Employing of CRISPR / Cas9 technology to knock out genes associated with flowering in aspen

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Populus tremula L. (Aspen) is a tree crop that is used in urban landscaping and forestry. Aspen is of great interest because of its high growth rate, and to improve its economic qualities, , it is profitable to create and further cultivate trees of this species improved by gene editing techniques. there are restrictions in the use of genetically edited plants due to the fear of horizontal gene transfer from the edited plants into natural ecosystems. To overcome these limitations and to test gene editing techniques on woody plants, a CRISPR/Cas9 system will be used to knock out aspen flowering-related genes.

The aim of the study

Create aspen plants with the sterility trait fixed in the genome.

Materials and methods

Callus cultures of *Populus tremula* will be used as a target. Callus cultures will be treated with the bioballistic method.

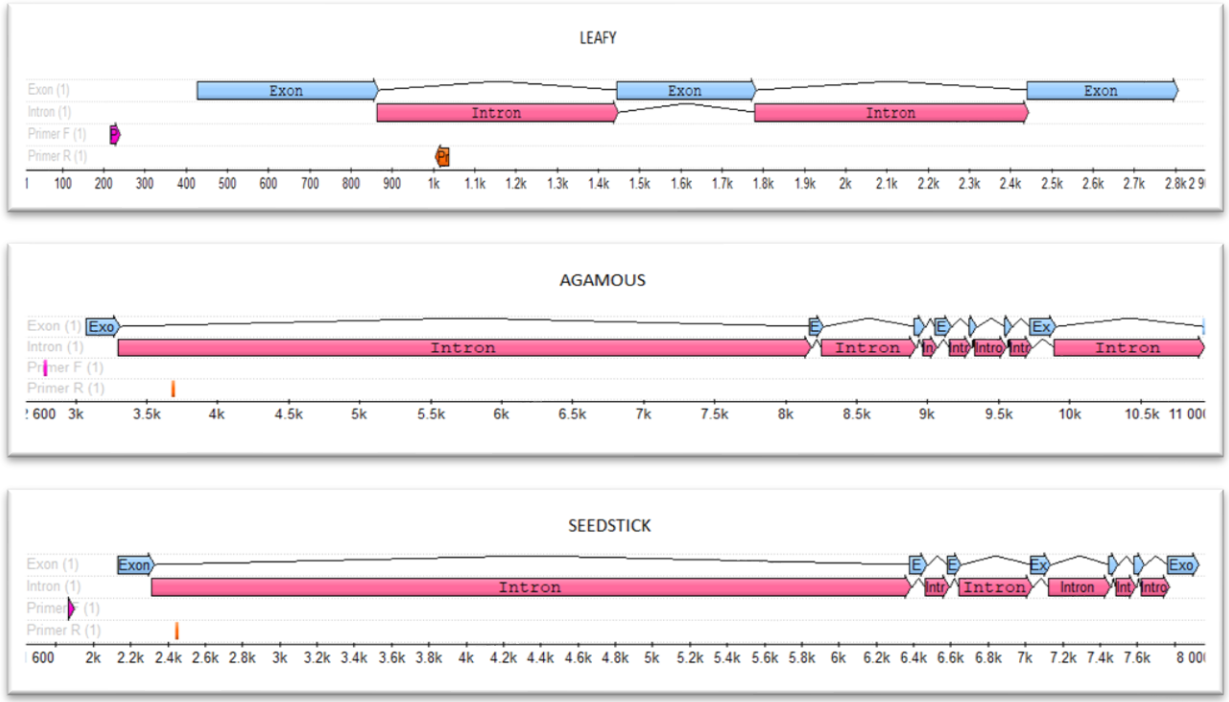
Three genes related to flowering were selected as target genes: *LEAFY, AGAMOUS* and *SEEDSTICK.*

LEAFY gene encodes a transcription factor that triggers the cascade of major genes responsible for cell differentiation into floral meristem.

The *AGAMOUS* gene belongs to the family of genes encoding the MADS-box transcription factor involved in the regulation of stamens, carpels, and ovules formation (group C genes according to the ABCDE model of floral organ development).

