

IMPROVE APPLE SCAB RESISTANCE VIA CISGENIC APPROACH

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Apple scab is one of the most common and most serious diseases that effects apple trees worldwide, caused by *Venturia inaequalis* which can be reduced by unique resistance genes. At present, we are cloning several apple scab genes. One of them, the apple scab Rvi 12 (Vb), originating from Siberian crab apple 'Hansen's baccata #2 has been mapped previously and a candidate gene, named Rvi12_Cd5, belonging to LRR receptor-like serine/threonine-protein kinase family was identified. In this study, the DNA sequence of native promoter was extended and characterized by in-silico analysis which showed homology with major defense related transcription factor binding sites such as ERF, bZIP, bHLH, MYBs, NAC and WRKY. The coding sequence of Rvi12_Cd5 controlled by the native promoter and 35S promoter along the selectable marker gene nptII was introduced by agrobacterium-mediated transformation in the apple scab susceptible 'Gala' cultivar. Successful 45 independent transgenic lines were characterized for copy number and transcription of the candidate resistance gene. The transformed lines with 1 or 2 integrations, expressing the candidate gene were acclimatized to the greenhouse for scab inoculations. The phenotypic characterization showed significant reduction in scab susceptibility in several transformed lines compared to control 'Gala' plants. Additionally, the NGS method was performed for the identification of T-DNA integration points in transformed 'lines carrying the vector with the native promoter, resulting in 5 chromosomal positions in the apple genome. Moreover, these plants were subjected to T-DNA removal using the heat-shock inducible FLP/FRT recombination system and confirmed by quantifying marker gene nptII. The real-time PCR showed complete exogenous DNA elimination in several independent lines. Currently, these results are being validated by

sequencing.