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Making RNAi mutants of apple with high-efficiency by use of multi-vector transformation.

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To facilitate high-efficiency generation of RNAi-mutants for determination of function of candidate genes in resistance of apple to Erwinia amylovora (fire blight), the M.26 apple genotype was transformed with a mixture of five RNAi EST-silencing vectors in each transformation experiment to allow selection of up to five types of mutants from a single experiment. ESTs associated with response to E. amylovora, identified by bioinformatics analysis, were used to create RNAi-silencing constructs. These constructs were transferred to Agrobacterium tumefaciens strain EHA105pCH32. The five transformed A. tumefaciens strains were mixed, and the mixture used to transform leaf-slice explants. Regenerants were selected on M.26 regeneration medium with 100 mg/L kanamycin, and screened by PCR using universal primers to determine integration of a silencing construct. In most lines PCR showed only single genes had been inserted. Because amplicons from some transgenics co migrated, to better determine the identity of the ESTs contained in the silencing-insertion, the PCR fragments were cut with 4-cutter restriction enzymes. Thus far ESTs from genes in six functional categories, general metabolism (1), photosynthesis (2), nucleic acid metabolism (1), protein metabolism (3), signaling (4), and defense/stress (4), have been subjected to this protocol. Young plantlets will be inoculated with E. amylovora to assayed phenotype reaction.
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