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DEVELOPMENT OF A WALNUT BLIGHT PROTECTING ROOTSTOCK

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PROJECT OBJECTIVES:

1. The overall objective of this research is to reduce/eliminate walnut blight disease in all susceptible cultivars by grafting to an engineered Paradox rootstock that secretes PGIP and suppresses crown gall disease. This will be accomplished by:
2. Develop an in vitro shoot assay to validate PGIP expression and walnut blight disease suppression.
3. Develop Paradox walnut shoot lines that efficiently secrete PGIP and that suppress crown gall disease.

BACKGROUND

Walnut Blight (WB) caused by X. arboricola pv. juglandis (Xaj) is the most serious above ground disease affecting all scion cultivars of walnuts reducing their productivity by up to 40% especially in wet years. This pathogen secretes a polygalacturonase (PG) enzyme that it uses to break down the protective plant cell wall barriers leading to a dampening of the plant counteractive defense response and successful colonization of the pathogen. We have previously shown that plants can defend themselves via the expression of a polygalacturonase inhibitory protein (PGIP) that successfully block the activity of the pathogen PG preventing colonization and the development of Pierce's Disease in grapevines. We are developing a crown gall suppressing (CGS) rootstocks that overexpress PGIP protein such that it is secreted into the xylem enabling it to traverse past the graft union accumulating in scion tissues to block pathogen secreted PG thus protecting the scion cultivar from developing blight disease. The deliverable in this proposal is a walnut blight protecting rootstock. Copper-based pesticides are the current management approach for walnut blight control. Despite their effectiveness, pesticide residues can persist in crops, and pests are developing resistance. Thus, we aim to develop walnut blight resistant rootstock to decrease the use of copper-based pesticides that not only induce resistance, but it is also toxic to the environment. Our strategy is based on suppressing the disease rather than focusing on killing or degrading the pathogen in the orchard environment.

KEY FINDINGS

- We identified two important proteins that play a central role in Walnut Blight (WB) disease, caused by the pathogen Xanthomonas arboricola pv. juglandis (Xaj).. The first is PGIP (polygalacturonase inhibitory protein), a defense protein produced by plants to protect against cell wall degradation that favors pathogen colonization. The second is ePG (endo-polygalacturonase), a protein secreted by the pathogen Xaj, which breaks down the host natural defenses to enable infection. Different studies show that constitutive expression of PGIP confers resistance against phytopathogens and may also aid in defense against herbivorous beetles. Understanding these proteins helps us uncover how the disease develops and how we can prevent it.
- To combat walnut blight, we are working to identify the most effective form of the PGIP protein. By testing different versions, we aim to select the one that is most readily secreted in walnut vascular tissues, offering the strongest protection against the disease. Since Paradox walnut rootstocks are extremely susceptible to crown gall disease, we first developed Paradox J1 embryo lines that efficiently suppress crown gall disease using the previously described vector pDE00.0201. We have successfully modified the best crown gall suppressing walnut embryo line (CGS1) by transforming them a second time with the PGIP vector pDU94.0928 to overexpress PGIP, creating 12 new lines that are now being propagated and tested for their ability to suppress both crown gall and WB.
- To monitor the success, we are developing specific antibodies to allow us to distinguish between the natural PGIP produced by walnuts (JrPGIP) and that introduced by us. This will help us identify walnut lines with the highest levels of the introduced protein and assess their natural defense capabilities. This strategy will enable the incorporation of both sets of genes in a single transformation allowing for better control on the selection of unique events that can be evaluated further. Development of CGR J1 lines were carried out and transient transformation of shoots inoculated with Agro A281 containing PGIP vectors will be used for selecting the best PGIP line for transformation in CRG J1 lines. To simplify and streamline our approach, we are developing a single genetic package, or vector, that combines crown gall suppression with PGIP production. This all-in-one solution will make the process more efficient and easier to manage while enhancing disease resistance. Elite walnut rootstock lines that suppress crown gall and blight will be identified through testing with shoot cultures.
- As part of our testing, we conducted an in-lab experiment to mimic WB symptoms seen in the field. The in vitro inoculation assay involves a vacuum infiltration inoculation protocol and evaluation one week after the infiltration. Using walnut twigs with young nutlets, we tested how various walnut genotypes respond to the disease. The result corroborates that Xaj colonization is a key feature of the disease emphasizing the importance of developing a walnut rootstock that secretes PGIP in scion tissues to counteract and inhibit the PG activity preventing pathogen colonization and blight disease development. These findings bring us closer to developing walnut rootstocks that can naturally resist WB without the need for

harmful pesticides. By enhancing the host natural defenses and combining resistance to multiple diseases, we aim to provide growers with a sustainable and environmentally friendly solution. This approach has the potential to transform walnut farming, reducing costs while protecting both the environment and crop yields.