventh Session
## Hanover, Germany, November 21 to 23, 2001

DEVELOPMENT OF SSR MARKERS AND IDENTIFICATION OF PEARS

*prepared by experts from Japan*

# Development of SSR Markers and Identification of Pears

Tetsuya Kimura[1], Toshiya Yamamoto[2], Yoshiyuki Ban[1]

[1]National Center for Seeds and Seedlings, Ibaraki 305-0852, Japan
[2]National Institute of Fruit Tree Science, Ibaraki 305-8605, Japan

## SUMMARY

Thirty-five SSRs were developed in pear by using 3 different approaches, RAHM (random amplified hybridization microsatellites), 5' anchored PCR methods and an enriched genomic library. The SSR markers obtained could be utilized on 5 tested *Pyrus* species. All SSR primers could produce 1 or 2 discrete amplified fragments in all accessions. Fifty-seven pear varieties from 5 species were successfully differentiated together using a total of 142 alleles. The number of alleles per locus ranged from 4 for KA4b to 19 for NH014a, with an average value of 12.9. Thirty-two individuals of the Japanese pear cultivar 'Choujuurou' from different sources showed the same genotypes with 11 SSR loci. The database of SSR genotype is now under development for native accessions and registered cultivars in Japanese pear.

Keywords: microsatellites, *Pyrus* spp., simple sequence repeat

## INTRODUCTION

Recently, SSRs (simple sequence repeats, also designated as microsatellites) have become the markers of choice in both animal and plant species because of their abundance, high degree of polymorphism and suitability for automation. In fruit tree species, SSR markers have been isolated and used for the cultivar identification of species belonging to the family Rosaceae, such as apple (*Malus* x *domestica*, Guilford *et al*., 1997; Gianfranceschi *et al*., 1998) and *Prunus* spp. (Cipriani *et al*., 1999). In the previous meeting (the 6th BMT), we reported that SSRs obtained from apple were efficiently utilized to identify pear cultivars and the parentage analysis in pear. Therefore, these SSR markers could be applied for DUS test.

In this study, we developed SSR markers in pear, and used them for characterization of pear varieties and estimation of uniformity within a cultivar.

## MATERIALS AND METHODS

### SSR isolation

Three different SSR isolation methods, an enriched genomic library, RAHM (random amplified hybridization microsatellites, Cifarelli *et al*., 1995) and 5' anchored PCR (Fisher *et al*., 1996), were applied for the isolation of SSRs from the Japanese pear 'Housui' and the European pear 'Bartlett' (Yamamoto *et al*., 2001b, 2001c).

**Identification of pear varieties**

Fifty-seven pear accessions were used, including 22 accessions of Japanese pear (*Pyrus pyrifolia* Nakai), 20 accessions of Chinese pear (*P. bretschneideri* Rehd., *P. ussuriensis* Maxim.), 12 accessions of European pear (*P. communis* L.) and 3 wild relatives (*P. calleryana* Decne.). All the varieties were obtained from the National Institute of Fruit Tree Science (Ibaraki, Japan) and the National Repository of Pear and Apple (Liaoning, P. R. China). Genomic DNA was isolated from young leaves using the modified CTAB protocol described in Yamamoto *et al*. (2001a). The SSR products were separated and detected using a PRISM 377 DNA sequencer (PE Applied Biosystems, USA). The size of the amplified bands was calculated based on an internal standard DNA with GeneScan software (PE Applied Biosystems, USA).

**Analysis of variation within cultivar**

Thirty-two individuals of the Japanese pear cultivar 'Choujuurou', including 20 individuals stored at NIFTS and 12 individuals collected from farmyards in Ibaraki prefecture, were analyzed by 11 SSRs to check variation within the cultivar. The SSR products were separated and detected using the same method as mentioned above.

**SSR database in pear**

A database of SSR genotype is now under development, composed of cultivar name, species, year of registration, parentage, genotypes of SSR markers and so on. Native accessions and registered cultivars in Japanese pear are mainly listed as the first step.

