

Unified Grant Management for Viticulture and Enology

1. Summary

Grapevine fanleaf virus (GFLV) is one of the most devastating viruses of grapevines worldwide. The virus is transmitted by the dagger nematode *Xiphinema index* and is primarily managed in diseased vineyards through the use of rootstocks that are resistant to *X. index*. Such rootstocks delay the debilitating effect of GFLV on vine health and production but do not prevent GFLV infection (Oliver and Fuchs 2011). In addition, no source of resistance to GFLV is known in wild or cultivated *Vitis* species (Oliver and Fuchs 2011). Therefore, the anti-viral pathways of RNA interference (RNAi), an innate plant defense system, could be exploited to confer resistance to GFLV infection in grapevine rootstocks. Different RNAi constructs derived from conserved regions of the GFLV genome, i.e. RNAi construct AB (corresponding that the RNA1-encoded 1A and 1B^{Hel}) and RNAi construct H (a concatenate construct of the GFLV RNA-encoded 1E^{Pol} and RNA2-encoded 2B^{MP} and 2C^{CP}), were cloned into a binary plasmid for expression *in planta*. The integrity of the RNAi constructs in the binary plasmid was verified by restriction digestion and sequencing. These constructs will be used in grape rootstock transformation experiments.

2. Technical Report

3. Project Title: Resistance to Grapevine Fanleaf Virus in Rootstocks

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5. Objectives and Experiments Conducted to Meet Stated Objectives

The research is designed to engineer resistance to grapevine fanleaf virus (GFLV) in transgenic grapevine rootstocks through RNA interference (RNAi). The specific objectives are to:

1. Develop RNA interference (RNAi) constructs from conserved genomic regions of GFLV
2. Test RNAi constructs for reduction of GFLV accumulation in transient assays
3. Transfer promising RNAi constructs into grapevine rootstock embryogenic calli and develop transgenic clones
4. Initiate phenotyping of transgenic RNAi grapevine rootstock clones by agroinfiltration with infectious GFLV constructs
5. Disseminate information to stakeholders through presentations at conventions and workshops

To address objective #1 - Develop RNA interference (RNAi) constructs from conserved genomic regions of GFLV -, the sequence of the complete bipartite RNA genome of GFLV the genus *Nepovirus* in the family *Secoviridae* was mined for short conserved nucleotide regions. Search parameters were 25 nts stretches in length for which 85% of the nucleotide positions are conserved amongst at least 95% of the

sequences. Search outputs revealed 10 conserved regions throughout the GFLV genome. These conserved nucleotide stretches of 100-300 nucleotides in size are located on RNA1 (five) and RNA2 (five). Conserved regions were amplified by PCR using specific primers and concatenate constructs resulting from the ligation of PCR products from different coding regions were produced. RNAi construct H comprises conserved regions of the RNA1-encoded 1E^{Pol} and the RNA2-encoded 2B^{MP} and 2C^{CP}. RNAi construct AB was comprised conserved regions of the RNA1-encoded 1A and 1B^{Hel}. The integrity of the H and AB constructs was verified by restriction digestions and by sequencing. They will be further used for grape rootstock transformation.

7. Outside Presentations of Research

n/a

8. Research Success Statements

The award was fully executed on March 8, 2017. Therefore, the research is progressing slowly because a postdoctoral research associated has not been hired yet to spearhead the project.

9. Funds Status

Funds were spent for salaries of key personnel (technicians) involved in the research and for supplies in molecular biology and tissue culture.

Literature Cited

Oliver, J.E. and Fuchs, M. 2011. Tolerance and resistance to viruses and their vectors in *Vitis* sp.: A virologist's perspective of the literature. *American Journal of Enology and Viticulture*, 62:438-451.