

University of California
Division of Agricultural Sciences

PROJECT PLAN/RESEARCH GRANT PROPOSAL

Project Year 2009-2010 Anticipated Duration of Project 2 years

Project Leader Abhaya M. Dandekar Location Plant Sciences/UC Davis

Cooperating Personnel David Tricoli, Gale McGranahan, Chuck Leslie

Project Title Crown gall resistant walnut rootstocks: analysis for scion transmitted genetic materials

Keywords

Commodity(s) Walnut, Juglans regis Relevant AES/CE Project No. 5271-H

Problem and its Significance:

English Walnut (*Juglans regia*) is one of California's top 20 agricultural commodities, grown on 216,000 acres with an annual production valued at of more than \$550 million. One hundred percent of U.S. walnut production is in California, and CA walnuts are a major export crop (CA Department of Food and Agriculture, 2007). Walnut trees suffer from a number of diseases that limit their profitability including *Phytophthora*, black line, nematodes, blight and crown gall disease. Crown gall disease, which is caused by the bacterium *Agrobacterium tumefaciens*, is an example of one disease that is a source of significant economic losses in California walnut orchards and nurseries. Profits are reduced in the form of unsalable nursery stock, lowered productivity from galled trees, and increased susceptibility of infected plants to pathogens and adverse environmental conditions. The increased use of Paradox rootstock (which is known to have a high susceptibility to crown gall) has exacerbated the crown gall problem.

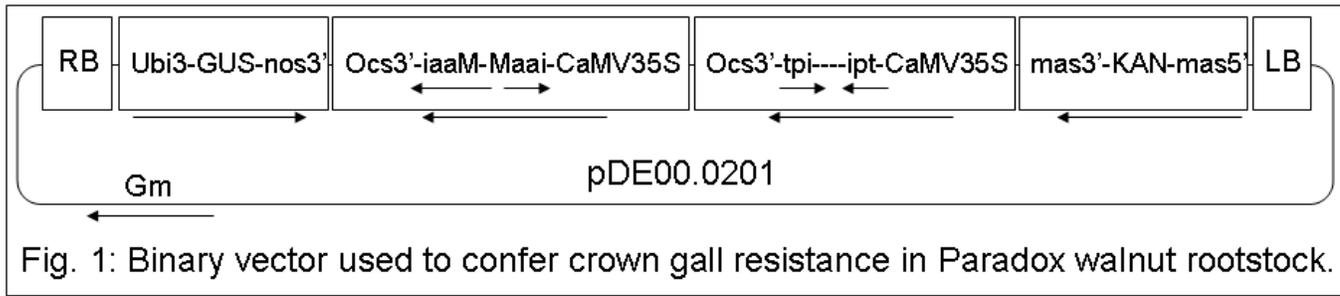
Central to the pathogenicity of *Agrobacterium tumefaciens* is the expression of the *ipt*, *iaaM* and *iaaH* oncogenes in infected plant cells, leading to uncontrolled cell proliferation and the crown gall phenotype. By introducing self-complimentary constructs of the *iaaM* and *ipt* genes into a Paradox-hybrid walnut rootstock, Escobar *et al.* (2001; 2003) were able to block the expression of these genes resulting in the suppression of tumor formation. Now that the success of this technology has been demonstrated with a Paradox hybrid genotype, the next step is to further evaluate the crown gall resistant material. A number of additional studies need to be complete. This includes research aimed at demonstrating the rigor of the resistance under various environmental conditions is study is currently underway. Further analysis of the resistant rootstocks with grafted wild type scion varieties need to be conducted to demonstrate the lack of movement of any genetic materials from the rootstocks into the wild type scion materials and the accumulation of regulatory data required for possible de-regulation of the material.

Objectives:

1. Analysis of grafted scions on transgenic walnut rootstocks for the transfer of genetic materials.
2. Consult with USDA APHIS, EPA and FDA regarding the regulatory requirements needed to de-regulate transgenic crown gall resistant walnut rootstocks.

