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S-genotypes determining based on S-RNase PCR systems in Korean-bred apple cultivars

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Objectives

The aim of this study was to determine the S-genotypes of 8 Korean apple cultivars and 3 apple cultivars by molecular analyses.

Materials and Methods

1. Material

Plant - Eight Korean-bred apple cultivars and those parents (6 cultivars) were used in this study

2. Methods

Genomic DNA was isolated using the DNeasy Plant Mini Kit (QIAGEN, Germany) and we designed common primers (ASPF3 + ASPR3) based on the conserved sequence of apple 15 S-RNases. Using the primers, we performed PCR amplification. Amplified PCR products were cloned by the pGEM-T Easy (Promega, USA) Vector. The nucleotide sequences of several clones were determined by the ABI PRISM 377 DNA sequencer. And than DNA sequence were aligned manually by using GENETIX-WIN 5.0 and analyzedusing the BLAST programs for searching the homology among other nucleotide sequences. Southern blot analysis was performed according to a DIG application protocol. Furthermore, we performed S-allele-specific PCR analysis by specific primer pairs for the S3- and S5-RNases which were designed from the variable regions.

Results and Discussion

The S-RNase PCR fragments of 13 apple cultivars could be obtainedby new common primers. PCR fragments of known S-genotypes were compared. We supposed that the S-genotype of Hwahong is S3S9 and Hwarang is S1S9. However, the S-genotypes of 13 apple cultivars were not determined by amplifiedPCR fragments analysis only, because the S-genotypes of parental cultivars had not determined yet and PCR fragments of the same size could not be determined which one is S-RNase. Through cloning and sequencing of the amplified PCR fragments, we obtained seven S-alleles from 13 apple cultivars and determined the nucleotide sequences of exon and intron regions. The intron region of S2- and S9-RNase showed the high homology (89.2%). The S-genotypes of 8 Korean bred apple cultivars and those parents were determined by the results of PCR and sequence analyses Hongro (S1S3), Kamhong (S1S9), Saenara (S1S3), Chukwang (S3S9), Hwahong (S3S9), Seokwang (S3S5), Hwarang (S1S9), SunHwang (S3S9) S.E.B. (S1S19), S.G.D. (S2S3), Molis (S3S7). However, as a PCR results, S3- and S5-RNase fragments expressed as one band like Seokwang (S3S5). Therefore, we synthesized new S3- and S5-allele specific primers for amplification of S-allele specific fragments, and using as specific gene markers. Using these primers, we have succeeded to obtain S3- and S5-RNase specific fragments from 15 apple cultivars. Furthermore, we confirmed the characteristic of S-genotypes of 14 apple cultivars by PCR-Southern blot analysisusing S1-, S2-, S3-, S5-, S7-, S9- and S19-RNases as probes.

Results show that the molecular techniques are availablefor determination of S-genotypes of 8 Korean apple cultivars to select pollination trees and identifying the S-genotypes of progeny in breeding programs. Developed molecular markers can be used to determine theself-incompatibility genotypes of other apple varieties.

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