## RESULTS AND DISCUSSION

**SSR isolation**

The 35 SSR markers, 9 obtained from RAHM, 7 from 5' anchored PCR and 19 from an enriched genomic library, were established from the Japanese pear 'Housui' or the European pear 'Bartlett' (Yamamoto *et al*., 2001b, 2001c). Among them, 11 SSRs were chosen and used in this study (Table 1). Ten SSRs included perfect or imperfect repeats of AG/TC, ranging from 10 to 20 in repeat number. One SSR KA14 obtained by the RAHM method contained the AC/TG repeats. All SSRs could produce the fragments of the expected size for their original cultivar 'Housui' or 'Bartlett'. The number of alleles per locus ranged from 4 for KA4b to 19 for NH014a, with an average value of 12.9 (Table 1).

Table 1. Eleven SSRs used in this study

| SSR name | SSR isolation method | Origin | Expected product size (bp) | No. putative alleles |
|---|---|---|---|---|
| | RAHM | | 107 | 4 |
| | RAHM | | 180 | 8 |
| | RAHM | | 137 | 16 |
| | RAHM | | 248 | 10 |
| | 5' anchored PCR | 'Bartlett' | 124 | 7 |
| BGT23b | 5' anchored PCR | 'Bartlett' | 203 | 18 |
| | enriched genomic library | | 113 | 16 |
| | enriched genomic library | | 182 | 14 |
| | enriched genomic library | | 219 | 15 |
| | enriched genomic library | | 86 | 19 |
| | enriched genomic library | | 136 | 15 |

**Identification of pear varieties**

All SSR primers could produce 1 or 2 discrete amplified fragments in all tested accessions. Fifty-seven pear varieties were successfully differentiated together using a total of 142 alleles (Fig. 1, Kimura *et al*., 2001). Variation of genotype was observed within species as well as among species. The use of 11 SSR markers was sufficient to differentiate all pear varieties.

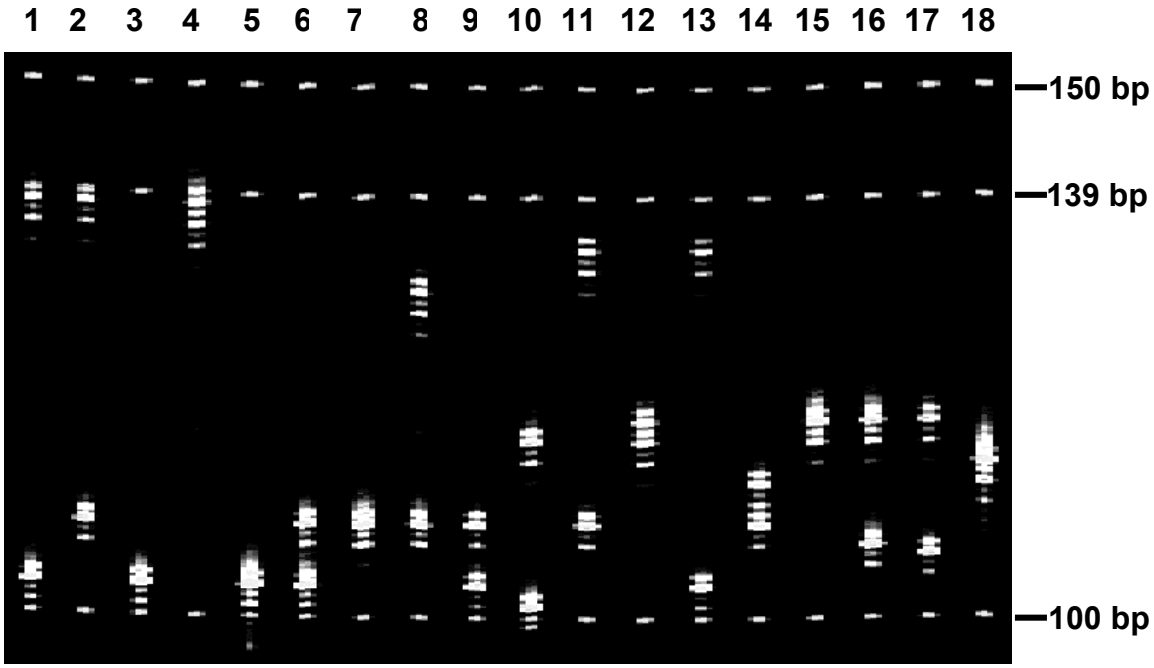


Fig. 1. Gel image of SSR products obtained from 18 cultivars with NH015a.
Lanes 1 to 18 are amplified products of the following varieties. Lane 1: Housui; 2: Ishiiwase; 3: Kousui; 4: Niitaka; 5: Nijisseiki; 6: Ohshuu; 7: Okusankichi; 8: Balixiang; 9: Iwateyamanashi; 10: Jingbaili; 11: Kuerlexiang; 12: Laiyangcili; 13: Mili; 14: Yali; 15: Comice; 16: La France; 17: Bartlett; 18: Mamenashi14.

