2020 MCBC RESEARCH REPORT

PROPOSAL TITLE**:** ***"GRAPEVINE COLD HARDINESS: INTEGRATING ENVIRONMENTAL CUES AND VINE PHYSIOLOGY."***
GRANT 190000002121

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Summary of the research

Sustainability of Michigan grape industry is limited by climatic constraints, primarily freezing temperatures. In 2014-15, the two "polar vortex" caused $8 million losses to Michigan vineyards, causing a crop reduction up to 70% for major varieties grown in the state. Therefore, there is a need to develop novel strategies to increase freezing tolerance (FT) of grapevines. This project investigated the molecular, biochemical, and physiological mechanisms underpinning the acquisition of FT. Ultimately, the findings from this research will enhance the economic and environmental sustainability of grape production in Michigan and the East of US. Previous studies demonstrated that the freezing tolerance (FT) of grapevine is improved by foliar application of foliar abscisic acid (ABA), a treatment with the potential to be incorporated into a cultural practice to reduce cold damages in vineyards. During the first year of the project a study was conducted to characterize the effects of foliar applied ABA on greenhouse-grown on Pinot noir (a moderate cold sensitive cultivar) and an American-hybrid Marquette (cold resistant cultivar) grapevines. Compared to control, ABA decreased stomatal conductance and improved 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) responsible for cold resistance. These new findings from this study have improved our understanding of the role of ABA in grapevine FT and during the second year of the project focus will be on the impact of ABA foliar application on the expression of raffinose and galactinol synthase genes. At the end of the project, several strategies to protect grapevines from cold damage will be proposed to our grape industry.

1. Use of the financial support from MGWIC

The funding requested to the MCBC supported technical staff undergraduate students' costs, laboratory analyses and all the cost related to vine maintenance in the greenhouse. Preliminary data will be presented in 2020-21 to state extension, regional and national meetings.

2. Objectives of the proposal and results for the first-year project

The goal of this research is to advance our understanding of the molecular and physiological mechanisms linking endogenous abscisic acid (ABA) and carbohydrates accumulation to FT in grapes. **Our hypothesis is that the different sensitivity to cold stress in grape genotypes is related to a diverse soluble sugar accumulation in buds, which is in turn modulated by an ABA-dependent pathway**.

- **2019 Objective 1**: Evaluate transcriptional, physiological, and biochemical responses to acute freezing stress in greenhouse-grown grapevines genotypes with varying levels of FT in presence or absence of ABA foliar application. We hypothesize that ABA mimics environmental cues (short day length and low temperature) and induce cold acclimation responses including sugar accumulation.
- **2020 Objective 2**: Elucidate the expression pattern, promoter activity and responsiveness to cold stress of VvMSA genes from grape genotypes with varying levels of FT by heterologous expression in Arabidopsis plants under ABA and cold treatments. We hypothesize that differences in VvMSA coding sequence or cis-acting elements confer altered sensitivities to stress, hormones and sugar signals, implicated in cold stress response.

3. Plant material, experimental design, and growth conditions

A cold sensitive cultivar, V. vinifera 'Pinot noir' and a cold hardy cultivar American-hybrid "Marquette clone FPS11, of economic importance in Michigan, was used in this project. Two-year-old grapevines were cultivated in 7.6 L pots filled with sterilized growing medium. Prior to the start of the experiments, uniform vines (similar shoot length and number of leaves per shoot) were selected and randomly assigned to each treatment. All clusters were removed. The greenhouse conditions were set at 22 °C/19 °C (day/night) with no supplemental lighting. Treated vines were sprayed with a foliar application of 500 mg· L-1 S-ABA using ProTone® SG (Valent BioSciences Corporation,Libertyville, IL) which contains 20 % (w/w) S-ABA as active ingredient. Control vines were sprayed with deionized water.

4. Physiological measurements

Photosynthesis and stomatal conductance were measured on fully developed leaves using a LI-COR 6400XT (LI-COR Biosciences, Lincoln, NE) with CO_2 mixing system and red/blue LED light source. The LI-COR 6400 XT was set at a CO_2 concentration of 400 ppm., photosynthetically active radiation of 1000 µmol m^{-2} s^{-1} and 20°C air temperature and ambient relative humidity. Measurements were taken between the hours of 11:00 am and 12:00 pm on fully expanded main leaves. Bud water content was determined using buds from node position three to eight. They were collected and weighed before and after placing in a drying oven at 80 °C. Bud water content was calculated as the difference between dry and fresh weight and expressed as percentage of fresh weight. Bud dormancy was evaluated based on the dormancy assay that consisted of pruning vines to two buds and monitoring budburst for 30 days under greenhouse conditions and expressed as the number of days to 50 % budburst. Freezing tolerance (FT) of buds was measured on buds with subtending tissues, excised and loaded on each thermoelectric module. The loaded modules were placed in a programmable freezer (Tenny Inc., New Columbia, PA) and subjected to a controlled freezing rate of 4 °C/h by lowering the temperature from −2 °C to the lowest temperature that kills 100 % of the bud tissues. Differential thermal analysis was used to determine the low temperature exotherms (LTE) detected at the ice nucleation temperature of the primary buds.

