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Development of SSR markers to assess molecular diversity in *Liriope platyphylla* WANG et TANG

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맥문동에서 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 analyses of genetic diversity and phylogenetic relationships in the accessions of *Liriope platyphylla.*

Materials and Methods

- ○ Materials
  - The accessions of *Liriope platyphylla* 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 758 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 terminator kit (Applied Biosystems). SSR identification within cloned sequences and primer design were carried out using the SSR MANAGER program.

Results

An enriched library was successfully constructed by using a modified biotin-streptavidin capture method to develop a SSR marker system in *Liriope platyphylla,* WANG et TANG. Of 758 sequenced clones, we found that 76 clones (10.03%) were redundant and 642 clones were having microsatellite repeating motifs.

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Sequence analysis of all SSR-containing clones revealed a predominance of di-nucleotide SSRs (98.90%) over tri-nucleotide SSRs (1.10%). Among the di-nucleotide type, the AG/GA class of repeat motif was most frequently identified (33.97% of the total di-nucleotide SSRs), followed by the CT/TC class (33.77%).

Among the tri-nucleotide SSRs, the CTG/TGC/GCT class of repeat motifs was predominant (36.36%), followed by CCT/CTC/TCC and CTT/TTC/TCT. Finally, we designed 296 primer pairs from the flanking sequences of SSR containing clones. We are undertaking the analysis of polymorphisms using the diverse collected accessions of *Liriope platyphylla* now. This newly developed SSR marker set shall provide a very useful tool for implementing molecular diversity assessment and population structure studies of *Liriope platyphylla* onward.

Table 1 Screening summary of microsatellite-enriched library of *Liriope platyphylla*

Screening steps	Numbers (percentage)
Sequenced clones	758
Redundant clones	76 (10.03%)
Unique clones	682 (89.97%)
SSR clones	643 (84.83%)
Primer design	296 (39.05%)

Table 2 Characteristics of the enriched library of *Liriope platyphylla* in terms of different identified microsatellite sequences

Repeat unit	Repeat class	Numbers	(%)
	AC/CA	56	5.65
	AG/GA	337	33.97
	AT/TA	83	8.37
	CG/GC	25	2.52
	CT/TC	335	33.77
	GT/TG	156	15.73
Total		992 (98.90%)	
Tri-nucleotide			
	AAC/ACA/CAA	1	9.09
	AGC/GCA/CAG	1	9.09
	CCT/CTC/TCC	2	18.18
	CGT/GTC/TCG	1	9.09
	CTG/TGC/GCT	4	36.36
	CTT/TTC/TCT	2	18.18
Total		11 (1.10%)	
Total Repeat motifs		1003	