**Analysis of variation within cultivar**

Thirty-two individuals of 'Choujuurou' obtained from different sources showed the same pattern with 11 SSRs (Fig. 2). We could not find differences among individuals of 'Choujuurou' in spite of propagation for more than one hundred years. It was suggested that SSR marker is very reliable and can be applied on an evaluation of uniformity. Information of the genotype based on SSR markers can be complementary to the DUS report.

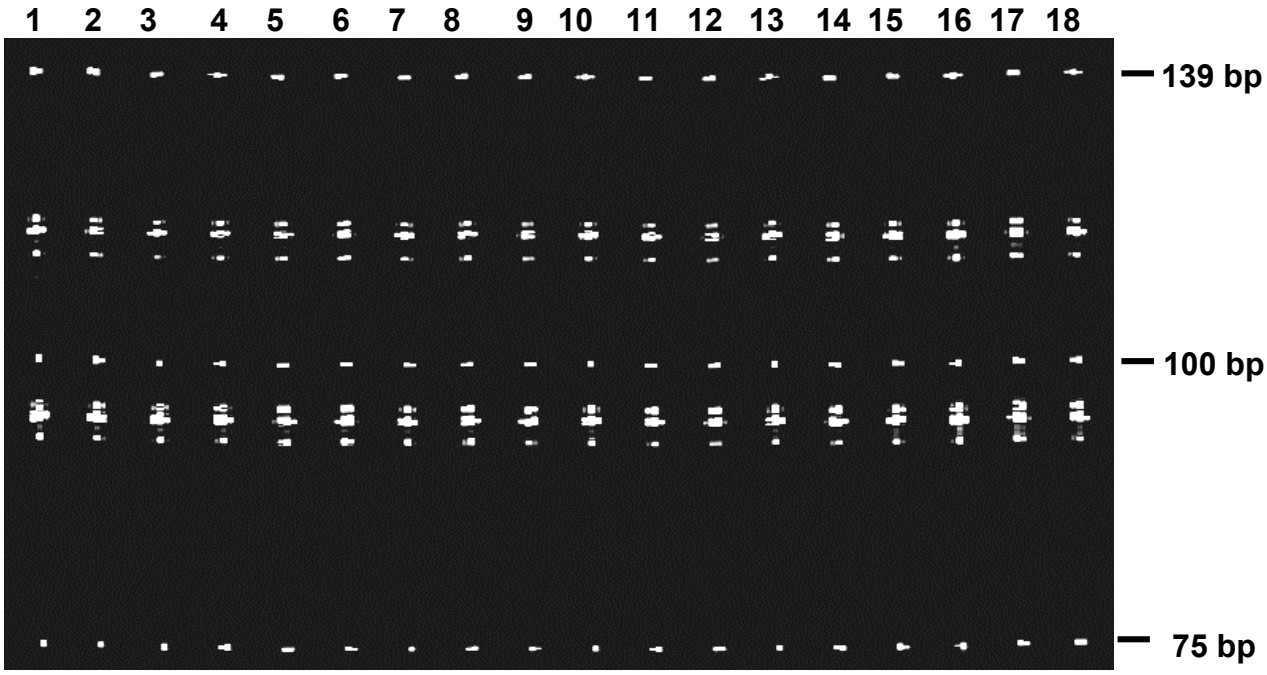


Fig. 2. Gel image of PCR products of 18 individuals of 'Choujuurou' with NH014a SSR locus.

**SSR database in pear**

The database of SSR genotype is now under development for native accessions and registered cultivars in Japanese pear and a part of the database is shown in Table 2. About 100 pear accessions have been surveyed with 11 SSR loci.

