Improved Detection and Evaluation of the Biological Significance of Grapevine Vitiviruses

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***This is an ongoing project from a prior year Agreement.**

Project Summary/Abstract

Briefly describe the long-term objectives for achieving the stated goals of the project.

Currently, nine different viruses are formally classified as vitiviruses (genus Vitivirus, family Betaflexiviridae) and known to infect grapevine: grapevine virus A (GVA), grapevine virus B (GVB), grapevine virus D (GVD), grapevine virus E (GVE), grapevine virus F (GVF), grapevine virus G (GVG), grapevine virus H (GVH), grapevine virus I (GVI) and grapevine virus J (GVJ). Some of these vitiviruses are associated with the etiology of rugose wood
(RW) disease in grapevine and are vectored by mealybugs (family Pseudococcidae) and soft-scale insects (family Coccidae); however, the main route of their transmission is by propagation using infected plant material. In addition, vitiviruses are frequently detected in coinfection with members of the family Closteroviridae (i.e. grapevine leafroll-associated viruses 1, 2 and 3), resulting in synergistic interactions that can lead to lethal effects in several scion and rootstock combinations. Through the application of high throughput sequencing (HTS), two new viruses were discovered in grapevine during the last two years and have been proposed as members in the Vitivirus genus. These new viruses were tentatively named grapevine virus L (GVL) and grapevine virus M (GVM). In contrast with long-known vitiviruses (i.e. GVA to GVF), the biological significance of the novel vitiviruses (i.e. GVG to GVM) remains largely unknown, including effects on vine performance and mechanisms of transmission. In 2018, a limited survey was launched to determine the prevalence of GVG, H, I, J and L in California; as a result, all five viruses were detected across different grapevine populations via conventional PCR. This project will update existing detection assays and in the case of the novel vitiviruses, design new reverse transcription quantitative PCR (RT- qPCR) assays to replace the current conventional PCR-based assays. This will increase the reliability and efficiency of vitivirus detection in grapevines. Another objective of this project is to investigate the biological significance of novel vitiviruses. We plan to conduct a field trial to determine if the novel vitiviruses (GVG, H, I, J, L and M) can cause RW-like symptoms (i.e. wood marking symptoms on the trunk) on commonly used indicator hosts LN 33, St. George, Kober 5BB and Cabernet franc. Inoculated grapevines will be examined for wood symptom development periodically. Finally, information generated from this project will be shared with nurseries and diagnostic labs to prevent a future negative impact to the grapevine industry.

Scope of Work

Describe the goals and specific objectives of the proposed project and summarize the expected outcomes. If applicable, describe the overall strategy, methodology, and analyses to be used. Include how the data will be collected, analyzed, and interpreted as well as any resource sharing plans as appropriate. Discuss potential problems, alternative strategies, and benchmarks for success anticipated to achieve the goals and objectives.

Project's Benefit to Nursery Industry

Previously known grapevine vitiviruses (GVA, B, D, E and F) are widespread and have been reported from all major grapevine-producing regions of the world, with GVA especially being one of the most regularly detected viruses. The grapevine-infecting vitiviruses are reported to be associated with the RW complex, which includes several important diseases that result in modifications to the woody cylinder, cambium tissue, and bark of vines (Figure 1). Symptom development depends on the virus-host combination and on environmental conditions. GVA, GVB, GVD are putative agents of Kober 5BB (V. berlandieri × V. riparia) stem grooving, corky bark in LN 33 (Couderc 1613 × V. berlandieri) and growth reduction in Freedom, respectively; however, the complete etiologies have not been resolved for any of these disease complexes. GVF was associated with graft incompatibility of Cabernet Sauvignon, resulting in death of plants. Lastly, there is no reported disease caused by GVE in grapevine. In contrast, the potential pathogenic role of new grapevine vitiviruses (GVG, H, I, J, L and M) is still unknown.

