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DEVELOPMENT OF SSR MARKERS AND THEIR APPLICATION FOR IDENTIFICATION IN ROSE

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# DEVELOPMENT OF SSR MARKERS AND THEIR APPLICATION FOR IDENTIFICATION IN ROSE

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## SUMMARY

1. Thirteen SSR markers were developed in rose (*Rosa hybrida* L.) from an enriched genomic library. Ten out of 13 markers showed high polymorphism and produced 4 to 12 alleles per locus, with an average of 8.4 in diploid rose *R. multiflora*. The average values of observed and expected heterozygosities among these 10 loci were 0.62 and 0.82, respectively. Furthermore, 12 SSR markers, except for RA003a, produced 7 to 26 genotypes (with an average of 14.0) per marker in 33 rose varieties. The average value of the power of discrimination (PD) among the markers was 0.82, ranging from 0.47 to 0.96. The 12 SSR markers were used for identification of 33 rose varieties. All rose varieties were successfully distinguished using 168 SSR genotypes produced from 12 SSR markers. Genetic relatedness of 33 rose varieties was analyzed based on SSR genotypes. The resulting dendrogram enabled the identification of 2 major groups, corresponding to the wild species and the cultivated species. The developed SSR markers were highly polymorphic and could be utilized as reliable tools for variety identification in rose.

Keywords: identification, rose, SSR markers

## INTRODUCTION

2. Modern garden roses belong to the genus *Rosa*, which contains more than 150 species (Shepherd 1978). Modern garden roses have been derived from only 8 or 10 species (Stewart 1969). Almost all garden roses are tetraploid. More than 10,000 varieties have been produced since 1867. Varieties were identified on the basis of morphological characteristics, which are often affected by environmental conditions and human judgment.

3. SSR markers are highly polymorphic and reproducible, and have an advantage of providing codominant inheritance in PCR analysis. SSR markers have been successfully used in the discrimination of many plant varieties. In previous BMT sessions (BMT/8, 9, 10), we reported that SSRs markers were efficient for identification of pear and quince varieties and for parentage analysis in pear. Therefore, SSR markers could be applied in DUS testing.

4. In this study, we isolated and developed 13 polymorphic SSRs from enriched genomic libraries of rose varieties. Genetic identification of 33 rose varieties was performed by using established SSR markers.

## MATERIALS AND METHODS

### Plant materials and DNA extraction

5. Thirty-three modern garden rose varieties (*Rosa hybrida* L.) were used and obtained from the National Institute of Floricultural Science (Ibaraki, Japan) and 12 individuals of *R. multiflora* collected within a 5 km radius from the Institute. Genomic DNA was isolated from young leaves with Genomic DNA Buffer G2 and Genomic-tip20/G (Qiagen, Germany) or with a DNeasy Plant Mini Kit (Qiagen, Germany) according to the manufacturer's instructions.

### SSR isolation and PCR amplification of SSRs

6. A genomic library enriched for (AG)/(TC) sequences was constructed from 'Asami Red' (*Rosa hybrida* L.) by using the magnetic bead method (Yamamoto *et al*. 2002a, b, c). SSR primer sets were designed using the software Oligo ver. 6.0 (Molecular Biology Insights, Inc., USA). SSR amplification was performed in a 20-µL solution of 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin, 0.2 mM each dNTP, 10 pmol of each forward primer labeled with fluorescent chemical (Fam or Vic or Ned) and unlabelled reverse primer, 10 ng genomic DNA, and 0.5 units of Taq polymerase (Invitrogen, USA). Amplification was performed in 35 cycles at 94 °C for 1 min, 55-60 °C for 1 min, and 72 °C for 2 min. The PCR products were separated and detected in a PRISM 3100 DNA sequencer. The size of the amplified bands was calculated based on an internal standard DNA (400HD-ROX, Applied Biosystems, USA) with GeneScan software (Applied Biosystems, USA).

### Statistical analysis

7. The heterozygosity of 12 individuals of diploid wild rose *R. multiflora* was estimated for SSR loci. The observed heterozygosity ($H_O$) and the expected heterozygosity ($H_E$) were calculated using CERVUS ver. 2.0 software (Marshall et al., 1998). $H_O$ was calculated as the number of heterozygous genotypes at a given locus divided by the total number of genotypes scored at that locus. $H_E$ is calculated using an unbiased formula from allele frequencies assuming Hardy-Weinberg equilibrium (Nei, 1987). The power of discrimination (PD) was calculated from 33 rose varieties based on the frequency of each genotype according to the formula : $PD=1-\Sigma p_i^2$ where $p_i$ is the frequency of the $i^{th}$ genotype of each SSR locus (Kloosterman et al., 1993). A phenogram of 33 rose varieties was constructed by using UPGMA (unweighted pair-group method using arithmetic averages) based on Nei's genetic identity (Nei and Li, 1979). The program NTSYS-pc, ver. 2.01 was used to construct the phenogram (Rohlf, 1998).

