Grapevine Molecular and Physiological Responses Under Low Temperature Stress

Thesis

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By

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Abstract

Sub-zero freezing temperatures cause 5-15% of annual crop losses to worldwide grapevine cultivation. Based on their cold hardiness, i.e., the ability to survive under low temperature conditions, grapevine genotypes can be classified as a) cold sensitive, such as *Vitis vinifera* (-18℃ to -22℃ critical range), and b) cold hardy, such as *Vitis labrusca* (-26℃ to -29℃). During spring, late-spring frost conditions can cause injury to young shoots emerging from dormant buds, affecting grapevine yield and wine quality. Enhancing cold hardiness and frost tolerance can improve grapevine's survivability under extreme low temperature conditions. Cold hardy wild grapevine species, such as native North American *Vitis labrusca*, are being utilized for the development of cold hardy hybrid cultivars, however, most of these species have low-chilling requirements, leading to early budburst in spring. Therefore, despite being cold hardy as dormant buds, it is unknown if the young shoots of *Vitis labrusca* have higher frost tolerance than those of *Vitis vinifera* cultivars. Our goal was to determine the difference in frost tolerance and transcriptomic response related to low temperatures between young shoots of cold hardy *V. labrusca* acc. 'GREM4' and cold sensitive *V. vinifera* cv. 'Cabernet Sauvignon'. Results showed that 'GREM4' shoots had significantly higher frost tolerance than those

of ‘Cabernet Sauvignon’. Transcriptomic analysis for chill (4°C) and freeze (-2°C) stress revealed that 'GREM4' shoots exhibited upregulation of genes encoding cell-wall-associated *receptor kinases* and *extensin* proteins under both chill and freeze stress. Moreover, genes encoding *3-ketoacyl-coenzymeA synthase* (*KCS*), a key enzyme involved in wax biosynthesis, and genes related to sugar transport and metabolism were differentially expressed between ‘GREM4’ and ‘Cabernet Sauvignon’. Interaction analysis between species and temperature treatments revealed that the gene encoding abscisic acid (ABA) degrading enzyme *ABA 8’-hydroxylase 3* was significantly upregulated in ‘GREM4’ shoots under chill and freeze stress, indicating ABA’s involvement in frost tolerance. These findings suggests that changes in cell-wall properties or synthesis of very-long-chain fatty acids (VLCFAs) by *KCS* genes might contribute to enhancing frost tolerance of ‘GREM4’ young shoots.

Cold hardiness of dormant buds prone to sub-zero temperatures can be improved by adopting management practices such as spraying cryoprotectants. Potassium has been considered to play a role in cryoprotection as its accumulation can increase cell solute concentration and decrease osmotic potential, ultimately lowering the freezing point of intracellular water. *Vitis spp.* ‘Chambourcin’, a commonly grown grapevine cultivar in Ohio, is considered moderately cold sensitive. Our research focused on determining if foliar potassium application is effective in improving the cold hardiness of ‘Chambourcin’, along with its effect on yield and fruit quality. This study showed that potassium application during the growing season can improve cold hardiness, with no

significant effect on yield. Instead, the foliar application increased berry sugar levels without affecting juice pH.

Overall, these findings will help improve the survivability of grapevines prone to sub-zero temperature conditions during winter and spring, thereby decreasing the negative impact of low temperature stress on yield and fruit quality and increasing the overall profitability of grapevine growers.

Dedication

To my dear brother, Harmanjot Singh.

Thank you for always being my source of inspiration and strength throughout this journey. I dedicate this work to you with all my love and gratitude.

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Fields of Study

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Chapter 1 - Physical and Molecular Responses to Low Temperature Stress in *Vitis spp.*

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Abstract

Freezing temperatures are one of the potential threats to the grape industry, impacting the yield and fruit quality. One of the several measures to mitigate the adverse effects of low temperatures is to develop grapevine cultivars with desirable traits enhancing cold hardiness. Here we provide a comprehensive overview of the effects of cold stress on grapevine morphology and physiology. Two molecular pathways involved in low temperature stress tolerance, the CBF-dependent pathway and the ABA-regulated pathway, will be discussed, as well as the genes involved in regulating these two pathways. An increase in the production of cryoprotectants, including soluble sugars, anti-freeze proteins, and other regulatory metabolites, regulates various physiological and morphological changes leading to either enhancing dormancy or suppressing growth to avoid freeze injury. Further, the prospects of grapevine breeding for developing more cold hardy cultivars along with other advanced strategies to understand the dynamics of cold acclimation and deacclimation are discussed. Overall, a comprehensive understanding of grapevine cold tolerance mechanisms is provided which is essential for developing resilient cultivars to mitigate the impact of sub-freezing temperatures on grape production and wine quality.

