IMPROVING OUR UNDERSTANDING OF SUBSTANCE TRANSPORT ACROSS GRAFT UNIONS

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ABSTRACT
Researchers seeking to genetically-engineer grapevine rootstocks in order to affect Pierce's disease (PD) resistance in scion cultivars know very little about the transport of substances produced by foreign genes across the graft union. Our project seeks to understand how protein size and concentration may affect protein transport from a rootstock to a scion. We possess genetically engineered lines of Chardonnay, Merlot and Chancellor that produced proteins ranging in size from 29 to 97 kDa. These proteins can be readily detected by established techniques. Lines will be identified with low and high protein production potential in their root tissues, and graft combinations will be created with non-transgenic Chardonnay scions. Xylem sap will be collected from the scion and tested for the presence of the transgenic proteins. Given that Xylella fastidiosa causing plugging of xylem tissues, the results of xylem sap testing will be directly applicable to efforts to develop PD resistance inducing rootstocks.

INTRODUCTION
One approach being utilized to develop a long-term solution to Pierce's disease is the development of transgenic PD resistant versions of important wine and table grape varieties. The development of each transgenic cultivar will require a concentrated effort and significant amounts of technical expertise, testing, and funding. To bring each successful product to market, and to pass regulatory agency approval for transgenic crops, also will require a great deal of time and funding. This would be required for each of dozens of scion varieties.

A rootstock-based approach provides a potentially excellent alternative. In theory, a transgenic rootstock would confer PD resistance to its non-transgenic scion. Advantages include: 1) many fewer rootstocks will need to be transformed as compared to the dozens of table grape and wine grape varieties that would need to be altered, 2) consumers might be more accepting of wines produced from non-transgenic scions even if they are grafted on transgenic stocks; and 3) in general, it has been technically easier to transform rootstocks than scion varieties. Before this approach is successful, however, our understanding of the biology of the graft union and the types of substances that can be successfully transported from rootstocks to scions must be improved.

Water, mineral nutrients, hormones, carbohydrates, and other compounds are all known to move, via both xylem and phloem, from rootstocks across graft unions into scions of woody plants. To date, however, there is little evidence available to show whether a transgenic protein can move from the rootstock into the scion in a grafted woody plant. In recent work with grapevines, Meredith and Dandekar (2003) showed that pear polygalacturonase inhibiting protein (PGIP), with a size of 36.5 kDa, could be detected in xylem sap of non-transgenic scions grafted on transgenic stocks engineered to produce this protein. Of great relevance to this proposal, we noted that protein movement into the xylem occurred even without a specific signal targeting it to the extracellular spaces or to the xylem. Imidacloprid (a small compound with molecular weight of approximately 0.25 kDa) and other systemic insecticides applied to the soil are taken up by the roots of grapevines and move from root systems into the scion (Toscano et al. 2003). The present project will investigate aspects of plant physiology critical to determining the potential for deploying transgenic rootstocks for PD management.

It is possible that the size of a transgenic protein produced in a rootstock influences its transport to the scion. For example, large proteins might be less likely to be transported than small proteins. Understanding the relationship between size and movement will allow us to more efficiently test anti-PD compounds. If transgenic proteins are transported across the graft union, their concentration in the roots might be higher than their concentration in the scion. Since there is likely to be a threshold concentration for PD control provided by a given compound, it will be critical to understand the relationship between concentration in the rootstock and concentration in the scion.

By studying non-transgenic scions grafted on transgenic rootstocks in the course of this project, we expect to learn whether the transgenic proteins can move from the rootstock to the scion, whether molecule size affects transport, and whether substance concentration in the rootstock affects levels found in the scion.

OBJECTIVE
Determine the relationship between protein molecule size and concentration in grapevine roots and its ability to move from a grapevine rootstock to a scion across a graft union.

RESULTS
This project is just getting underway, thus, rather than present non-existent research results, an outline of our research plan is presented here.