5. Sugar extraction and quantification

Targeted metabolomic analyses were conducted to quantify specific sugars, including: myo-inositol, galactinol, stachyose, raffinose, sucrose, glucose and fructose. Sugars were extracted and then quantified via capillary electrophoresis. Five buds were excised with a razor blade, flash-

frozen in liquid nitrogen immediately after collection and stored at −80 °C until analysis at the MSU Core Metabolomic facility.

6. Statistical analysis

Data were subjected to two-way analysis of variance (ANOVA) with significant difference set at alpha = 0.05. Multiple comparisons were made using Fisher' least significant difference test (LSD) when ANOVA revealed significant difference.

7. Preliminary results

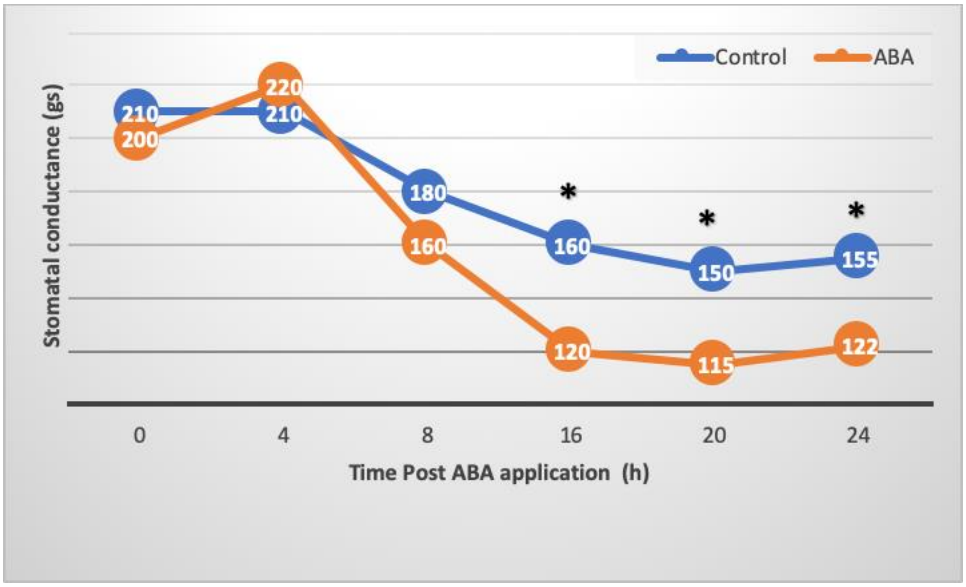

Fig 1. Effect of foliar applied ABA on grapevine leaf stomatal conductance (gs). Significant difference of treatments is indicated by '*' at $P \leq 0.05$. Mean of the two cultivars.

(A) Time (hours)	Control	ABA
8	53 a	55 a
168 (1 week)	38 a	30 b

(B) D50BB	Control	ABA
0	12 a	10 a
336 (2 weeks)	18 b	32 b

	Control	ABA
0	-12 a	-13 a
672 (4 weeks)	-10 a	-15 b

Tab. Effect of exogenous ABA on bud water content, dormancy and bud freezing tolerance). A) bud water content; B) dormancy, expressed in days to 50 % of budburst (D50BB). Higher D50BB indicate deeper dormancy; C) bud freezing tolerance, expressed in LT50 (lethal temperature that

kills 50 % of tissue). Means with different letters between rows indicate significant difference between time points in LSD. Mean of the two cultivars.

8. Preliminary conclusions

Application of foliar ABA impacted vine physiology parameters modifying cold acclimation and bud dormancy. The decrease of leaf gs induced changes to bud water content (lower in treated vine) a signal of early dormancy in grapevines. The increase of bud FT suggested an accelerated transition of grapevine physiology from growth to early stages of cold acclimation. We are currently working in the laboratory on the sugar samples that should confirm all the data we collected so far. In fact, lower water content, deep dormancy and higher freezing tolerance should correlate with an increased accumulation of sugars, including galactinol (precursor), sucrose, and myo-inositol.

This year we will work on understanding ABA impact on sugar pathways. However, increased synthesis may not be the only mechanism contributing to increased sugar accumulation post-ABA treatment. It is also possible that ABA facilitates the translocation of sugar from other plant tissues to buds, resulting in increased accumulation and higher cold tolerance.