Introduction

Grapevine (*Vitis vinifera* L.) is one of the prominent fruit crops cultivated worldwide. Originally domesticated in the Mediterranean, *Vitis vinifera* is well adapted to mild but not severe winter conditions (Londo et al., 2018). Freezing injury is a major problem in

grapevine production, causing an average of 5-15% annual crop loss worldwide (Zabadal et al., 2007). Temperate-to-cool regions, such as northern Europe, the northeast US, and Canada, experience harsh mid-winters and late-spring frost events that pose a threat to grapevine yield and wine quality, inducing “vintage effect”, which refers to the yearly variations in yield and quality (van Leeuwen & Darriet, 2016).

Within the United States, grape production holds significant economic value, amounting to 6 million tons of grapes produced, with an annual revenue of $5.5 billion (USDA, 2022). The economic contribution from the midwestern and eastern grape industries has been increasing in recent years, however, there is a high risk to grape cultivation in these regions due to late spring frosts (Loseke et al., 2015). The eastern United States, in particular, has experienced major spring frost events since 2007, resulting in an estimated annual loss of $250 million, as reported through insurance claims by grape and other fruit growers (Poni et al., 2022). Following that, the Polar Vortex in 2014 caused a significant impact, causing damage to 29%, 57%, and 97% of Ohio's American, hybrid, and *V. vinifera* grape varieties, respectively, resulting in a financial loss of $4 million (Dami, 2014). In addition to impacting crop growth and development, winter injury significantly affects wine quality. This dual effect results in grape growers experiencing both direct losses in crop yield and indirect repercussions due to reduced wine sales (Zabadal et al., 2007).

Freezing injury of buds can be mitigated by some long-term and short-term management practices (Dami, I., 2022). Long-term practices include appropriate site selection and choosing appropriate cultivars for the growing region. Mid-term mitigation

strategies tend to prevent tissue injury by delaying the budburst or extending the period of dormancy. These include double and delayed pruning, cover crop management, evaporative cooling, sprayable cryoprotectants, dormant oils, growth regulators, and ice-nucleation inhibitors. Short-term freezing protection methods include the use of wind machines or heaters prior to or during the freezing or frost event. These short-term and mid-term strategies can help avoid winter injury to some extent, but genotype selection is of prime importance.

Wild *Vitis spp.* have a wide range of chilling requirement (De Rosa et al., 2021), and the level of cold hardiness varies between, as well as within, the species (Londo & Johnson, 2014). Some of these species have been utilized for the development of cold climate interspecific hybrid grapevine (CCIHG) cultivars to boost the viticulture industry in regions most affected by cold damage, such as the Midwestern US (North et al., 2021). The CCIHG cultivars have been developed to combine the desirable fruit quality traits of *V. vinifera* with the midwinter cold-hardiness traits of wild *Vitis* species. These cultivars have acquired the ability to deacclimate at lower temperatures which extends the growth period, however, this also increases the risk of late-spring frost injury to the crop. Therefore, it becomes imperative to understand cold-hardiness dynamics in *Vitis* and how it is being regulated by different genetic and metabolic pathways.

In this review, we aim to provide an overview of grapevine dormancy and how it is being affected by different environmental conditions. We specifically discuss important genetic and metabolic pathways involved in improving cold hardiness or alleviating freezing damage in different grapevine tissues, especially dormant buds and

young shoots or leaves, and how this information can be used to develop breeding strategies to avoid grapevine freezing injury.

Grapevine Bud Dormancy

Apart from genotypic effects, external environmental conditions, especially temperature, greatly affect grapevine phenology, including its cold hardiness and deacclimation (De Rosa et al., 2021; Pagter & Arora, 2013). Being a woody-temperate perennial, grapevine undergoes dormancy to survive subfreezing temperatures. Grapevine (*Vitis vinifera* L.) has two buds that develop in every leaf axil, i) a simple lateral bud axillary to the leaf and ii) a compound bud axillary to the basal prophyll of the lateral bud (Morrison, 1991). The simple lateral bud does not underdo dormancy during winter, but in summer, lateral shoots originate from it during the growing season. On the other hand, the compound bud consists of a central primary bud and a smaller secondary and tertiary bud. The compound bud undergoes dormancy during winter, and thus is also referred as the dormant or latent bud (Morrison, 1991).

