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Development of EST-SSR and genomic-SSR markers to assess genetic diversity in *Liriope*

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맥문동속의 유전다양성 분석을 위한 DNA 마커개발

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Objectives

Liriope and *Ophiopogon* distributed in subtropical and temperate regions, Korea, China, Japan, and India. The two genera are closely related and vegetatively similar- the most significant differences are in floral morphology. Both are now placed in the Ruscaceae. *L. platyphylla* produces steroidal glycosides in tubers which are used as expectorants and tonic in herbal medicine and making tea in China and Korea. However, the understanding of this medicine plant remains very limited and little genomic research has been done. We used EST-SSRs and G-SSRs to analyze the genetic relationships among 68 accessions of *Liriope* and *Ophiopogon*.

Materials and Methods

○ Materials

Sixty-eight accessions representing four species, *Liriope platyphylla* (53), *Liriope spicata* (9), *Ophiopogon japonicus* (4), *Ophiopogon jaburan* (2). The two *Liriope spicata* accessions from Sichuan, Zhejiang province, China and the other materials were obtained from National Institute of Horticulture & Herbal Science, Rural Development Administration.

○ Methods

Ophiopogon EST sequences were obtained via the ENTREZ search tool of the EST database at the NCBI. The SSR-ESTs were reassembled using CAP3 program, and primers pairs were designed from SSR flanking sequences with the help of PRIMER 3. The variability at each locus was measured in terms of the number of alleles, heterozygosity (H), major allele frequency (M_{AF}), Gene diversity (GD), and polymorphic information content (PIC). These variables were measured by calculating the shared allele frequencies using PowerMark 3.25. The UPGMA algorithm was used to construct an unrooted phylogram from a distance matrix using MEGA4 software embedded in PowerMarker. The genetic distance between each pairwas determined using POPGENE Version 1.31.

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Results

In total, 20 EST-SSRs and 5 G-SSRs polymorphic markers were developed among the *Liriope* accessions. The EST-SSR markers comprised 56.2% di-nucleotide repeat, 41.6% trinucleotide repeats, 1.1% tetra-nucleotide repeats. The 20 EST-SSRs resided in genes that involved mainly in biological and metabolic processes. A total of 78 polymorphic alleles were detected with an average of 3.12 per locus. The average heterozygosity was 0.61; the mean genetic diversity and polymorphic information content were 0.47 and 0.38, respectively. We identified 25 EST-SSR and G-SSR in *Liriope*, which will be useful tools for genotype identification, germplasm conservation, molecular breeding, and assessments of genetic diversity and population structure of *Liriope.*

Table 1. The list of newly developed simple sequence repeats markers in this study.

Primer name	NA[a]	M_{AF}[b]	GD[c]	HE[d]	PIC[e]
KNU-L08	5	0.63	0.48	0.72	0.39
KNU-L12	4	0.50	0.59	0.89	0.51
KNU-ON1	3	0.48	0.55	1.00	0.44
KNU-ON2	2	0.51	0.50	0.98	0.37
KNU-ON3	2	0.52	0.50	0.96	0.37
KNU-L26	3	0.43	0.61	0.85	0.53
KNU-L39	3	0.50	0.51	1.00	0.39
KNU-L45	5	0.40	0.66	0.76	0.58
KNU-L31	3	0.57	0.50	0.08	0.38
KNU-L34	5	0.50	0.58	1.00	0.49
KNU-L36	2	0.95	0.10	0.07	0.09
KNU-L37	2	0.95	0.10	0.10	0.09
KNU-L39	4	0.50	0.52	1.00	0.41
KNU-L40	5	0.44	0.66	0.22	0.59
KNU-L43	5	0.76	0.40	0.04	0.38
KNU-L44	4	0.68	0.46	0.46	0.39
KNU-L45	4	0.54	0.63	0.38	0.58
KNU-L46	2	0.56	0.49	0.03	0.37
KNU-L53	2	0.93	0.12	0.04	0.12
KNU-L55	2	0.63	0.47	0.74	0.36
KNU-L56	2	0.59	0.48	0.81	0.37
KNU-L57	3	0.50	0.53	1.00	0.43
KNU-L67	2	0.57	0.49	0.86	0.37
KNU-L68	2	0.60	0.48	0.79	0.36
KNU-L72	2	0.84	0.27	0.33	0.24
mean	3.12	0.61	0.47	0.61	0.38

[a]: Number of alleles, [b]: Major allele frequency,
[c]: Gene diversity, [d]: Expected heterozygosity
[e]: Polymorphism information content

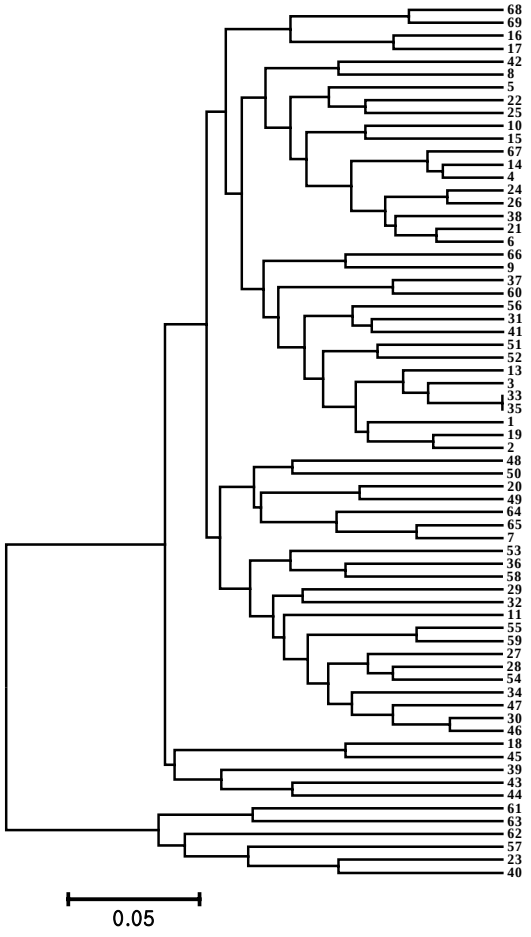

Fig. 1. UPGMA dendrogram showing phylogenic relationships among the 68 *Liriope* accessions.