Summary Final Report for CDFA Agreement Number 18-0296-000-SA: "STRUCTURE-FUNCTION STUDIES ON GRBaV TO ELUCIDATE DISEASE ETIOLOGY"

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The etiology of *Grapevine Red Blotch Virus* (GRBV) on the host plant is completely unknown. The PD/GWSSB is addressing the GRBV threat to stakeholders by investing in applied research on vectors, epidemiology, ecology, and field transmission. We submit that studies on understanding the viral gene functions and disease mechanism are necessary to effectively combat Red Blotch disease. Understanding how GRBV causes disease can present cogent strategies for mitigating this threat to a multibillion-dollar industry. Degradation of viral transcripts (RNA silencing) has evolved as a major host defense mechanism against invasive pathogens. Viruses counter the plant defense mechanisms by evolving one or more "silencing suppressor" proteins. The efficacy of host silencing versus viral silencing suppression results in resistance/tolerance or susceptibility to the pathogen. The anthocyanin levels in dicot leaves are under a tightly controlled regulatory mechanism involving endogenous small RNAs. The red patches in the interstitial lamina of GRBV-infected leaves and in petioles and veins are caused by deranged anthocyanin accumulation, a well-known stress response in plants. We hypothesize the viral suppressor protein(s) of GRBV interfere with the anthocyanin regulatory pathways and result in uncontrolled anthocyanin accumulation in vegetative tissues, thus serving as a visual cue for feeding by the assumed arthropod vector capable of transmitting the viruses. Thus, identifying the GRBV viral suppressor proteins and host target proteins is an essential objective to develop disease resistance strategies involving genetic engineering and/or breeding for virus resistance going forward.

Towards this we have accomplished the following at the end of one year of support from CDFA:

1. Sub-cloning of all six open reading frames under a plant promoter and terminator
2. Cloning of all six viral gene cassettes in a plant transformation vector
3. Agroinfiltration of *Nicotiana benthamiana* 16c transgenic GFP plant was performed with all six viral genes to evaluate silencing suppression
4. C2 and V2 are identified to be candidate GRBV suppressor protein candidates
5. Molecular analysis confirms C2 and V2 as GRBV suppressor proteins
6. RNA and small RNA libraries were prepared from GRBV-infected and healthy leaf samples from Temecula and Sonoma Co., CA and southern Oregon and sent for deep sequencing.
7. Transgenic anthocyanin expressing tobacco plants for super-transformation with suppressor proteins are established in tissue culture conditions.