Typically, dormancy has been categorized into three types, namely, paradormancy, endodormancy, and ecodormancy (Lang, 1987). Paradormancy is regulated by physiological factors of other tissues within the plant, for example, apical dominance. During late-summer or fall, as the plants experience shorter photoperiods, apparent growth ceases due to complex genetic signaling (Cooke et al., 2012; Fraire-Velázquez et al., 2011). This physiological stage of internal molecular inhibition is known as “endodormancy” (Kühn et al., 2009). The transition from paradormancy to

endodormancy occurs through a process called “acclimation”, which is necessary for plants to withstand low temperature conditions, as well as to acquire synchronous growth upon the onset of climatic conditions conducive to growth during spring. Endodormancy is followed by an increase in cold hardiness of the plants, which is induced by non-freezing low temperatures. However, the time required to acclimate and acquire complete endodormancy, commonly known as the “chilling requirement”, differs among fruit crops, as well as within species (Bañuelos et al., 2008). Once the chilling requirement is fulfilled, the buds become ecodormant, i.e., remain dormant due to external environmental conditions. Once favorable conditions for growth prevail in the spring, grapevines deacclimate and initiate budburst as a physically manifested sign of resuming growth and development (Ault et al., 2013; Byun et al., 2014; Gu et al., 2008; Kovi et al., 2016).

Effect of low temperature stress on grapevine

During fall, plants acclimate to enter endodormancy, however, the requirements to enter endodormancy may differ among *Vitis* species. For example, *Vitis vinifera* requires both short-photoperiod and low temperature to enter endodormancy, whereas *Vitis labrusca*, a wild grapevine species, requires only short-photoperiod (Zabadal et al., 2007). Upon reaching critical day-length, growth ceases as leaf abscission occurs and periderm formation is initiated from the basal portion of the shoot towards the growing tip after four to six weeks of short-daylength photoperiod (Grant et al., 2013; Rubio et al., 2016). The cork cells of periderm release a waxy substance making it nearly impervious to

water, protecting acclimated cells interior to the periderm layer of the stem (Zabadal et al., 2007). Along with that, endodormancy is induced in latent compound buds and the buds are isolated from the vascular system in the canes and trunks (Rubio et al., 2016; Zabadal et al., 2007).

At the cellular level, endodormancy induced by short photoperiod increases starch content and cell wall thickening in meristematic cells. Growth cessation leads to a decrease in water and nutrient uptake from the soil. These physiological and structural changes are required for grapevine to become cold hardy in winter (Rubio et al., 2016). Thus, endodormancy of buds is crucial for achieving a certain degree of cold hardiness (Pérez & Rubio, 2015).

Under low temperature conditions, the osmotically inert starch accumulated during endodormancy is degraded into sugars (osmotically active solutes) simultaneously occurring in different tissues of grapevine, including dormant buds (Fennell, 2004; Guy, 1990; Hamman et al., 1996; Jones et al., 1999; Keller, 2015). These sugars are utilized to generate energy to compensate for the decline in photosynthetic activity, while others get used in the formation of cryoprotectants (Druart et al., 2007; Keller, 2015). Once sugars are transported to other tissues, the outer cork cells get completely suberized, hindering phloem loading and transport. As a result, the interior cells of different tissues are protected from being in contact with the external water and the water content of the tissues is reduced to 42-45% (Keller, 2015).

Prolonged chilling or sub-zero temperatures cause freezing injury to grapevine tissues, and it is greatly affected by the external temperature and the water content in the

