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Development of SSR markers to assess molecular diversity in *Paeonia lactiflora*

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작약에서 분자유전학적 다양성 평가를 위한 SSR 마커 개발

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Objectives

The purpose of our study was to develop and characterize novel microsatellite markers for the analysis of genetic diversity and phylogenetic relationships in the accessions of *Paeonia lactiflora*

Materials and Methods

○ Materials
- The accessions of *Paeonia lactiflora* were obtained from National Institute of Horticultural & Herbal Science, Rural Development Administration (RDA).

○ Methods
- Construction of SSR-enriched library: A microsatellite-enriched library was constructed by using a modified biotin-streptavidin capture method.
- Characteristics of an enriched library, primer design and marker development: A total of 759 recombinant clones were randomly picked from the primary transformation plates containing ampicillin, X-gal, and IPTG. Plasmid DNA was sequenced using an ABI 3730xl DNA sequencer with a BigDye terminater kit(Applied Biosystems). SSR identification within cloned sequences and primer design were carried out using the ARGOS 1.46 SSR MANAGER program.

Results

An eriched library was successfully constructed by using a modified biotin-streptavidin capture method to develop a SSR marker system in *Paeonia lactiflora*. From the 759 sequenced clones, we found that 35 clones (4.6%) were redundant and 724 clones were having microsatellite repeating motifs. Sequence analysis of all SSR-containing clones revealed a predominance of di-nucleotide SSRs (86.9%) over tri-nucleotide SSRs (11.6%). Among the di-nucleotide type, the AG/GA class of repeat motif was most

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frequently identified (36.6% of the total di-nucleotide SSRs), followed by the GT/TG class (24.1%), and the CT/TC class (21.8%). Among the tri-nucleotide SSRs, the AGA/GAA/AAG class of repeat motif was predominant (23.9%), followed by the GAG/AGG/GGA, GTT/TTG/TGT class (17.4%). Finally, we designed 212 primer pairs from the flanking sequences of SSR containing clones. We are undertaking the analysis of polymorphisms using the diverse collected accessions of *Paeonia lactiflora* now. This newly developed SSR marker set shall provide a very useful tool for implementing molecular diversity assessment and population structure studies of *Paeonia lactiflora* onward.

Table 1 Screening summary of microsatellite-enriched library of *Paeonia lactiflora*

Screening steps	Numbers (percentage)
Sequenced clones	759
Redundant clones	35 (4.6%)
Unique clones	724 (95.4%)
SSR clones	321 (42.3%)
Primer design	212 (27.9%)

Table 2 Characteristics of the enriched library of *Paeonia lactiflora* in terms of different identified microsatellite sequences

Repeat unit	Repeat class	Numbers	(%)
Di-nucleotide			
	AG/GA	126	36.6
	GT/TG	83	24.1
	CT/TC	75	21.8
	AC/CA	45	13.1
	AT/TA	15	4.4
Total		344 (86.9%)	
Tri-nucleotide			
	AGA/GAA/AAG	11	23.9
	GAG/AGG/GGA	8	17.4
	GTT/TTG/TGT	8	17.4
	CAG/AGC/GCA	6	13
	GCT/CTG/TGC	5	10.9
	CAA/AAC/ACA	3	6.5
	TGA/GAT/ATG	2	4.3
	CCA/CAC/ACC	2	4.3
	CTT/TTC/TCT	1	2.2
Total		46 (11.6%)	
Others		6 (1.5%)	
Total Repeat motifs		396	