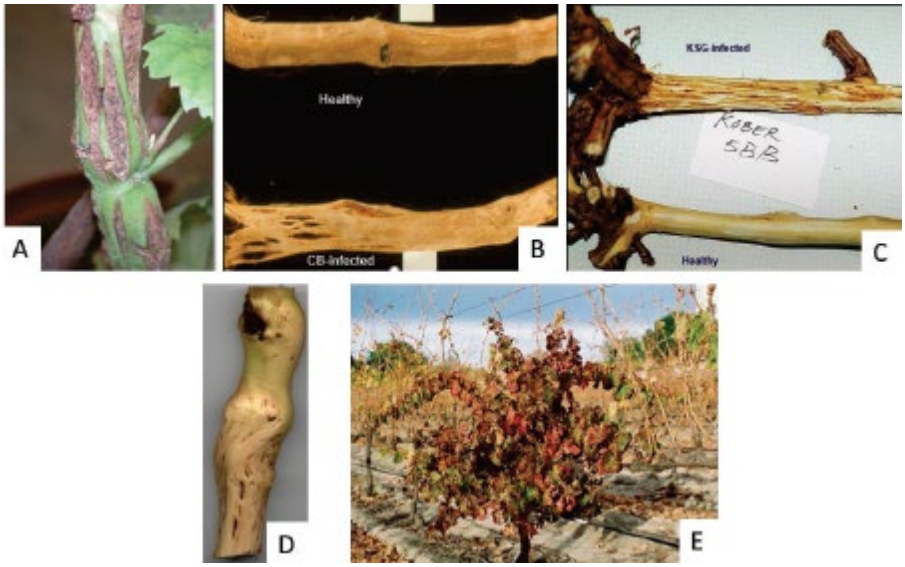

Figure 1. Grapevine disease symptoms putatively associated with vitivirus infection. A Corky bark and swelling symptoms on young LN 33 vine; B, Corky bark symptoms on LN33 wood; C, Kober Stem Grooving symptoms on Kober 5BB wood; D, Stem pitting and grooving on rootstock wood, under the grafting point associated with vitivirus infection; E, Shiraz disease symptoms on a Merlot vine (natural infection in the vineyard) at the end of the vegetative season. Nearby vines unaffected by the disease showed normal hardening of their canes and normal loss of foliage (Minafra et al., 2017).

Other viruses infecting grapevine are the grapevine leafroll-associated viruses (GLRaV; family Closteroviridae), which are frequently detected in coinfections with vitiviruses. Even when vitiviruses by themselves do not cause symptoms on common grapevine scion and rootstock combinations, a mixed infection of a vitivirus with a member of the family Closteroviridae may create a serious problem in the field, especially if one of the susceptible rootstocks has been used for propagation. Our observations in California have shown that Cabernet Franc and Chardonnay vines propagated on Freedom rootstock die within 1-2 years after inoculation when inoculated with a virus source containing GLRaV-1 or GLRaV-3 and GVA. Reports from South Africa indicate that Shiraz disease is caused by a co-infection of GVA and GLRaV-3. GVG, H, I, J and L were detected recently during an initial study using conventional PCR at Foundation Plant Services (FPS, University of California-Davis), which represents the first identification of such viruses in the US. New advances in qPCR have significantly improved the detection of pathogens, allowing quick, more sensitive and precise quantification compared to conventional PCR. This project will design new RT-qPCR assays for detection of the previously mentioned vitiviruses. In the case of GVA, B, D, E, F, we will evaluate the broad-range detection capacity and specificity of currently available RT-qPCR assays, and update these assays if they are inadequate. Genetic diversity and recombination are common among viruses, which can compromise the reliability of PCR-based assays. Since FPS works closely with the California Department of Food and Agriculture (CDFA) to register and certify grapevines and is the source of foundation planting material for California nurseries, FPS just developed a new RT-qPCR assay for the reliable detection of GLRaV-3 (Project: "Survey and analysis of grapevine leafroll-associated virus-3 genetic variants and application towards improved RT-qPCR assay design") and currently is working on the update and development of new assays for the common Prunus and pome fruit viruses (Project: "Development and validation of real time quantitative PCR assays for the detection of fruit tree viruses"). Consequently, we anticipate the continued shift to qPCR-based methods for virus detection given the improvement in accuracy and high throughput sample processing efficiency. Any assay we develop as a result of this project will be made available to CDFA and commercial diagnostic labs and will facilitate the production of certified propagation material and the effective control of vitiviruses in California vineyards.

