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Molecular characterization of two new S-RNases ('S_{31}' and 'S_{32}') in apple (*Malus domestica* Borkh.)

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Objectives

The objectives of this study were to identify and characterize the new S-RNases and develop systematic evaluation using the S-RNase specific PCR method.

Materials and Methods

1. Material

Plant - Eight apple cultivars were collected at Morioka Apple Research Center in Japan.

2. Methods

In this work, we designed new common primers (ASPF3 + ASPR3) based on the conserved sequence of 18 apple S-RNases. Also we designed new primers (ASR5'F1 + SAR3'R1) for amplification of ORF region of new S-RNases from 5'- and 3'-flanking regions. Amplified PCR products were subcloned using pGEM-T Easy Vector. The nucleotide sequences of several clones were determined by the ABI PRISM 377 DNA sequencer. Specific fragments of the new S-RNases (S31- and S32-RNases) were amplified by S-allele specific primers were designed from the variable regions.

Results and Discussion

We obtained two new S-RNases from apple cultivars viz.'York Imperial' and 'Burgundy' by new common primers. The accession numbers of apple S-alleles were elucidated by Broothaerts (2003) and Matsumoto et al. (2003). Among those accession numbers, two new S-RNases were designated as S31 of 'York Imperial' and S32 of 'Burgundy'. We also succeeded amplifying the ORF regions of the two new S-RNases. The amplified PCR fragments of ORF regions were cloned and sequenced. The size of S31-RNase ORF fragments is 958 bp and S32-RNase is 838 bp. Furthermore, the comparison of the deduced amino acid sequences of two S-RNases with 15 S-RNases of apple, we found that two new S-RNases contained two essential histidine residues for RNase activity and the eight conserved cysteine residues are specific to S-RNase in apple. We also compared the amino acid sequence similarity of the exon regions among 17 apple S-RNases. The new S31 show a high homology with S20 (94%), and the new S32 shows 58 (S24) to 76% (S25) homology. Also, the S-RNase genotypes of 'York Imperial' and 'Burgundy' apple cultivars were determined to be S2S31 and S20S32by PCR and sequence analyses. And we also succeeded in amplifying the S31- and S32-RNase specific fragments by the S31- and S32-allele specific primers, respectively.

The two new S-alleles have conserved eight cysteine residues, and two histidine residues essential for RNase activity. Moreover they showed high homology with other S-RNases of apple. Thus, we justified those as a new S-RNases. The two new S-RNases (S31: DQ135990, S32: DQ135991) were updated to GenBank (NCBI). This database information is useful to find new self-incompatibility types and to contribute to breeding system. The developed methods are applicable to select pollinator for improvement of pollination effect and production of qualified fruit.

However, the other molecular markers have been developed because the S-genotypes are not fully identified. Also, self-incompatibility is not only controlled by pistil S-genes. Thus we have to identify pollen S-gene for understanding the self-incompatibility mechanism and application.

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