## RESULTS AND DISCUSSION

### SSR marker development

8. We screened 83 positive clones from 192 colonies by colony hybridization. After sequencing, 55 sequences contained 7 to 30 microsatellite repeats of (AG)/(TC), ca. 16.1 on average. The average insert size of the obtained clones was 489 bp (range, 106–1169 bp). Three sequences showed duplication. Out of the 52 independent sequences, 47 contained complete (AG)/(TC) repeats; the others had interrupted repeats or combined motifs of (AG)/(TC) and other units. Three sequences showed 2 repeat regions apart from each.

9. Out of 20 primer pairs designed, 17 primer pairs designed could successfully amplify the target fragments of the expected size for the original cultivar 'Asami Red'. Ten markers except for RA020a, RA027a and RA042a showed high polymorphism and produced 4 to 12 alleles per locus, with an average of 8.4 in *R. multiflora* (Table 1, Kimura *et al*., 2006). The average values of observed and expected heterozygosities among these 10 loci were 0.62 and 0.82, respectively. Furthermore, 33 rose varieties were analyzed by 12 SSR markers (Table 1). These 12 SSR markers except for RA003a showed 7 to 26 genotypes (with an average of 14.0) per marker in 33 rose varieties. The average value of the power of discrimination (PD) among the markers was 0.82, ranging from 0.47 to 0.96. It was found that 12 markers were very useful for the cultivar identification in modern garden roses.

Identification of rose varieties

10. Thirty-three rose varieties were successfully differentiated together using a total of 168 SSR genotypes (Fig. 1). Surprisingly, all of 33 varieties could be discriminated by using only 2 SSR markers; RA032b and RA034a.

11. Genetic relatedness of 33 rose varieties was analyzed based on SSR genotypes. The resulting dendrogram identified 2 major groups, corresponding to the wild species and the cultivated species. *R. chinensis* was included with the cultivated species (Fig. 1). The varieties 'Peace' and 'Royal Highness' showing a parent-offspring relationship were grouped at a very close position in this study. The SSR markers developed were highly polymorphic and could be utilized as reliable tools for variety identification in rose.

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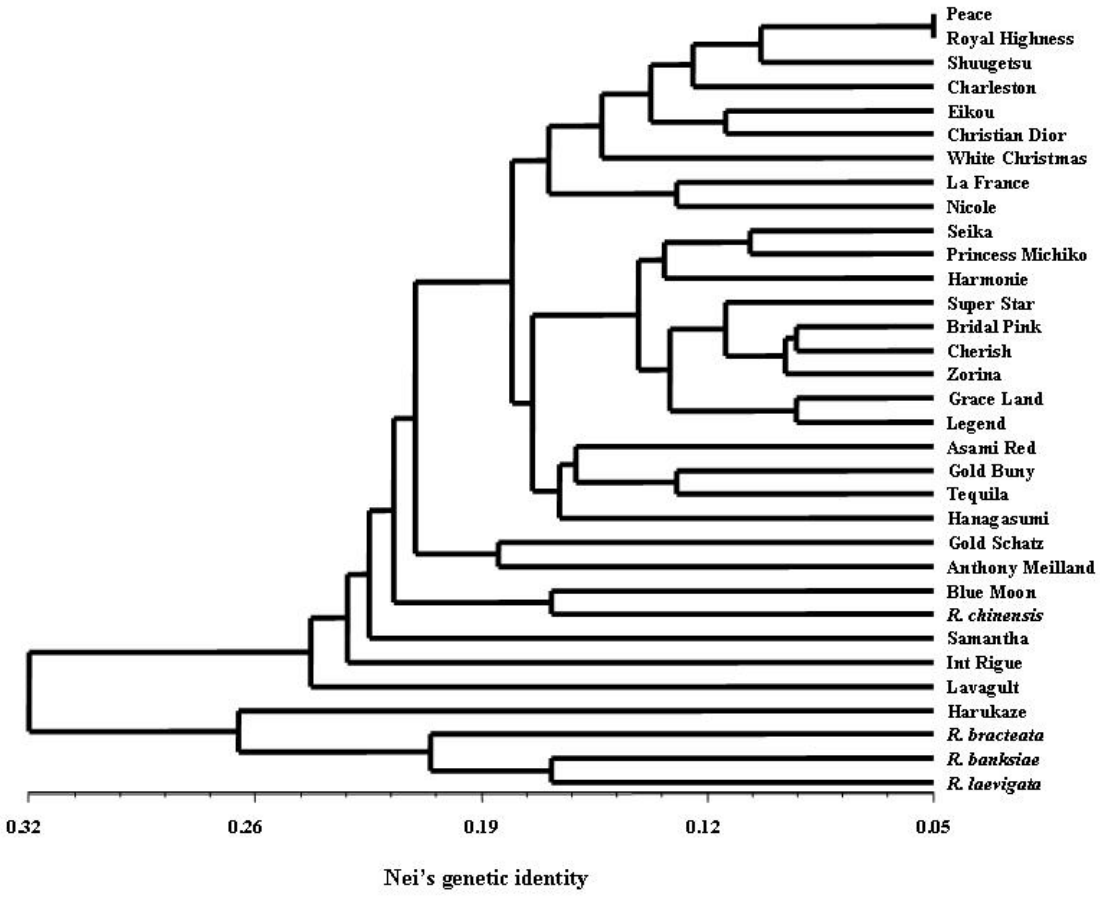