As discussed previously, to date there is no scientific data available to indicate symptoms caused by novel grapevine- infecting vitiviruses. The grapevine bioassay is a test for viruses based on a panel of grapevine selections that serve as indicator hosts. The bioassay detects those disease agents that are designated as of particular agronomic significance. The selections include Cabernet franc, which generates a diagnostic interveinal reddening and down-rolling of leaf margins as an indicator of leafroll disease caused by members of the family Closteroviridae; Kober 5BB, which expresses stem grooving disease associated with infection by GVA; LN 33, which develops corky-bark symptoms associated with infection by GVB; and St. George (V. rupestris), which develops diagnostic leaf symptoms due to infection by grapevine fanleaf virus or grapevine fleck virus, and stem pitting symptoms from infection by grapevine Rupestris stem pitting-associated virus. We note that these are not the only grapevine viruses that can be detected with these indicator cultivars. For example, Cabernet franc is also an indicator for grapevine red blotch virus and St. George is an indicator for grapevine asteroid mosaic associated virus. Consequently, we plan to conduct a field trial to investigate symptomatology caused by GVG, H, I, J, L and M on the four different indicator plants. In these woody indicators, we will verify their infection status using the improved RT-qPCR assays (generated during the project) and assess their

reaction to novel vitiviruses. Finally, results of this research will be shared with growers and other stakeholders involved in the grapevine industry.

Objectives

Objective 1: Construct new or improve individual RT-qPCR assays for all known grapevine vitiviruses.

Objective 2: Screen select grapevine populations for vitiviruses and validate improved RT-qPCR assays.

Objective 3: Evaluate the biological effects of GVG, H, I, J, L and M on the common grapevine indicators.

Objective 4: Disseminate research progress and results.

Work Plans and Methods

Objective 1: Construct new or improve individual RT-qPCR assays for all known grapevine vitiviruses.

Comparison of previously developed RT-qPCR assays of GVA to GVF to new sequence data from GenBank showed that there was cross reaction between GVA and GVF assays. We will design new assays for these two viruses to improve their specificity. Development of assays for GVG to GVM will be completed. In case of GVG, it is believed that the virus is endemic of Croatia and has several genetic variants. To cover the GVG diversity in the new assay, we are closely collaborating with Dr. Darko Vončina from University of Zagreb (Croatia). Once designed, the new assays will be analyzed in silico using data available in GenBank to assess the specificity.

Objective 2: Screen select grapevine populations for vitiviruses and validate improved RT-qPCR assays. Grapevine populations with a historical incidence of vitiviruses will be tested by the newly developed RT-qPCR assays

(Objective 1) to make sure that each system has reliability as expected. The efficiency of each system will also be compared with previous assays (when available). These populations include: The USDA National Clonal Germplasm Repository (NCGR) in Davis, CA; the FPS domestic and quarantine material; the University of California-Davis Virus Collection (DVC); and select commercial vineyards in California.

To date, 387 grapevine samples have been collected at the DVC. Additionally, 500 stored total nucleic acids extracts originated from the NCGR; the FPS pipeline; and select commercial vineyards in California are ready to be tested using the new RT-qPCR assays.

Objective 3: Evaluate the biological effects of GVG, H, I, J, L and M on the common grapevine indicators. Previously, selected grapevine accessions tested positive for novel vitiviruses (GVG, H, I, J, L and M) were graft inoculated into four woody indicators (LN 33, St. George, Kober 5BB, Cabernet franc) to determine the effects on these plants. This year, grafted grapevines will be observed for symptom development on leaves and trunk. Likewise, leaf petioles will be collected from all grafted plants, total nucleic acids will be extracted from each sample and later analyzed by RT-qPCR to test for the presence of the viruses inoculated into each plant. This information will let us correlate the presence of the virus with the symptoms recorded.

Objective 4: Disseminate research progress and results.

