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NEMATODE-RESISTANT WALNUT ROOTSTOCKS: BIOTECHNOLOGICAL SOLUTIONS FOR A SUSTAINABLE CALIFORNIA WALNUT INDUSTRY

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Project status in 2024: Year 1 of 1

PROJECT OBJECTIVES:

1. Generate a high-quality genome assembly of Pratylenchus vulnus to identify RNAi targets.
2. Develop and optimize soaking-based RNAi techniques to evaluate candidate genes for nematode control.

BACKGROUND

Walnut orchards in California face severe damage from the root lesion nematode (Pratylenchus vulnus), a migratory parasite that feeds on roots, causing cellular damage, reduced tree growth, and diminished yields. Once established, nematode populations persist in the soil, making management extremely challenging. Current control strategies, such as fumigation, are becoming less viable due to regulatory restrictions, and no commercially available walnut rootstocks offer true nematode resistance. RNA interference (RNAi) provides a promising alternative by silencing genes critical to nematode survival and parasitism. High-quality genome assemblies are essential for identifying target genes, offering insights into key pathways involved in nematode motility, feeding, and reproduction. This project aims to use RNAi to combat P. vulnus infestations, with the goal of implementing Host-Induced Gene Silencing (HIGS), a sustainable strategy where walnut trees produce RNA molecules targeting nematode genes directly.

KEY FINDINGS

This year, we achieved a major milestone by assembling the first chromosome-scale genome for P. vulnus. The genome spans 62 Mb, organized into six chromosomes, and provides a foundational resource for understanding nematode biology. Using computational analyses, we identified 13 critical genes associated with essential pathways, such as cell wall degradation and motility. To ensure specificity and avoid unintended effects, we selected gene regions with no homology to non-target organisms, minimizing the risk of off-target silencing. Preliminary RNAi experiments are now underway. Nematodes are being soaked in double-stranded RNA solutions to silence expression of these 13 genes, and infection assays will soon evaluate the effects of gene silencing on nematode survival and ability to infect roots. These efforts lay the groundwork for transitioning to HIGS, enabling walnut trees to combat nematode infestations autonomously.

In parallel, we optimized a rapid nematode infection assay to evaluate resistance traits in walnut rootstocks. This assay quantifies infection rates as nematodes per milligram of root tissue in small growth tubes, providing a reliable method for assessing susceptibility. Among the rootstocks tested, AD317 +demonstrated enhanced resistance, with significantly lower nematode counts compared to commercially available rootstocks like VX211 and Grizzly.