tissues or cells (Keller, 2015). There are two mechanisms by which grapevines survive winter conditions: a) desiccation of cell cytoplasm in cane and trunk tissues due to extracellular ice formation and b) the supercooling ability of dormant buds to prevent freezing of intracellular water (Zabadal et al., 2007). Deep-supercooling is a complex process, involving changes in cell membrane structure, sugar concentrations, and the production of osmoprotectants (dehydrin proteins), proline, organic acids, etc. (Hébert-Haché et al., 2021; Pérez & Rubio, 2015; Pierquet & Stushnoff, 1980; Quamme, 1991; Zheng et al., 2018). It is well known that solutes help in lowering the freezing point of water. Intercellular spaces in the apoplast and cell wall regions have higher water content and lower solute concentration, thus, the freezing point of water in intercellular space is higher, compared to intracellular content (Keller, 2015; Wisniewski et al., 2014; Xin & Browse, 2000). Therefore, on experiencing low temperatures, ice formation is initiated in apoplast, however, this ice formation is non-lethal as it does not affect the intracellular components. As ice formation continues, the solutes in the intercellular spaces get separated, generating a water potential gradient, and intracellular water tends to move outside the cells. As a consequence, the solute concentration within the cells increases, which contributes to lowering the freezing point to some extent (Guy, 1990; Keller, 2015; Pearce, 2001; Steponkus, 1984; Thomashow, 1999). Turgor pressure of a cell is lost due to a very high water potential gradient which causes the cell to shrink and the symplast to dehydrate (Browse & Xin, 2001). Ice-formation expansion in the apoplast can rupture the cell membranes, causing freezing injury observed in the form of cell death and plant tissue browning (Guy, 1990; Keller, 2015; Thomashow, 1999; Xin & Browse, 2000).

Molecular mechanism during low temperature stress

Low temperature stress tolerance is a multigenic trait involving the regulation of different transcription factors controlling growth metabolism and enhancing dormancy (Mahajan & Tuteja, 2005). The stress-responsive genes activated under low temperatures are broadly divided into early-induced and late-induced genes. Early-induced genes include transcription factors such as *dehydration-responsive elements* (*DRE*) or *C-repeats* (*CRT*), *ABA-responsive element* (*ABRE*), and *MYB recognition sequence* (*MYBRS*). These transcription factors do not require the synthesis of new proteins, and thus, they are activated within minutes after receiving cold stimulus (Mahajan & Tuteja, 2005). On the other hand, late-induced genes consist of major stress responsive protein-encoding genes, for example, late-embryogenesis abundant (*LEA*) proteins, antioxidant enzyme systems, membrane stabilizing proteins, etc. Some of the important *Vitis* genes involved in low temperature stress tolerance are mentioned in Table 1.1.

Low temperatures induce two major types of regulatory mechanisms in plants: the CBF-transcription factor dependent pathway and the ABA-regulated (CBF-independent) pathway (Wu et al., 2023). Both of these pathways consist of early-induced and late-induced genes, the regulation of which determines the cold tolerance of the tissues.

A. CBF-transcription factor dependent pathway

The CBF-transcription factor-dependent pathway is the most studied low temperature response pathway in different crop species. Prior research on various plant species

suggests low temperatures induce a genetic signal which is similar to that initiated during drought, heat or biotic stresses (Kovaleski & Londo, 2019). On sensing low temperatures, calcium (Ca^{2+}) influx regulates important down-stream genes involved in cold response, such as *MAPK* (Mitogen-activated protein kinase), *ICE* (Inducer of *CBF* Expression) family factors, and *PLD* (Phospholipase D). *CDPKs* (Calcium-dependent protein kinases) and *CNGCs* (Cyclic non-gated ion channels) are involved in Ca^{2+} signaling. Kovaleski and Londo (2019) reported downregulation of nuclear-localized Ca^{2+} channel protein, *CNGC15*, in different wild and cultivated *Vitis* species during deacclimation, suggesting its role in grapevine bud dormancy. Another study on wild species *V. amurensis* showed the role of *VaCPK20* in cold stress response (Dubrovina et al., 2015). Cytosolic Ca^{2+} accumulation is also influenced by membrane rigidification as plant cytoskeletons (including microtubules) are also involved in perceiving external stimuli (Nick, 2013). In conjunction with *CNGC15*, the downregulation of *FATTY-ACID DESATURASE 5* (*FAD5*) may contribute to alterations in membrane fluidity (Kovaleski & Londo, 2019). All these genes could alter the physical properties of the plasma membrane, thus, influencing the calcium signaling to regulate downstream cellular processes.