An important aspect of this project will be to communicate opportunities, progress, and results to growers, stakeholders, and scientific peers. We will use growers' meetings organized by the UC Cooperative Extension, symposiums, and scientific meetings to accomplish our objective. Information will also be disseminated in printed form through news articles, research reports, and peer-reviewed scientific papers. In addition, for broader dissemination of information at the national level, results will be shared with the National Clean Plant Network.

Literature Review

Virus infection is of particular concern in grapevine. At least 85 different viruses have been reported in this host and many are proven to be spreading in the field by different natural vectors. Furthermore, in the past few years several new viruses have been discovered and more await their discovery, especially with the new HTS technology which generates a large amount of sequence information from the pathogenic and non-pathogenic agents present in a plant (Al Rwahnih et al., 2009). These discoveries may lead to better understand of the disease problems found in the vineyards and find procedures to remedy or reduce damages caused.

The genus Vitivirus in the family Betaflexiviridae formally includes GVA (Conti et al., 1980), GVB (Goszczynski et al., 1996), GVD (Choueiri et al., 1997), GVE (Nakaune et al., 2008), GVF (Al Rwahnih et al., 2012), GVG (Blouin et al., 2017), GVH (Candresse et al., 2017), GVI (Blouin et al., 2018) and GVJ (Diaz-Lara et al., 2018). This class of grapevine viruses is usually transmissible to several herbaceous hosts including Nicotiana benthamiana, N. occidentalis and Chenopodium species; additionally, vitiviruses are transmitted by members of several insect genera of mealybugs and scale insects (Pseudococcus, Planococcus, Heliococcus, Neopulvinaria, Parthenolecanium, Cavariella and Ovatus) in a semi-persistent manner (La Notte et al., 1997; Rosciglione et al., 1983). The hosts and vectors of vitiviruses are all shared with members of the family Closteroviridae (i.e. GLRaV-1, -2 and -3), which are frequently detected in coinfections with vitiviruses. Two recently discovered viruses, GVL (Debat et al., 2018) and GVM (Alabi et al., 2019) are proposed as members of the Vitivirus genus.

Several studies have linked the presence of some Vitivirus species with the RW disease in grapevine (for a review Minafra et al., 2017). RW is a disease complex with world-wide distribution (Martelli, 2014) and is associated with different syndromes affecting the bark, cambium tissue, and woody cylinder of grapevines (Minafra et al., 2017). In contrast, these studies have only been successful in showing the association of a particular virus with a specific host. GVA was associated with Kober 5BB stem grooving on Kober 5BB (Chevalier et al., 1995; Credi, 1997), GVB was identified as the putative causal agent of corky bark in LN 33 (Bonavia et al., 1996), and GVD was implicated in growth reduction in Freedom (Rosa et al., 2011). More recently, GVF was associated with graft incompatibility of Cabernet Sauvignon. The potential pathogenic role of other vitiviruses, including proposed members is still unknown. In this project, we will investigate the biological significance (symptoms) of novel vitiviruses using indicator grapevines.

In 2018, a limited survey was launched to determine the incidence of novel grapevine-infecting vitiviruses in California

(Diaz-Lara et al., 2019). Results of this work revealed that all five vitiviruses, GVG, H, I, J and L, were present in California, predominantly occurring as mixed infections with GVA. Furthermore, grapevines carrying up to six different vitiviruses were identified.

Efficient and reliable laboratory diagnostic tests are critical in determining viral infection in grapevines. While a number of diagnostic methods are available for viral detection, advantages of using qPCR to detect viral pathogens has been reported. The development of qPCR-based methods leads to superior sensitivity, speed, reproducibility, and limited risk of contaminations compared to conventional PCR (Osman et al., 2017); such characteristics often make it the method of choice in routine diagnostics. qPCR has been used successfully for the detection of several grapevine viruses, including GLRaV-3 and grapevine pinot gris virus (Diaz-Lara et al., 2018b; Morán et al., 2018). Our research aims to evaluate and improve existing grapevine vitivirus assays. In the case of GVA, B, D, E and F this means updating our current RT-qPCR assays based on newly available sequence data in GenBank and FPS. For the novel vitiviruses, this will require designing and evaluating new RT-qPCR assays.