Malus genome editing via CRISPR/Cas9 to develop sustainable and pest free apples

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Background

Apple is one of the ten most valuable crops worldwide with a total production of about 84.6 million tons per year

Pathogens causing powdery mildew, fire blight, and scab represent serious threats to apple cultivation

The control of these pathogens requires pesticides with a negative impact on the environment and human health

Suppression of susceptibility genes (S-genes) could confer durable and sustainable resistance to apples but past attempts to mutate S-genes mostly relied on transgenic techniques

Limitations to the cultivation of transgenic plants are present in many countries and these plants are scarcely accepted by customers

CRISPR/Cas9-mediated genome editing can be used to target multiple genes with both DNA-based and DNA-free approaches

Robust protoplast-to-plant regeneration protocols are not available for apple

Inducible recombination systems can be couple with the CRISPR/Cas9 system to eliminate editing components from edited plants

Project objectives

Develop multi-resistant apples via CRISPR/Cas9-mediated knockout of S-genes

Minimize the amount of non-apple DNA left in the edited plant genome by combining CRISPR/Cas-9 to different DNA delivery systems and post-editing recombination-based methods

Develop a protoplast-to-plant regeneration procedure to use DNA-free methods for genome editing

Materials and Methods

1. **Preparation and delivery of the genome-editing cassette**

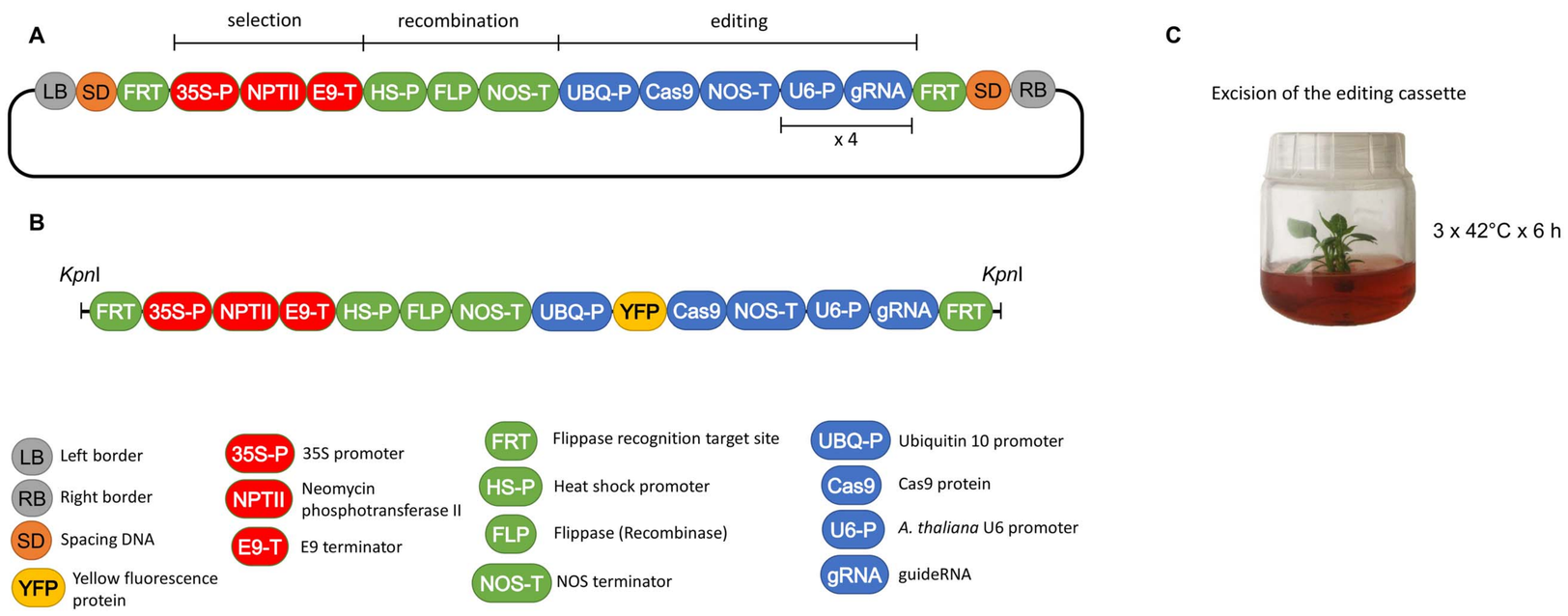
Single guide RNAs to target 4 known S-genes are designed using CRISPOR tool and inserted between AtU6 promoter and scaffold gRNA sequences. guideRNAs, Cas9, flippase (Flp), kanamycin expression cassettes are assembled between Flp recombination sites. YFP expressing cassette is added to the 5' of Cas9 to monitor and optimize the delivery of editing components to plants. *Agrobacterium tumefaciens* and the particle gun system are used to deliver the editing cassette to plant leaves and/or callus. Different conditions for particle gun delivery are tested

