P-25

Molecular identification of Korean ginseng cultivars (*Panax ginseng* C. A. Meyer) using EST-SSR markers

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Objectives

Recently, new ginseng cultivars having superior agricultural traits have been developed in Korea. For newly developed plant cultivars, the identification of distinctiveness is very important factors not only in plant cultivar management but also in breeding programs. Thus, thirty EST-SSR primers were applied to detect polymorphisms among nine Korean ginseng cultivars and two foreign ginsengs.

Materials and Methods

○ Preparation of samples

Korean ginseng cultivars; Chunpoong, Yunpoong, Gopoong, Kumpoong, Sunpoong, Sunwoon, Sunwon, Chungsun and Sunhyang

Foreign ginsengs; *P. quinquefolius* and *P. notoginseng*.

○ DNA extraction

Total genomic DNAs were extracted from fresh leaves of 3 year old plants of each cultivars and foreign ginseng 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% (w/v) agarose gel, after which it was adjusted to 10 ng/μl.

○ PCR amplification with EST-SSR primers

PCR primers for EST-SSR analysis were obtained from Northeast Forestry University, Harbin (China). EST-SSR PCR was performed in total 30μl reaction volume containing 10ng of DNA template, 30 pmole of primer, 2.5 mM of $MgCl_2$, 0.25mM of dNTPs, and 0.5U of Taq polymerase (Neurotics, Deajeon, Korea). The reaction conditions for PCR consisted of initial denaturation at 95℃ for 3min, followed by 35 cycles of amplification at 95℃ for 30sec, annealing at 55, extension at 72℃ for 1min , and a final extension at 72℃ for 5min. PCR was conducted by using T professional thermocycler (Biometra, Göttingen,

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Germany). The PCR amplification
products were analyzed by electrophoresis on 8% polyacrylamide gels. The amplified DNA bands were stained with ethidium bromide solution and visualized on UV-transilluminator.

Results

Three species of *P. ginseng* including Korean ginseng cultivars, *P. quinquefolius*, and *P. notoginseng* discriminated most tested primers. P26, P35, P57 and P58 primers generated polymorphic bands among nine Korean ginseng cultivars, and could distinguish them from foreign ginsengs. In addition, the cultivar specific banding patterns of Kumpoong and Sunhyang were generated at P57 and P26 primers, respectively. These results will serve as useful DNA markers for identification of Korean ginseng, especially Kumpoong and Sunhyang cultivars, seed management, and molecular breeding program supplemented with marker-assisted selection.

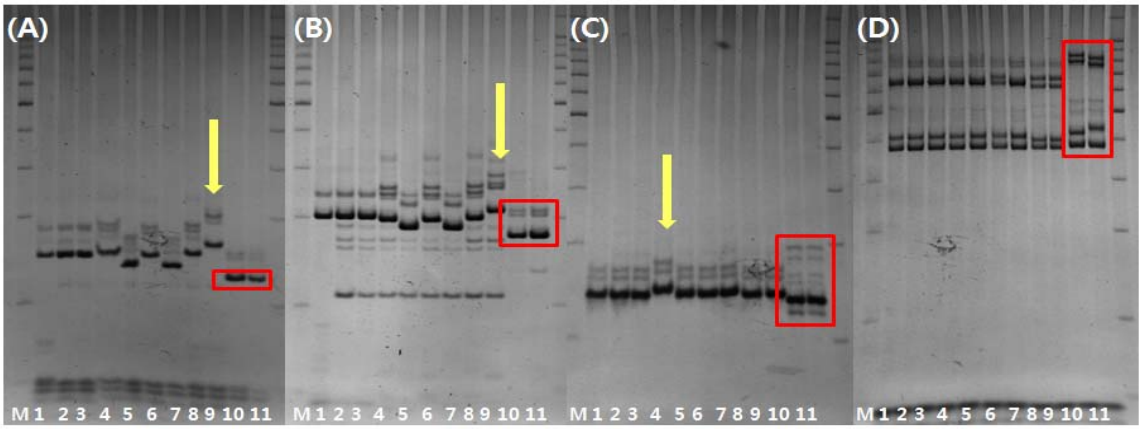

Fig. 1. Polymorphisms by the EST-SSR primers between Korean ginseng cultivars and foreign ginseng, (A) 'Sunhhyang'-specific polymorphism detected by P26 primer, (B) 'Sunhhyang'-specific polymorphism detected by P35 primer, (C) 'Kumpoong'-specific polymorphism detected by P57 primer, (D) Polymorphisms by the P58 primer between Korean ginseng cultivars and foreign ginseng, lane 1, Chunpoong; lane 2, Yunpoong; lane 3, Gopoong; lane 4, Kumpoong; lane 5, Sunpoong; lane 6, Sunwoon; lane 7, Sunwon; lane 8, Chungsun; lane 9, Sunhyang; lane 10, *P. quinquefolius*; lane 11, *P. notoginseng*; M, 100bp molecular weight marker (Promega).