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BMT/11/14 Add.
ORIGINAL: English
DATE: September 27, 2008

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# INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

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

## Eleventh Session
## Madrid, September 16 to 18, 2008

ADDENDUM

DEVELOPMENT OF SSR MARKERS AND THEIR APPLICATION FOR IDENTIFICATION IN ROSE

*Document prepared by experts from Japan*

# Development of SSR Markers and Their Application for Identification in Rose

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## The Number of Rose Varieties Registered in Japanese Registration System.

**Ornamental Plants 13,100**

1. Chrysanthemum 1,887 (14.4%)
2. Rose 1,824 (13.9%)
3. Carnation 1,196 ( 9.1%)

**Modern garden roses (*Rosa hybrida* L.)**

- economically important ornamental plants.
- belonging to the genus *Rosa*, which contain more than 150 species.
- derived from 8 or 10 species.
- tetraploid for almost all garden roses.
- produced for more than 10,000 cultivars since 1867.
- time-consuming for the judgment based on the morphological characteristics in DUS test.
- a little information of variety identification and DNA based fingerprinting techniques.

## In our previous studies

**Characteristics of SSR markers**

- High polymorphism
- High reproducibility
- Codominant inheritance

**pear apple quince**

- Development of SSR markers
- Genetic Identification
- Parentage analysis
- Application across genera

- DUS test
- PVP (Plant Variety Protection )

# In this study

## 1. Development of SSR Markers in Rose

**Polymorphic SSRs were isolated and developed from enriched genomic libraries**

## 2. Identification of Rose by SSRs

**Genetic identification of 33 rose varieties was performed by the developed SSR markers.**

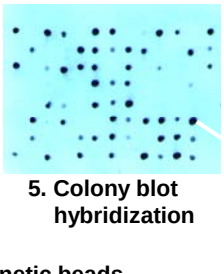
## 1. Development of SSR Markers in Rose

**An enriched genomic library - the magnetic bead method -**
**(Yamamoto *et al*. 2002. *Mol Ecol Notes* 2: 14–16)**

**A genomic library enriched for (AG)/ (TC) sequences was constructed from 'Asami Red' (*Rosa hybrida* L.) by using the magnetic bead method.**

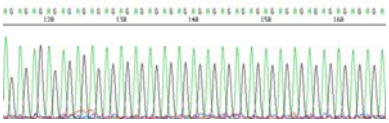
### Procedure

1. Digestion and ligation
2. Pre-PCR
3. Hybridization of PCR products with 3′-biotin -labeled (AG)15 oligonucleotides.
4. Enrichment using magnetic beads



5. Colony blot hybridization

6. Sequencing



**Positive plasmids were sequenced in a PRISM 3100 DNA sequencer**

**SSR clones from a rose variety**

| Variety | No. of obtained clones | No. of SSR clones | Average repeat number (ranges) |
|---|---|---|---|
| Asami Red | 192 | 55 | 16.1 ( 7 – 30 ) |

**Forward primer**

```
  1: GTTTCTTCAGGTGAAGAAGAGAAGG :  25
 26: GTGTTTTTCTGCGAGGTATCTGCAT :  50
 51: GAATCAGAGAGAGAGAGAGAGAGAG :  75
 76: AGAGAGAGAGAGAGAGAGAGAGGAA : 100
101: GAAGAACAAGAAAGAGAGTTGGAGA : 125
126: TGATTGAAATGAACTGAGG       : 144
```

**Reverse primer**

## Sequence of SSR marker RA016a

**AG repeats of SSR region were indicated by yellow.**

**Out of 20 primer pairs designed, 13 SSR markers could successfully amplify the target fragments for the original cultivar 'Asami Red'.**