The following transgenic grapevines are available for use:
1. Two lines of Chancellor transformed with an NPT-II/GUS gene fusion producing a fused protein product. One line strongly expresses the *gus* reporter gene (*uidA*) in all tissues, while the other line shows no GUS expression, even though the gene is present.
2. Multiple lines of Chardonnay and Merlot producing both NPT-II and endochitinase.
3. A series of lines of Chardonnay producing NPT-II along with one of three antimicrobial peptides (AMPs).

All of these lines produce transgenic products under control of constitutive promoters. In cases 1 and 2 above, the CaMV 35S promoter was employed, whereas in case 3, NPT-II was downstream of an *Arabidopsis* ubiquitin promoter. The CaMV 35S promoter was used by Meredith and Dandekar (2003), who showed that PGIP protein from rootstocks could be detected in xylem sap. The NPT-II/GUS gene fusion product in Chancellor was shown to express in root tissues (Striem et al. 2000), but will require re-testing to make sure that protein production has not been lost since these tests were run. We will need to test the other lines (2 and 3 above) to determine the transgenic protein concentration in their roots. The size of the transgenic product molecules varies: NPT-II is ~280 amino acids (aa) (29 kDa); endochitinase is 424 aa (42 kDa); the NPT-II/GUS bifunctional fusion protein has 885 aa (97 kDa).

We will examine root tissues from separate lines of each of the three types of transformed vines listed to determine gene transcription and transgenic protein concentration via established procedures. To test for gene transcription we will use semi-quantitative RT-PCR (Vidal et al. 2003). Transgenic protein concentrations will be determined using standard methods already in use in our lab. We will identify lines with high and low concentrations of transgenic proteins for further use in this project.

The transgenic lines with high and low concentrations of transgenic proteins, along with negative controls, will be bench grafted as rootstocks to non-transgenic Chardonnay scions. The grafted vines will be grown in a greenhouse. Once the grafted vines have been established and their shoots have grown to 50 cm, the non-transgenic Chardonnay scions will be examined for presence of transgenic proteins. Leaf tissue as well as xylem sap will be tested. Samples will be collected under sunny, warm conditions conducive to transpirational pull through the xylem.

Outline of rootstock/scion combination planned:
13 rootstock/scion combinations planned, including control
10 vines of each combination x 13 combinations = 130 vines total planned
Control rootstock: Non-transgenic Chardonnay (to be grafted to non-transgenic Chardonnay)

Experimental rootstocks:
(Each rootstock will be grafted to non-transgenic Chardonnay scions.)
Chancellor, high NPT-II/GUS fused protein product concentration in roots (35S promoter)
Chancellor, transformed vine with no GUS expression in roots (35S promoter)

Chardonnay, high NPT-II concentration in roots (Nos promoter)
Chardonnay, low NPT-II concentration in roots (Nos promoter)

Chardonnay, high NPT-II (*Arabidopsis* ubiquitin promoter)
Chardonnay, low NPT- II (*Arabidopsis* ubiquitin promoter)

Chardonnay, high endochitinase concentration in roots (35S promoter)
Chardonnay, low endochitinase concentration in roots (35S promoter)

Merlot, high NPT-II concentration in roots (Nos promoter)
Merlot, low NPT-II concentration in roots (Nos promoter)

Merlot, high endochitinase concentration in roots (35S promoter)
Merlot, low endochitinase concentration in roots (35S promoter)

Additional controls will include own-rooted transgenic vines to be used to test for presence of foreign protein in the xylem sap.

CONCLUSION

The success of this project will rest on the careful, methodical characterization of foreign gene products. This project will not involve the speculative and lengthy creation of novel transgenic grapevines, but rather uses pre-existing transgenic grapevines in order to investigate the potential for transgenic rootstocks to deliver proteins to their non-transgenic scions.

Based on the evidence from the movement of imidacloprid and PGIP in grafted grapevines, it is likely that transgenic grapevine rootstocks will transmit transgenic proteins to their non-transgenic scions. However, it is premature to speculate concerning the time frame for reduction to practice in the form of a novel PD management strategy. We emphasize that this study is intended to investigate the biological principles of protein transport via xylem in grapevines, a topic that has been studied very little in the past. By understanding the potential of a transgenic grapevine rootstock to move proteins into a non-transgenic scion, scientists will be better equipped to investigate and develop novel PD management strategies.

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