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Development of STS-CAPS marker for the identification of Korean ginseng cultivars (*Panax ginseng* C. A. Meyer)

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Objectives

We have recently collected ninety nine clones from genomic DNA libraries of *P. ginseng* cv. Yunpoong. These libraries were constructed, in which clone inserts were derived from the hypomethylated regions of Korean ginseng genome. A total of ninety nine STS primer sets was designed from clones of gDNA libraries. Amplification products were tested for latent polymorphism across seven cultivars of *P. ginseng* using six endonucleases recognizing four-bases. STS-CAPS marker sets described here will be useful for marker-assisted selection, genome mapping and cultivar discrimination of Korean ginseng.

Materials and Methods

○ Preparation of samples

Korean ginseng cultivars; Chunpoong, Yunpoong, Gopoong, Kumpoong, Sunpoong, Sunwoon, Sunwon.

○ DNA extraction

Total genomic DNAs were extracted from fresh leaves of each *P. ginseng* cultivars by using Dneasy Plant Mini Kit (QIAGEN, Germany). The concentration of DNA was then determined based on a comparison of the plant DNA samples with commercial standard lambda DNA on 1.5% (w/v) agarose gel.

○ PCR amplification with STS primers

STS-PCR was performed in total 25μl reaction volume containing 5ng of DNA template, 20pmole of primer, 2.5mM of $MgCl_2$, 0.25mM of dNTPs, and 0.5U of Taq

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polymerase (Neurotics, Deajeon, Korea). The reaction conditions for PCR consisted of initial denaturation at 94℃ for 5min, followed by 40cycles of amplification at 94℃ for 30sec, annealing at 65, extension at 72℃ for 1min , and a final extension at 72℃ for 7min. PCR was conducted by using T professional thermocycler (Biometra, Göttingen, Germany).

○ Restriction endonuclease treatment

we used several restriction endonucleases (*Alu*-I, *Hae*-Ⅲ, *Hinf*-I, *Rsa*-I, *Taq*-I, *Tsp*509-I) and analysed the digestion products by agarose gel electrophoresis. The fragments were stained with ethidium bromide solution and visualized on UV-transilluminator.

Results

In the CAPS analysis results, the majority of the cleaved PCR products also yielded additional latent polymorphisms among the Korean ginseng cultivars. Specific latent CAPS polymorphisms for cultivar Yunpoong, Sunwoon and Sunwon were detected from internal region amplified with KGY+274 primer by treating *Rsa*- I endonuclease [Fig. 1 (A)]. Chunpoong and Kumpoong were detected from internal region amplified with KGY+211 primer by treating *Rsa*- I endonuclease [Fig. 1 (B)].

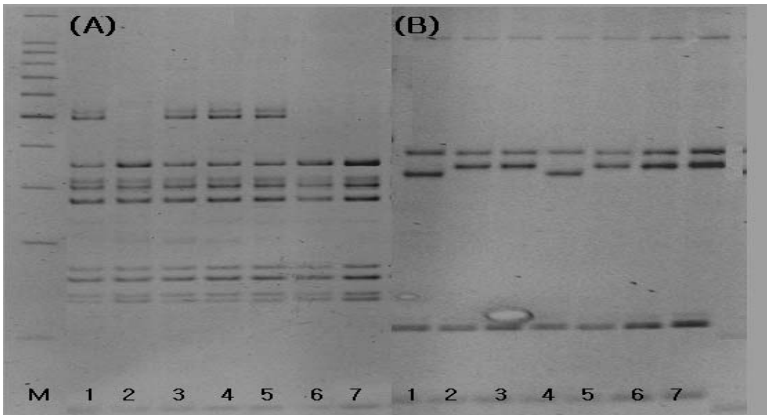

Fig. 1. PCR-RFLP patterns restricted by *Rsa*- I from seven Korean ginseng cultivars, (A) KGY+274 primer, (B) KGY+211 primer, lane 1, Chunpoong; lane 2, Yunpoong; lane 3, Gopoong; lane 4, Kumpoong; lane 5, Sunpoong; lane 6, Sunwoon; lane 7, Sunwon; M, 100bp molecular weight marker (Promega).