Plans and Procedures:

1. Analysis of grafted scions on transgenic walnut rootstocks for the transfer of genetic materials: Elite transgenic paradox lines displaying complete resistance to crown gall will be grafted to wild type Chandler scions. Grafted plants will be grown in the greenhouse and the scion tissues from these grafted plants will be analyzed for the transmission of genetic materials expressed in the transgenic rootstocks. Shown below in Fig 1 is the vector used for the transformation of transgenic Paradox rootstocks.



Analysis genomic DNA of the scion for the movement of genetic materials from the rootstock: We will test for the movement of the 4 genes present on this vector, the GUS, *iaaM*, *ipt* and Kan genes. Young leaf tissues will be harvested from each of the grafted plants and total DNA will be isolated. Primers for these 4 genes will be used to detect the presence of any positive genetic materials using PCR. If this is negative, a more sensitive quantitative real time PCR (QRT-PCR) method will be used. A set of specific primers will be constructed for each of these 4 genes to conduct this analysis. An endogenous control single copy gene (*actin/tubulin*) will also be included to validate the QRT-PCR as a positive control.

Analysis expressed RNA of the scion for the presence of mRNA sequences corresponding to genes expressed in the rootstock: We will test for the presence of mRNA sequences corresponding to each of the 4 genes present on this vector, the GUS, *iaaM*, *ipt* and Kan genes. Young leaf tissues will be harvested from each of the grafted plants and total RNA will be isolated. Primers for these 4 genes will be used to detect the presence of any positive mRNA after converting the RNA to cDNA using reverse transcriptase and then amplifying cDNA using PCR. If this is negative, a more sensitive quantitative real time PCR (QRT-PCR) method will be used. A set of specific primers developed for the DNA sequence analysis will be used to detect the presence of any mRNA species corresponding to any of these 4 genes expressed in the rootstock. An endogenous control mRNA corresponding to a housekeeping gene (*actin/tubulin*) will also be included to validate the QRT-PCR as a positive control.

2. Consult with USDA APHIS, EPA and FDA regarding the regulatory requirements needed to de-regulate transgenic crown gall resistant walnut rootstocks: We will begin consultation with APHIS to develop the regulatory package necessary for the de-regulation of transgenic walnut rootstocks resistant to crown gall. Plans will be developed to conduct this analysis to create a data package for de-regulation.

References:

- Escobar, M.A., Civerolo, E.L., Summerfelt, K.R. and Dandekar, A.M. RNAi-mediated oncogene silencing confers resistance to crown gall tumorigenesis. In: Proceedings of the National Academy of Sciences of the United States of America. November 6, 2001. 98(23):13437-13442.
- Escobar, M.A., Uratsu, S.L., Civerolo, E.L., McGranahan, G.H., Leslie, C.A., Polito, V.S. Pinney, K.A. and Dandekar, A.M. 2003. Development of a crown gall resistant walnut rootstock. In: Walnut Research Reports, 2002. Walnut Marketing Board, Sacramento. pp. 57-67.

BUDGET REQUEST

Budget Year 2009-2010

Funding Source

Salaries and Benefits

Postdocs/RA's

SRA's

SRAlII (0.17 FTE)

\$ _____

\$ 10,988.00

Lab/Field Assistance

\$ _____

Subtotal

Sub 2

\$ _____

Employee benefits

Sub 6

\$ 3,066.00

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SUBTOTAL

\$ 14,054.00

Supplies and Expenses

Sub 3

\$ 4,000.00

Equipment

Sub 4

\$ _____

Travel

Sub 5

\$ 800.00

TOTAL

\$ 18,854.00

Department account number _____



Date Dec. 30, 2008

Originator's Signature

COOPERATIVE EXTENSION

County Director _____ Date _____

Program Director _____ Date _____

AGRICULTURAL EXPERIMENT STATION

Department Chair _____ Date _____

LIAISON OFFICER

_____ Date _____