2. Optimization of the delivery of plasmid DNA to apple leaves

Plasmids harboring the 35S::GUS and 35S::YFP expressing cassettes are delivered to plant cells to analyze transformation efficiency and YFP functionality, respectively. 35S::GUS is delivered with different parameters, bombarded leaves are GUS-stained one day after transformation and GUS expressing cells are counted with a binocular

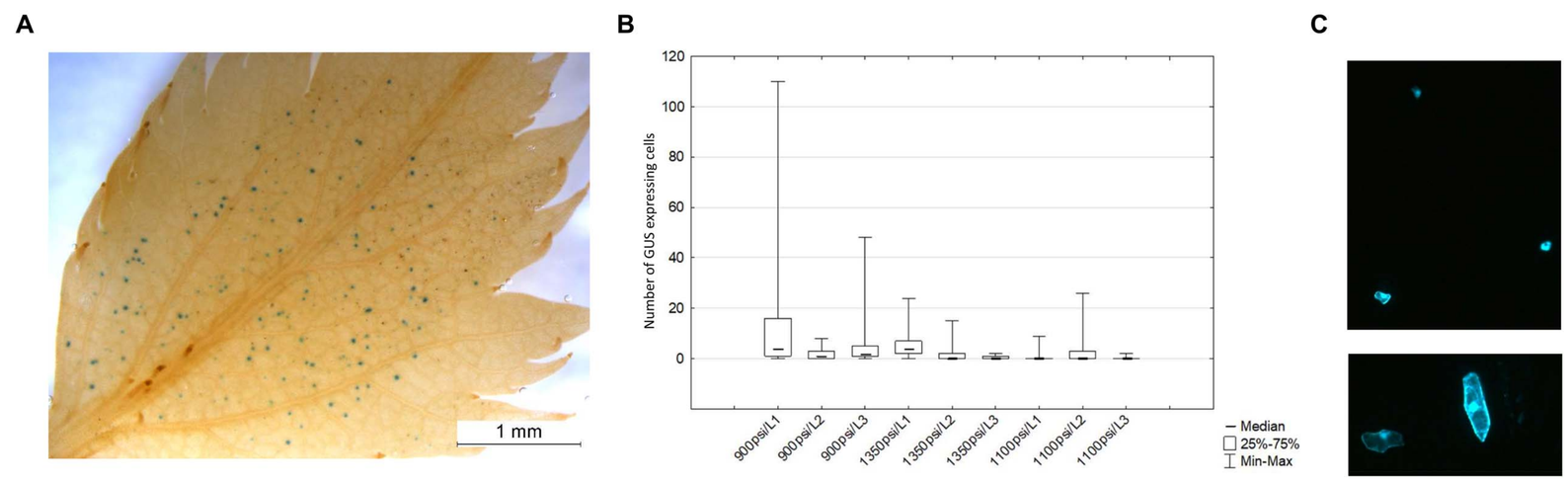
Preliminary Results

Constructs for genome editing and delivery to plant cells



(A) The CRISPR/Cas9 genome editing and recombination components are combined in a vector (see Materials and Methods 1) which is delivered to apple leaf strips by *A. tumefaciens*. The vector contains 4 gRNA expressing units to target DIPM1, DIPM4, HIPM and Mlo genes. After transformation leaf strips are transferred on MS plates supplemented with kanamycin and hormones for regeneration. (B) Alternatively, a linearized fragment of the editing vector containing only the sequence between FRT sites is delivered to cells via particle gun. Here, the coding sequence of YFP was fused at the 5' of the Cas9 to check for successful delivery and to monitor transformation efficiency. (C) Elimination of the editing components from the genome of edited plants is induced by the activation of the expression of the Flp gene using heat shock treatments

Conditions for biolistic transformation



Transformation conditions for the delivery of the editing DNA were optimized to increase the chance of regeneration. (A) Example of an apple leaf transformed with the 35S::GUS construct. (B) Box plot representing the number of GUS expressing cells obtained using different transformation parameters. Both the distance and type of rupture disc used influenced the number of transformed cells. (C) Examples of apple cells expressing YFP

Expected Results and Future Perspectives

Kanamycin-resistant regenerating shoots from *Agro-* and particle gun-mediated transformations will be analyzed for the presence of the excisable editing cassette. The genome sequence at the editing sites of the positive plants will be analyzed by Sanger and Illumina sequencing to check for effective editing of target genes. Copy number of the editing cassette will be determined by RT-qPCR and plants with single insertions will be submitted to heat shock treatments. Cassette-free edited plants will be scored for disease resistance and genetic stability

Available and new methods for protoplast preparation and regeneration will be tested using apple leaves and callus to establish a protoplast-to-plant regeneration procedure

References

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