**Fig. 1. A phenogram for 33 rose genotypes constructed by using the UPGMA method**

**Table 1.** Characteristics of 13 SSR markers derived from an enriched genomic library in rose. The PCR product size refers to sequenced clones from 'Asami Red'. $T_a$; annealing temperature; $H_O$ and $H_E$ denote observed and expected heterozygosities, respectively. PD denotes the power of descrimination in 33 roses cultivars excpt for RA003a (PD and No. of genotypes in 24 rose cultivars) . DDBJ accession nos are listed under respective SSR markers.

| Microsatellite markers | Primer sequence (5'-3') | Motif | $T_a$ (℃) | PCR product size (bp) | No. of alleles in *R. multiflora* | $H_O$ | $H_E$ | No. of genotypes in rose cultivars | PD |
|---|---|---|---|---|---|---|---|---|---|
| RA003a AB231934 | F: CAGAATTGGGTGTCCGTATG<br>R: CAATTTTCAAAGGATAATTTGG | $(GA)_{30}$ | 55 | 113 | 12 | 0.83 | 0.92 | (13) | (0.90) |
| RA013a AB211280 | F: GAGGGGAAAGAGATACACAAA<br>R: GTAAGACCTTGCGTGTTCATA | $(AG)_{13}$ | 55 | 149 | 8 | 0.75 | 0.81 | 12 | 0.81 |
| RA016a AB211281 | F: CAGGTGAAGAAGAGAAGGGTGT<br>R: CCTCAGTTCATTTCAATCATCTCC | $(AG)_{21}$ | 55 | 137 | 6 | 0.33 | 0.77 | 15 | 0.88 |
| RA019a AB211282 | F: CGTTAGAGATCCGAGGGGGTC<br>R: TGTCATGGTTGGGAAGTTGGCT | $(AG)_{11}(AC)_9$ | 55 | 129 | 4 | 0.08 | 0.68 | 9 | 0.65 |
| RA020a AB211283 | F: GTTAGAACCGAAGGCTCTAGT<br>R: CCCGCTAAGGTGGAGACATAC | $(AG)_{15}(AC)_{12}$ | 60 | 116 | ------- | ------- | ------- | 13 | 0.83 |
| RA023b AB211284 | F: CATCCTCGGTGTTGCGTTGA<br>R: TGTCTCCAGCAACCTTTTTTTCCC | $(GA)_{20}$ | 55 | 172 | 10 | 0.83 | 0.91 | 21 | 0.94 |
| RA027a AB211285 | F: ACCGTCCACAGTGTAAGAAAG<br>R: CCCTCAAGTCTAGTAAAACCA | $(AG)_{25}A(CAGAGA)_5$ | 55 | 165 | ------- | ------- | ------- | 13 | 0.85 |
| RA032b AB211286 | F: CGGCATCAAAGATATAGCTTCC<br>R: AGAAATGCAAAACGCCCCTATGA | $(GA)_{23}$ | 55 | 147 | 7 | 0.50 | 0.82 | 26 | 0.96 |
| RA034a AB211287 | F: GCATAGAGAACTCGGGAATCAC<br>R: TTCCGAAATGCCAACAACCAG | $(GA)_{22}$ | 57 | 91 | 6 | 0.42 | 0.55 | 12 | 0.82 |
| RA037a AB211288 | F: AGAGAGTATGTCGTTTGGAGGAG<br>R: CTGCCTAAAATACCCCAAGTCAT | $(GA)_{21}$ | 55 | 173 | 9 | 1.00 | 0.89 | 7 | 0.47 |
| RA042a AB211289 | F: CAGACTTATCAATGCGATCGTGCC<br>R: CAGCAATTCAGCAAGCCGTCTC | $(GA)_{26}GT(GA)_8$ | 55 | 122 | ------- | ------- | ------- | 11 | 0.82 |
| RA043a AB211290 | F: GCAACGTACTTCAATTTCCAC<br>R: CAAGCTCAGAACTGAGACAC | $(AG)_{17}$ | 55 | 144 | 10 | 0.83 | 0.92 | 19 | 0.94 |
| RA044b AB211291 | F: TAGACAGATAGATATTGGCAC<br>R: CAACTACAGATTTCTACCAACT | $(AG)_{14}$ | 57 | 92 | 12 | 0.58 | 0.92 | 10 | 0.82 |

Modified by Kimura et al., 2006. Molecular Ecology Notes, 2, 810-812

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