



# TRANSLATING GENOME EDITING TOOLS FOR WALNUT IMPROVEMENT

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## PROJECT OBJECTIVES:

1. To develop and implement a genome editing toolbox for the improvement of walnut scion and rootstocks:
2. Develop and deploy genomic tools to enhance the functional analysis of traits encoding resilience to disease, pests and environmental stressors.
3. Deploy transiently expressed and DNA free genome editing tools to recover edited walnut somatic embryos that are resilient to disease, pests and oxidative stress.
4. Functional validation of edited plants for resilience to disease, pests and oxidative stress.

## BACKGROUND

There is a strategic need to develop new walnut varieties resistant to disease, pest and/or various environmental stressors while maintaining their productivity, quality and profitability. Improving walnuts via traditional breeding methods can benefit from new tools and approaches. Recently developed genome editing tools have been adapted to enhance the efficiency of traditional breeding efforts in walnuts. This proposal addressed the following high priority PRAC research objectives. 1) Develop tools to enhance the efficiency of classical breeding efforts. 2) Molecular genetic characterization of products of the walnut genome to help better diagnose problems and to define traits. 3) Translate genomics and genetics to develop new treatments and cultural practices and 4) Develop genomics information that will assist both scion and rootstock development.

## KEY FINDINGS

- New additions were made to the web-based knowledge base (JuglansKB) to visualize and interpret walnut genomics data.
- Progress was made in genome sequencing of phased genomes, in which both chromosome sets are resolved. This year we added the phased genomes of rootstocks 'J1 Paradox' and 'RX1', as well as the red pigmented walnut 'Robert Livermore'.

- We have advanced development of genome editing tools, specifically based on the CRISPR system, aimed at precisely altering the walnut gene PPO2. This gene is responsible for important traits like disease resistance and environmental stress tolerance. PPO2 is a biomarker of early abiotic or biotic stresses such as blight, crown gall, root and crown rot and nematode infections, waterlogging, and drought stress, holding immense value for growers. Detecting stress at an early stage can play a crucial role in disease and pest management. Stressed trees tend to be more susceptible to a range of pathogens and pest invasions. Identifying stress using biomarkers enables timely interventions that effectively prevent or control these issues. Integrating these innovative tools and strategies into conventional breeding programs strengthens walnut breeders to amplify the efficiency and accuracy in developing improved varieties with desired traits. This integration significantly contributes to the sustainability and profitability of the walnut industry.