**Thirteen SSR markers derived from an enriched genomic library.**

| SSR markers | Primer sequence (5'-3') | Motif | $T_a$ (°C) | PCR product size (bp) |
|---|---|---|---|---|
| RA003a | F: CAGAATTGGGTGTCCGTATG<br>R: CAATTTTCAAAGGATAATTTGG | $(GA)_{30}$ | 55 | 113 |
| RA013a | F: GAGGGGAAAGAGATACACAAA<br>R: GTAAGACCTTGCGTGTTCATA | $(AG)_{13}$ | 55 | 149 |
| RA016a | F: CAGGTGAAGAAGAGAAGGGTGT<br>R: CCTCAGTTCATTTCAATCATCTCC | $(AG)_{21}$ | 55 | 137 |
| RA019a | F: CGTTAGAGATCCGAGGGGGTC<br>R: TGTCATGGTTGGGAAGTTGGCT | $(AG)_{11}(AC)_{9}$ | 55 | 129 |
| RA020a | F: GTTAGAACCGAAGGCTCTAGT<br>R: CCCGCTAAGGTGGAGACATAC | $(AG)_{15}(AC)_{12}$ | 60 | 116 |
| RA023b | F: CATCCTCGGTGTTGCGTTGA<br>R: TGTCTCCAGCAACCTTTTTTTCCC | $(GA)_{20}$ | 55 | 172 |
| RA027a | F: ACCGTCCACAGTGTAAGAAAG<br>R: CCCTCAAGTCTAGTAAAACCA | $(AG)_{25}A(CAGAGA)_{5}$ | 55 | 165 |
| RA032b | F: CGGCATCAAAGATATAGCTTCC<br>R: AGAAATGCAAAACGCCCCTATGA | $(GA)_{23}$ | 55 | 147 |
| RA034a | F: GCATAGAGAACTCGGGAATCAC<br>R: TTCCGAAATGCCAACAACCAG | $(GA)_{22}$ | 57 | 91 |
| RA037a | F: AGAGAGTATGTCGTTTGGAGGAG<br>R: CTGCCTAAAATACCCCAAGTCAT | $(GA)_{21}$ | 55 | 173 |
| RA042a | F: CAGACTTATCAATGCGATCGTGCC<br>R: CAGCAATTCAGCAAGCCGTCTC | $(GA)_{26}GT(GA)_{8}$ | 55 | 122 |
| RA043a | F: GCAACGTACTTCAATTTCCAC<br>R: CAAGCTCAGAACTGAGACAC | $(AG)_{17}$ | 55 | 144 |
| RA044b | F: TAGACAGATAGATATTGGCAC<br>R: CAACTACAGATTTCTACCAACT | $(AG)_{14}$ | 57 | 92 |

## Characteristics of 13 SSR markers in rose.

| SSR markers | DDBJ accessions | No. of alleles in *R. multiflora* | $H_O$ | $H_E$ | No. of genotypes in rose varieties | PD* |
|---|---|---|---|---|---|---|
| RA003a | AB231934 | 12 | 0.83 | 0.92 | (13) | (0.90) |
| RA013a | AB211280 | 8 | 0.75 | 0.81 | 12 | 0.81 |
| RA016a | AB211281 | 6 | 0.33 | 0.77 | 15 | 0.88 |
| RA019a | AB211282 | 4 | 0.08 | 0.68 | 9 | 0.65 |
| RA020a | AB211283 | ------- | ------- | ------- | 13 | 0.83 |
| RA023b | AB211284 | 10 | 0.83 | 0.91 | 21 | 0.94 |
| RA027a | AB211285 | ------- | ------- | ------- | 13 | 0.85 |
| RA032b | AB211286 | 7 | 0.50 | 0.82 | 26 | 0.96 |
| RA034a | AB211287 | 6 | 0.42 | 0.55 | 12 | 0.82 |
| RA037a | AB211288 | 9 | 1.00 | 0.89 | 7 | 0.47 |
| RA042a | AB211289 | ------- | ------- | ------- | 11 | 0.82 |
| RA043a | AB211290 | 10 | 0.83 | 0.92 | 19 | 0.94 |
| RA044b | AB211291 | 12 | 0.58 | 0.92 | 10 | 0.82 |

* PD denotes the power of discrimination in 33 roses varieties except for RA003a (PD and No. of genotypes in 24 rose varieties)
Modified from Kimura *et al*. 2006. Mole Ecol Notes, 2: 810-812

## 2. Genetic identification in rose varieties

### 33 rose varieties used in this study.

***Rosa hybrida* L.** **29***

- Anthony Meilland
- Asami Red
- Blue Moon
- Bridal Pink
- Charleston
- Cherish
- Christian Dior
- Eikou
- Gold Buny
- Gold Schatz
- Grace Land
- Hanagasumi
- Harmonie
- Harukaze
- Int Rigue
- La France
- Lavagult
- Legend
- Nicole
- Peace
- Princess Michiko
- Royal Highness
- Samantha
- Seika
- Shuugetsu
- Super Star
- Tequila
- White Christmas
- Zorina

***R. chinensis*** 1*
***R. bracteata*** 1*
***R. banksiae*** 1*
***R. laevigata*** 1*

All germplasms were from the National Institute of Floricultural Science (NIFS, Ibaraki, Japan)

