walnuts.org

GENOME EDITING TOOLS FOR WALNUT IMPROVEMENT

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Objectives

To develop and implement a genome editing toolbox for the improvement of walnut scion and rootstocks.

1. Improve the functional analysis and mapping of traits in the walnut genome.
2. Improve the genome editing and recovery of edited walnut somatic embryos without the stable incorporation of transgenes and/or editing components.
3. Field-testing of trees obtained from edited somatic embryos.

Background

The growing walnut industry relies on new walnut cultivars resistant to disease, pest, and/or various environmental stressors while maintaining their productivity, quality, and profitability. Development of new cultivars can take decades, and new biotechnological tools can help reduce this period, as well as perform precise genetic modifications without changing other desired traits already present in a cultivar.

We have recently demonstrated the CRISPR-Cas9 method on a 'Chandler' cultivar, in which we targeted a specific gene for inactivation. We are now expanding the functionality by developing a method to target multiple genes simultaneously. Another application will be the removal of a whole gene sequence instead of simply a few DNA bases, enabling the removal of transgenes for example. For this method to work properly, knowledge of the whole genome sequence is necessary, a milestone also recently achieved for walnut research. It is now possible to target any region in the genome with high precision while avoiding unwanted modifications in similar regions.

To test the performance of gene-edited cultivars in the field is also a challenge that can take many years. To expedite this process we are also developing propagation methods to reproduce the individuals vegetatively. The walnut microshoots can be used to generate clonal plantlets for field planting, in addition to serving as a quicker starting point for disease resistance evaluation at a very early stage. Taken together, these approaches are enabling a quicker and more precise path from gene discovery to commercial application, aggregating value to the walnut industry.

Results & Discussion

To make the best use of the walnut genomic resources, we are creating the Walnut Genomics Knowledge Base (JuglansGKB), accessible online and hosted on a UC Davis server. Private access to data not yet made public will also be available. JuglansGKB will host data generated in different projects as well as provide a set of tools

to help in data analysis and visualization. We believe this will help the discovery process of genetic traits of interest that can then be tested with gene-editing methods and validated. We are building the database in a modular structure, the first of which is the Genome Browser. This module will display the 16 chromosomes and let users zoom in at different levels, as well as display the chromosomal positions of a list of genes provided by the user. We are also developing the webpage structure for the individual “Gene Cards” that will gather all available information for any given gene/protein in the walnut genome.

Knowledge of the genomic sequence of an organism is just the initial step in learning how it responds to different conditions. Understanding the subset of genes active under certain conditions or tissues is a common procedure in the field known as Functional Genomics. The JuglansGKB module for gene expression analysis will help users find the biomarkers that best describe the differences among different conditions, such as nut maturity, tissue type, disease state, and many others to come. The tools for these analyses are already available, and we will bring them together in a convenient and user-friendly interface in the database. Another module we are planning will be useful for the gene-editing projects, with tools for RNAi and CRISPR-Cas9 analyses. This will help us standardize procedures and visualize potential off-targets.

A third front we are working on is the improvement of tissue culture methods to propagate plant material of high value. Gene-edited embryos can be propagated in vitro and germinated into microshoots. These can be clonally propagated and then rooted in an aeroponic system to generate plantlets that can be potted. After a few months of growth in the greenhouse the edited plantlets are ready to be transplanted to the field.