

# Purity Test Using SSR Markers in Self-fertilized Progenies of *Codonopsis lanceolata*

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## ABSTRACT

**Background:** Creation of pure lines is one of the basic manipulations for crop breeding. *Codonopsis lanceolata* is known to be a cross-fertilizing plant. In order to breed allogamous plants, it is necessary to confirm that the self-fertilized line is a genetically fixed pure line. This study was carried out to confirm the genetic purity of the self-fertilized lines of *Codonopsis lanceolata* using SSR (simple sequence repeats) markers.

**Methods and Results:** In order to develop a new variety of *Codonopsis lanceolata*, a pure lines were created by self-fertilization. Genetic analysis was performed using SSR markers to confirm whether genetic purity increased in the pure lines created. Among the previously developed markers, 8 SSR markers showing high polymorphism were selected and applied to this study. We obtained clearly amplified bands using 8 SSR markers from female line (FL) and self-fertilized line (SL). The polymorphisms of all samples were analyzed by GeneScanTM<sup>500</sup> LIZ<sup>®</sup> size standard. The mean values of genotype number ( $G_N=1.75$ ), number of alleles per locus ( $N_A=1.5$ ), expected heterozygosity ( $H_E=0.22$ ), observed heterozygosity ( $H_O=0.24$ ) and polymorphic information content (PIC=0.17) in SL were lower than those in FL ( $G_N=7.375$ ,  $N_A=4.5$ ,  $H_E=0.62$ ,  $H_O=0.48$ , PIC=0.55). These results indicate that the genetic purity of SL is higher than that of FL.

**Conclusion:** Our study showed that genetic analysis with molecular marker is useful tool to test purity of self-fertilized lines, suggesting that further phenotypic analysis combination with genetic analysis will be helpful in improving breeding efficiency for developing varieties of *Codonopsis lanceolata*.

## MATERIALS AND METHODS

### 1. Plant materials

To create a self-fertilizing line (SL), artificial crosses were performed using a landrace as a female line (FL). In this experiment, purity test was performed in the second generation of self-fertilization. Leaves from 20 plants of each FL and SL were collected and used for SSR marker analysis.

### 2. Data analysis

To analysis the SSR markers, the PCR amplified bands were scored based on presence/absence/miss (1/0/9). Diversity values of loci in FL and SL were calculated using PowerMarker software (version 3.25) for the following indices: major allele frequency ( $M_{AF}$ ), genotype number ( $G_N$ ), the number of alleles per locus ( $N_A$ ), expected heterozygosity ( $H_E$ ), observed heterozygosity ( $H_O$ ), and polymorphic information content (PIC).

## RESULTS

**Table 1.** Primer sequences of SSR markers used in this

No.	Name	Sequence	Fragment size
1	CL-SSR-2	F : PET-CACCACTCAATCATGCAAGC R : GTTTGACGCAGRRGCAGAAAAGAA	179-201
2	CL-SSR-14	F : VIC-CCACTGGAACAAAGATTACGG R : GTTTCATGGAATTTTCATCGACAAGA	185-188
3	YL-CL-0151	F : CTGTGTGTGCTGCTTGAGTG R : GCTGGTCTGTTGGATACG	184-190
4	YL-CL-0153	F : GAAGTGCTTGATCCATAGGC R : TCATGTGGGCTATCTTCACC	157-197
5	YL-CL-0795	F : GCCCTAAATGTCAACCCAC R : GATGATGATGGCTTTGGC	161-197
6	YL-CL-1056	F : GGCAAATGATCACCAC R : CCTCTGAGACTCGTAAATC	168-183
7	YL-CL-2461	F : CTGGAACAGCTTTTACCTG R : CTGGATGCCTAGAGGTACCATAC	148-188
8	YL-CL-2596	F : GAATTCGGAAATGGCTGC R : AGCAGACAAATACCTGGGTG	166-196

**Table 2.** Genetic diversity statistics for 8 SSR markers in female line (FL) individuals

Marker	$M_{AF}$	$G_N$	$N_A$	$H_E$	$H_O$	PIC
CLSSR2	0.33	10	5	0.77	0.6	0.73
CLSSR14	0.5	4	3	0.58	0.3	0.49
YL-CLtri0151	0.6	6	4	0.55	0.35	0.48
YL-CLtri0153	0.43	10	5	0.69	0.45	0.64
YL-CLtri0795	0.58	6	4	0.54	0.6	0.46
YL-CLtri1056	0.63	6	4	0.53	0.55	0.46
YL-CLtri2461	0.63	5	4	0.5	0.4	0.42
YL-CLtri2596	0.3	12	7	0.78	0.6	0.75
Mean	0.5	7.375	4.5	0.62	0.48	0.55

**Table 2.** Genetic diversity statistics for 8 SSR markers in self-fertilizing line (SL) individuals

Marker	$M_{AF}$	$G_N$	$N_A$	$H_E$	$H_O$	PIC
CLSSR2	0.8	2	2	0.32	0	0.27
CLSSR14	0.6	3	2	0.48	0.6	0.36
YL-CLtri0151	1	1	1	0	0	0
YL-CLtri0153	1	1	1	0	0	0
YL-CLtri0795	0.58	2	2	0.49	0.85	0.37
YL-CLtri1056	1	1	1	0	0	0
YL-CLtri2461	0.53	3	2	0.5	0.45	0.37
YL-CLtri2596	1	1	1	0	0	0
Mean	0.81	1.75	1.5	0.22	0.24	0.17

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