Table 2. Example of SSR database of pear

| No. | Cultivar name | Species | Origin | Year of registration | Parentage | SSR genotype | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | KU10 | BGT23b | NH011b | NH014a | NH015a |
| 01-0001-0001 | Akizuki | *Pyrus pyrifolia* | Tsukuba, Japan | under application | 162-29 x Hiratsuka17gou | 239/251 | 192/222 | 183/183 | 86/96 | 103/103 |
| 01-0001-0002 | Choujuurou | *Pyrus pyrifolia* | native, Japan | — | unknown | 251/253 | 192/202 | 175/183 | 70/86 | 103/138 |
| 01-0001-0003 | Housui | *Pyrus pyrifolia* | Tsukuba, Japan | — | unknown | 251/253 | 192/222 | 183/183 | 70/86 | 103/138 |
| 01-0001-0004 | Nijisseiki | *Pyrus pyrifolia* | native, Japan | — | unknown | 239/251 | 192/222 | 175/183 | 96/96 | 103/103 |
| 01-0001-0005 | Shinsei | *Pyrus pyrifolia* | Tsukuba, Japan | 1984 | Suisei x Shinkou | 239/253 | 192/222 | 183/185 | 70/96 | 103/138 |
| 01-0002-0001 | Yali | *Pyrus bretshneideri* | China | — | unknown | 251/251 | 188/192 | 185/185 | 70/84 | 109/113 |
| 01-0003-0001 | Balixiang | *Pyrus ussuriensis* | China | — | unknown | 251/251 | 188/196 | 185/185 | 88/104 | 109/130 |
| 01-0004-0001 | La France | *Pyrus communis* | France | — | unknown | 233/233 | 208/226 | 162/174 | 72/84 | 106/119 |
| 01-0004-0002 | Bartlett | *Pyrus communis* | England | — | unknown | 233/233 | 206/236 | 168/172 | 70/72 | 107/119 |

**ACKNOWLEDGEMENTS**

The authors are grateful to Drs. Y.Z. Shi, K. Kotobuki, N. Matsuta, T. Hayashi, H. Iketani and T. Manabe for their valuable suggestions. We are also grateful to Mrs. M. Osono, T. Saito, M. Shoda , Y. Sawamura, T. Imai and T. Iida for their useful discussions.

**Literature Cited**

Cifarelli, R.A., M. Gallitelli and F. Cellini. 1995. Random amplified hybridization microsatellites (RAHM): isolation of a new class of microsatellite-containing DNA clones. Nucleic Acid Res. 23: 3802-3803.

Cipriani, G., G. Lot, W.G. Huang, M.T. Marrazzo, E. Peterlunger and R. Testolin. 1999. AC/GT and AG/CT microsatellite repeats in peach [*Prunus persica* (L) Batsch]: isolation, characterization and cross-species amplification in *Prunus*. Theor. Appl. Genet. 99: 65-72.

Fisher, P.J., R.C. Gardner and T.E. Richardson. 1996. Single locus microsatellite isolated using 5' anchored PCR. Nucleic Acid Res. 24: 4369-4371.

Gianfranceschi, L., N. Seglias, R. Tarchini, M. Komjanc and C. Gessler. 1998. Simple sequence repeats for the genetic analysis of apple. Theor. Appl. Genet. 96: 1069-1076.

Guilford, P., S. Prakash, J.M. Zhu, E. Rikkerink, S. Gardiner, H. Bassett and R. Forster. 1997. Microsatellites in *Malus* x *domestica* (apple): abundance, polymorphism and cultivar identification. Theor. Appl. Genet. 94: 249-254.

Kimura, T., T. Yamamoto, Y.Z. Shi, K. Kotobuki, N. Matsuta, T. Hayashi and Y. Ban. (2001) The Japanese pear genome program I. development of SSR markers and identification of pears. International symposium on Asian pears (Kurayoshi, Japan) P39.

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

Yamamoto, T., T. Kimura, Y. Ban, M. Shoda, T. Hayashi and N. Matsuta. 2001b. Development of microsatellite markers in Japanese pear (*Pyrus pyrifolia* Nakai). Molecular Ecology Notes. 100 (1).

Yamamoto, T., T. Kimura, Y. Sawamura, T. Manabe, K. Kotobuki, T. Hayashi, Y. Ban and N. Matsuta. 2001c. Simple sequence repeats for genetic analysis in pear. Euphytica (in press).

[End of document]