Low temperature further influences the activity of the transcription factor *BASIC HELIX-LOOP-HELIX* (*bHLHs*) in both *V. vinifera* cv. 'Cabernet Sauvignon' and wild *V. amurensis* (Xu et al., 2014). The gene regulation cascade initiated by low temperature exposure induces the activity of *bHLH* factor family *INDUCER of CBF EXPRESSION* (*ICE*), which further activates the expression of *COLD BINDING FACTOR/DEHYDRATION-RESPONSIVE ELEMENT BINDING* (*CBF/DREB*)

transcription factors (Londo et al., 2018). Out of four *ICE* genes found in grapes (Rahman et al., 2014), *VaICE1* was strongly upregulated under freezing stress in grapevine roots, leaves, stems, and petioles. These *ICE* genes are also regulated by genes, such as *MYELOBLASTOSIS* (*MYB*), E3 ubiquitin ligase *HIGH EXPRESSION OF OSMOTICALLY RESPONSIVE* Gene (*HOS*), and *PLANT U-BOX PROTEIN* (*PUB*). *PUB24* positively regulates the expression of *ICE1* in *V. pseudoreticulata*, whereas *HOS1* competes with *PUB24* to break down *ICE1*. Additionally, genes involved jasmonic acid pathways may involve in regulation of *ICE* factors (Wang et al., 2021; Wu et al., 2014; Yao et al., 2017; Zhao et al., 2016).

Upon activation, *ICE* factors activate *CBF/DREB* transcription factors, that further induce cold-responsive genes. *CBF* is one of the *ETHYLENE-RESPONSIVE ELEMENT BINDING FACTOR* (*ERF*)/ *APETALA2* (*AP2*) super family transcription factors (Licausi et al., 2013; Mizoi et al., 2012), and there are at least seven *CBF* genes present in *V. vinifera* and *V. amurensis* (Carlow et al., 2017). These *CBF* genes may have evolved to function differently under cold stress as they activate different cis-acting elements (Carlow et al., 2017). Transcription factors, such as *MYBs*, *WRKYs*, and *NAC* might also be involved in regulating the expression of *CBFs/DREBs* to improve cold tolerance, as reported in *Arabidopsis* and different grapevine species (Agarwal et al., 2006; Diao et al., 2020; Li et al., 2010; Puranik et al., 2012; Sun et al., 2018; Wang et al., 2014; Zhang et al., 2019; Zhang et al., 2022).

CBF/DREB genes further activate *COLD-RESPONSIVE* (*COR*) genes by binding to their promoter regions (Mahajan & Tuteja, 2005). *CBFs/DREBs* mainly target

DEHYDRINS (*DHNs*) (Group II of late-embryogenesis abundant (*LEA*) proteins), which play an important role in cold acclimation and mitigating freezing injury (Sun et al., 2021; Wisniewski et al., 2014). Other *COR* genes encode cryoprotectants that further induce chaperone encoding genes or lead to an increase in soluble sugar accumulation to enhance low temperature stress tolerance (Mahajan & Tuteja, 2005).

Dehydrins (*DHNs*), a class of thermostable stress proteins, were found to accumulate in buds of *V. labruscana* L. cv. 'Concord' under cold stress (Salzman et al., 1996). These proteins play a role in cell membrane stabilization and activation of antioxidant enzyme systems in different crop species (Sun et al., 2021). Out of four *DHNs* genes identified in *V. vinifera* and *V. yeshanensis* (Yang et al., 2012), *DHN1* and *DHN2* were found to be induced by cold and ABA. *DHN1* was also induced by the application of salicylic acid (SA) and methyl jasmonate (MeJA), the phytohormones that play a role in imparting cold stress tolerance in plants (Raza et al., 2023).

Other cold-induced responses are involved in signal transduction, change in sugar levels, and formation of regulatory proteins, such as mitogen-activated protein kinase (*MAPK*) and the calmodulin-like proteins as found in *A. thaliana* (Kovaleski & Londo, 2019; Mahajan & Tuteja, 2005). Sugar metabolism has been reported to play a major role in enhancing grapevine cold hardiness as there is a strong correlation between total soluble solids accumulation and the lowest external temperatures (Jiang et al., 2014). It was suggested that sucrose plays a role in osmoregulation in grapevine tissues under low, but non-freezing, temperatures by reducing ice nucleation within the apoplast. Other sugars, such as, glucose, fructose, raffinose, and stachyose are also associated with cold