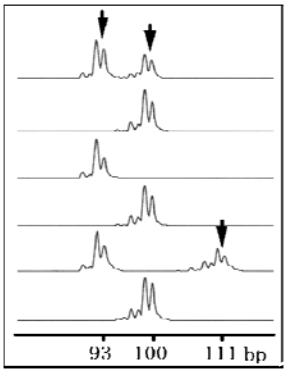
* No. of used varieties

## SSR analysis

Total genomic DNA

DNA Isolation

Analysis of SSR fragments



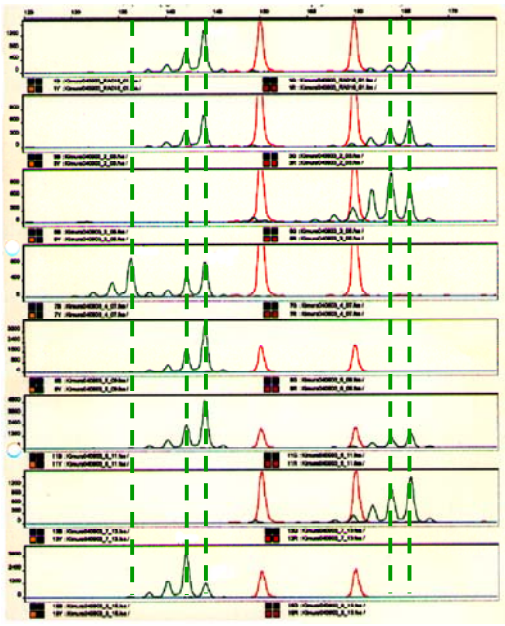

SSR fragments were amplified by 12 markers

94 °C, 2 min
94 °C, 1 min
55-60 °C, 1 min
72 °C, 2 min
35 cycles
72 °C, 7 min

PRISM 3100 DNA sequencer

## Amplified fragment patterns of the SSR RA016a

Peace
Charleston
Eikou
Harukaze
Royal Highness
Asami Red
Seika
Blue Moon

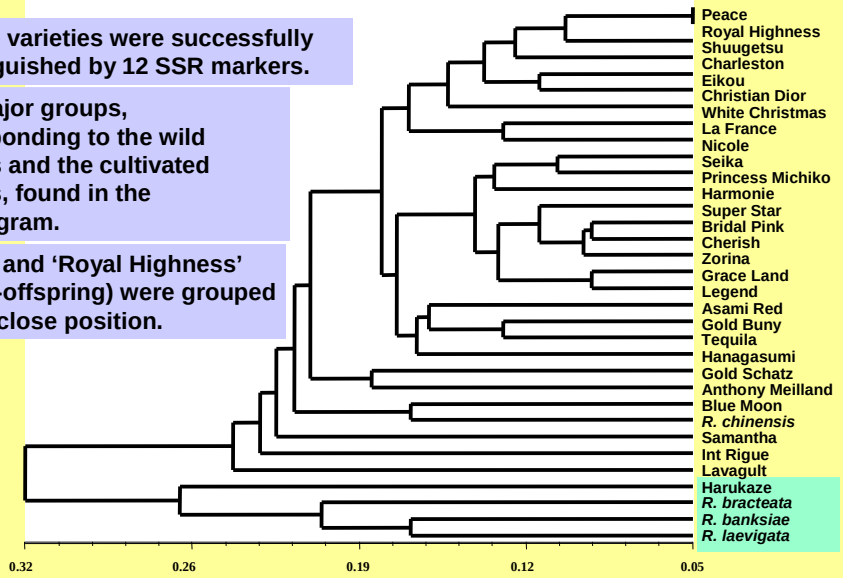


**A phenogram for 33 rose genotypes constructed by using the UPGMA method.**

All rose varieties were successfully distinguished by 12 SSR markers.

Two major groups, corresponding to the wild species and the cultivated species, found in the dendrogram.

'Peace' and 'Royal Highness' (parent-offspring) were grouped at very close position.




## Conclusion

1. Thirteen SSR markers were developed in rose (*Rosahybrida* L.) from an enriched genomic library.

2. Thirty-three rose varieties (*Rosa hybrida* L.) were successfully distinguished by 12 SSR markers.

3. The SSR markers developed were highly polymorphic and could be utilized as reliable tools for varieties identification in rose.

## *Acknowledgements*

***National Center for Seeds and Seedlings***

**M. Osono** **N. Asano**

***National Institute of Fruit Tree Science***

**H. Iketani** **C. Nishitani**

**T. Imai** **S. Terakami**

***National Institute of Floricultural Science***

**T. Onozaki** **M. Yagi**

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