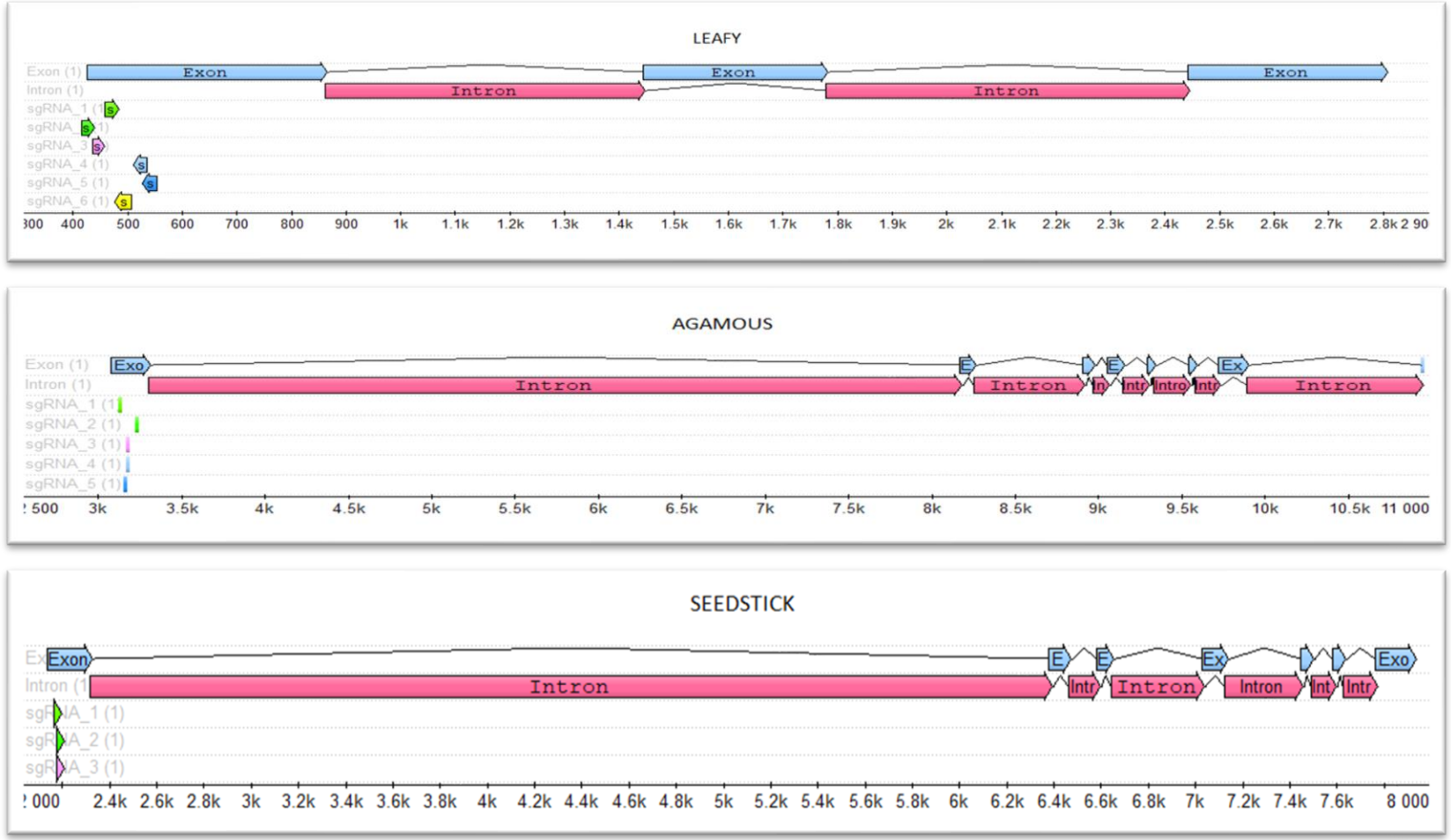
The *SEEDSTICK* gene belongs to the group D genes that regulate ovule formation.

Three primer pairs were created to study the target genes and select the most efficient CRISPR/Cas9 sgRNA.



Construction of sgRNAs.

In the course of our work, we created *in silico* 36 sgRNAs aimed at editing the first exon of each of the three target genes. In the course of laboratory experiments, the most efficient guide (hyde RNAs) will be selected among them through restriction of the PCR product by Cas9 nuclease. The locations of some of the sgRNAs are shown in the diagrams.



Conclusions

These guides will be used to edit aspen genes in callus cultures using the bioballistic method. Sterile regenerates will be obtained from *in vitro* callus cultures, which will serve as the basis for work in aspen gene editing to order highly productive sterile clonal aspen plantations.

Thank you for your attention!

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