hardiness to varying degree (De Rosa et al., 2022). Raffinose accumulation, in particular, was found to change at a faster rate in response to external temperature changes, however, genes involved in raffinose family oligosaccharides (RFOs) metabolism, such as *RAFFINOSE SYNTHASE* (*RafS)*, and *GALACTINOL SYNTHASE* (*GolS*) need to be further studied to understand its complexities (De Rosa et al., 2022; Sengupta et al., 2015). A study conducted on red grape interspecific hybrid UD 31-103 (Merlot × Kozma 20-3) showed upregulation of *VvRS* (*RAFFINOSE SYNTHASE*) during acclimation (De Rosa et al., 2022). As the temperature decreases further, the upregulation of *HEXOSE-TRANSPORTER VvHT5* and downregulation of *VvHT1* was correlated to an increase in cold hardiness of the buds which was enhanced by higher accumulation of hexoses (glucose). Interestingly, expression of *VvMSA* (*MATURATION, STRESS, ABA*) was found in coordination with *VvHT1*, suggesting sugar metabolism was regulated by hormones such as ABA (De Rosa et al., 2022). On the other hand, a GWAS study conducted by Wang et al. (2021) found a *PHOSPHOGLYCERATE KINASE* (*PGK*) gene associated with cold hardiness of grapevine buds. Another study found an increase in several metabolites, such as galactinol, proline, putrescine in chilling exposed leaves of *V. amurensis* as compared to those of *V. vinifera* cv. 'Muscat Hamburg'. Higher levels of these cryoprotectants could be responsible for higher cold hardiness of wild species *V. amurensis* (Chai et al., 2019).

B. ABA-regulated (CBF-independent) pathway

ABA, along with low temperature, has a synergistic effect on *CBFs/DREBs* in dormant grapevine buds (Rubio et al., 2019). ABA is not involved in inducing, but maintaining and releasing endodormancy (De Rosa et al., 2021). Studies in grapevine have shown a positive correlation between ABA accumulation and acclimation during winters, while deacclimation occurs due to the loss of ABA (Kovaleski & Londo, 2019; Rubio et al., 2019). A decrease in water availability in plant cells could lead to osmotic stress which could stimulate ABA synthesis. Studies in other crops suggest that ABA upregulation during acclimation could induce protein and sugar synthesis, and ROS scavenging system, ultimately leading to higher cold hardiness (Raza et al., 2023). The key enzyme involved in ABA biosynthesis is *9-cis epoxycarotenoid dioxygenase* (*NCED*), while *ABA8'OH* (*ABA 8'-hydroxylase*) is involved in catabolizing ABA, thus regulating dormancy release (Vergara et al., 2017). Londo et al. (2018) found downregulation of *NCED3* under acclimated chill (4°C) treatment, while it was upregulated under freeze and acclimated freeze-treated (-3°C) of *V. vinifera* leaves. The expression of ABA-degrading *VvAH8'-hydroxylase* was upregulated during budburst (Vergara et al., 2017).
ABA-signaling cascade consists of interaction between ABA-receptors and *PP2Cs* (a type *2C PROTEIN PHOSPHATASE*), which can affect the activity of the *ABA-RESPONSIVE ELEMENT BINDING* (*AREBs/ABFs*) transcription factors to regulate ABA-responsive genes. ABA and *PP2Cs* mediate the activity of *SNF-1 RELATED PROTEIN KINASE 2* (*SnRK2*), which further activates *AREBs/ABFs*, ultimately regulating ABA-responsive genes. *SnRK2* is generally inactivated by *PP2C* in the

absence of ABA. However, binding of ABA to *PYR/PYLs* (*PYROBACTIN-RESISTANCE LIKE*) receptors enables the interaction between *PP2C* and ABA-receptors, thus preventing *PP2C* regulation on *SnRK2*. As a result, induced ABA-responsive genes could increase the accumulation of proteins and soluble sugars to improve cold hardiness (Raza et al., 2023; Ren et al., 2023; Zhang & Dami, 2012).

