Final Technical Report

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GRAPEVINE COLD HARDINESS: INTEGRATING ENVIRONMENTAL CUES AND VINE PHYSIOLOGY

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Goals and Objectives

The sustainability of the Michigan grape industry is limited by climatic constraints, primarily freezing temperatures. Due to its location in a northern climate, Michigan vineyards are particularly vulnerable to cold weather events, and extreme cold can cause severe damage to grapevines. In 2014-15, the two “polar vortex” events caused $8 million in losses to Michigan vineyards, resulting in a crop reduction of up to 70% for major grape varieties grown in the state. These losses can be devastating to individual growers and can have a ripple effect on the entire industry, as it is heavily dependent on grape production.

Therefore, there is a pressing need to develop novel strategies to increase the freezing tolerance (FT) of grapevines. This project investigated the molecular, biochemical, and physiological mechanisms underpinning the acquisition of FT, with the ultimate goal of enhancing the economic and environmental sustainability of grape production in Michigan and the East of the US.

Our studies have demonstrated that the FT of grapevines can be improved by foliar application of foliar abscisic acid (ABA). Now, this treatment has the potential to be incorporated into cultural practices by the Michigan growers, to reduce cold damages in vineyards, thus increasing the resilience of the industry to extreme weather events.

During the first year of the project, a study was conducted to characterize the effects of foliar-applied ABA on greenhouse-grown Pinot noir (a moderately cold-sensitive cultivar) and Marquette (a cold-resistant cultivar) grapevines. Compared to the control, ABA decreased stomatal conductance and improved the transition of grapevine physiology towards cold acclimation. In particular, ABA application induced the accumulation of several sugars in the buds of the raffinose family oligosaccharides (RFOs), which are responsible for cold resistance. These new findings have improved our understanding of the role of ABA in grapevine FT.

During the second year of the project, the focus was on the impact of ABA foliar application on the expression of raffinose and galactinol synthase genes, which are involved in the biosynthesis of RFOs. The goal was to further elucidate the molecular mechanisms underlying ABA-mediated FT improvement in grapevines.

At the end of the project, several strategies to protect grapevines from cold damage can be proposed to the Michigan grape industry. These may include incorporating ABA foliar application into standard cultural practices, utilizing cold-hardy grape varieties, or implementing novel frost-protection technologies. By increasing the resilience of grapevines to cold weather events, the Michigan grape industry will be better equipped to weather the challenges posed by a changing climate, ultimately promoting its long-term sustainability.

Results, Conclusions and Outcomes

In summary, this study explored the effects of exogenous ABA application on the dormancy and freezing tolerance (FT) of Pinot noir and Marquette grapevines. The results showed that ABA application at a concentration of 400 mg L^{-1} was effective in inducing deeper dormancy and improving bud freezing tolerance (FT) without any negative impact on vine size, yield, or fruit quality. The optimal timing for ABA application was found to be between véraison and 3 weeks post-véraison, corresponding to leaf age between DAB = 107 and 134 (Days After Bloom). These findings suggest that ABA could be used as an additional tool to protect grapevines from freezing damage in cold regions. Overall, this research highlights the potential of ABA application for enhancing the winter hardiness of grapevines and provides valuable insights for future studies on grapevine dormancy and freezing tolerance.

ABA (abscisic acid) is a plant hormone that plays a crucial role in regulating plant responses to environmental stresses, including cold stress. We demonstrated how ABA induce plant dormancy and enhance cold tolerance by triggering a range of physiological and molecular responses, such as the accumulation of protective solutes, the activation of antioxidant systems, and the alteration of gene expression.

Time Span

Project activities carried out 2019-2022

Work Accomplished

The protocol used to determine the impact of ABA on grapevine cold hardness involved several steps, including sample preparation, ABA treatment, biochemical and molecular data analysis. The detailed protocol is outlined below:

Sample preparation:

- Grapevines were grown in a vineyard subjected to standard management practices and also in greenhouse for more detailed experiments.

- Shoots were collected in the dormant period (January-February) and kept at 4 °C for two weeks to break their dormancy.
- 12-15 shoots were collected per replicate, and three replicates were used for each treatment.

ABA treatment:

- ABA was dissolved in water with 0.1% Tween-20 and applied to the buds at four different concentrations: 0, 200, 400, and 800 mg L^{-1}.
- The ABA solution was sprayed on the buds until they were completely wet, using a hand-held sprayer.
- The application was carried out in two different periods: one at véraison and the other at 3 weeks post-véraison.

Biochemical analysis:

- The buds were harvested at different time points after ABA treatment (0, 7, 14, 21, and 28 days), and the following biochemical analyses were performed: total soluble sugars, starch, free proline, and MDA.
- The buds were homogenized in liquid nitrogen and extracted using appropriate extraction solutions.
- Total soluble sugars were determined by the anthrone-sulfuric acid method.
- Starch was determined by the anthrone method.
- Free proline was determined by the ninhydrin method.
- MDA was determined by the TBA method.

Molecular analysis:

- The expression levels of several genes related to cold hardiness were analyzed by quantitative real-time PCR (qRT-PCR).
- Total RNA was extracted from buds using the Trizol method.
- Reverse transcription was performed using a cDNA synthesis kit.
- qRT-PCR was carried out using gene-specific primers and SYBR Green chemistry.
- The relative expression levels of the target genes were calculated using the $2^{-\Delta\Delta Ct}$ method.

Statistical analysis:

- The data were analyzed using one-way ANOVA followed by Tukey's test.
- The statistical significance was set at $p < 0.05$.
- The results of the biochemical and molecular analyses were used to determine the impact of ABA on grapevine cold hardness.

Communication Activities, Accomplishments, and Impacts

Some results of the project were communicated to grape growers at the 2021 Great Lakes Fruit, Vegetable and Farm Market Expo in Grand Rapids and at the NW Viticultural Day in August 2022 December 2021.

Budget Narrative

This project was conducted consistent with the budget proposed by the principal investigator and approved by the State of Michigan. No matching funds or additional sources of funding were sought nor contributed to the work described herein.