CHARACTERIZATION OF LENGTH POLYMORPHISMS AT THE 3' UTR OF THE MALOIDEAE S-RNASE AND ITS APPLICATION FOR *S*-GENOTYPING IN EUROPEAN PEAR

Javier Sanzol
Unidad de Fruticultura, CITA de Aragón, Apartado 727, 50080 Zaragoza, Spain

Length polymorphisms are especially suited for genotyping purposes since multiple allelic variants can be evaluated with a single PCR reaction. In fruit tree species of the Maloideae subfamily, the variation in length exhibited by the single intron of the S-RNase gene has been widely exploited for *S*-genotyping. However, the distribution of the intron length frequencies within this gene subfamily strongly deviates toward a minimal intron size of approximately 150 (±10) bp, which often hampers the discrimination of a large number of alleles by conventional agarose gel electrophoresis. In this study, full length cDNA sequences including the 3' and 5' UTRs, corresponding to eleven apple and seven Japanese pear *S*-RNase alleles have been analysed. The alignment of these sequences has revealed a region at the 3' UTR highly variable in sequence and length, flanked upstream by the protein coding sequence and a short stretch of conserved nucleotide positions downstream. The convenience of this region for S-genotyping has been evaluated by analysing length polymorphisms in European pear cultivars with known *S*-RNase alleles, after genomic PCR using a pair of consensus primers flanking the variable region. Overall, the proportion of alleles amplified is lower than with consensus primers commonly used to amplify the intron containing region. However, instance of alleles which are undistinguishable based on intron size variation but are polymorphic at this new region has been detected, making promising its application for S-genotyping. Moreover, the characterization of new product sizes in a group of European pear cultivars with unknown S-genotypes has allowed identifying three still unreported S-RNase alleles. At present, the applicability of this new approach for routinely S-genotyping in European pear is being explored. Resolving two main drawbacks will be needed, which are: lack of amplification for a substantial number of S-alleles and, multiple secondary amplifications maybe due to the repetitive nature of the conserved sequence used for designing the reverse primer at the 3'UTR.