In response to cold (4℃) stimulus*, VaPYL1*, *VaPYL4*, *VaPYL5,* and *VaPYL13* were significantly upregulated in *V. amurensis* (Ren et al., 2022). In another cold-stress (4℃) response study in grapevine, only *VvPP2C1* (out of a total six *PP2Cs*) was found to be upregulated in leaf tissues in response to low temperature, since different *PP2Cs* may be specifically expressed in different organs or stresses (Boneh et al., 2012a). Similarly, Zhang et al. (2021) found upregulation of *VvPP2C14* under low temperature stress, while other *PP2Cs* (*VvPP2C59*, *VvPP2C60*, and *VvPP2C66*) were significantly downregulated. As a result of the downregulation of *PP2Cs*, *SnRK2* gets phosphorylated, and it further activates *AREBs/ABFs* to induce ABA-responsive genes. Among six different *SnRK2s*, *VvSnRK2.1* was highly upregulated in leaves of *V. vinifera* after ABA and cold (4℃) treatment (Boneh et al., 2012b). Under sub-zero temperature conditions, ABA-regulated transcription factors, such as *AREB2* and *ABF4* (*AREB4*) get upregulated, while the *WRKY* expression was different between acclimation and freeze-treated (-3℃) *V. vinifera* leaves (Londo et al., 2018). In another study, it was found that ABA represses the *CELL CYCLE GENES* (*CCG*) in somatic embryo and shoot apex tissue of *V. vinifera* cv. 'Thompson seedless' (Vergara et al., 2017). These results showed that ABA-responsive transcription factors might be directly or indirectly involved in inhibiting plant growth to

avoid freezing injury. Interestingly, in a study conducted on buds of grapevine hybrid Merlot, UD 30-103, a strong correlation was found between the expression of *HEXOSE TRANSPORTERS* (*VvHT1* and *VvHT5*) and *VvMSA* (*MATURATION, STRESS, ABA*), suggesting the role of sugar-hormonal interaction in regulating bud dormancy (De Rosa et al., 2022).

Other transcription factors

In addition to *CBFs/DREBs*, other transcription factors, such as *MYBs* and *WRKYs* are induced under low temperatures to regulate grapevine bud dormancy (Ren et al., 2023). *MYB* in conjunction with *bHLH* are involved in controlling secondary metabolism (Min et al., 2017). *MYB* genes are involved in different pathways, such as stilbene synthesis, phenylpropanoid pathway, and flavonoid synthesis that may play a role under different abiotic stresses by regulating plant growth (Bogs et al., 2007; Czemmel et al., 2009; Deluc et al., 2008; Duan et al., 2016; Höll et al., 2013). Genes involved in stilbene synthesis were upregulated in young shoots of *V. vinifera* cv. 'Sangiovese' subjected to freeze and acclimated freeze conditions (Londo et al., 2018). Additionally, *VvMYB24* has been reported to negatively regulate gibberellin (GA) metabolism involved in plant development (Zhu et al., 2022). On the other hand, ABA has an antagonistic effect on GA biosynthesis, as higher ABA during endodormancy correlates with a decrease in GA metabolism (Zheng et al., 2018). Reducing GA allows the accumulation of growth-suppressing *DELLA* proteins in the cell nucleus, thus enhancing the cold hardiness with a mechanism yet unknown (Keller, 2015).

WRKY transcription factors have been reported to be involved in cold hardiness, sugar conversion and accumulation, and ABA metabolism (Huang et al., 2021; Ren et al., 2023). Wang et al. (2014) found *VvWRKY24* was upregulated under cold stress. Similarly, Li et al. (2010) and Zhang et al. (2019) found higher cold tolerance in *V. pseudoreticulata* and *V. amurensis* due to over-expression of *VpWRKY2* and *VaWRKY12*, respectively. On the other hand, *WRKY70* was found to regulate the changes in defence hormone salicylic acid (SA) in cold-treated buds of *V. vinifera* cv. 'Flame seedless' (Orrantia-Araujo et al., 2021). Salicylic acid has been reported to modulate soluble sugar levels in different crop species (Raza et al., 2023). In different grapevine species, exogenous application of SA has been found to increase the antioxidant enzyme activity, soluble sugar levels, proline, and the expression of *CBFs* (Aazami & Mahna, 2017; Li & Wang, 2021).

Future Prospects and Conclusion

Aberrant environmental conditions, especially in the context of climate change, negatively affect crop yield which could have a drastic effect on food sustainability. In grapevine, low temperatures during mid-winter or later in spring can damage the vegetative plant parts as well as the inflorescences, which could result in damaging the crop for one or more seasons, depending on the level of damage (Scurlock, 2014). Although the prediction of the occurrence of future frost or freezing events lacks consistency among different studies (De Rosa et al., 2021; Kartschall et al., 2015; Mosedale et al., 2015; Santos et al., 2020), it is still an important challenge that grapevine

researchers need to tackle either through long-term strategies or short-term management practices (Dami, 2022).

From a grower's perspective, it is important vines are cold hardy enough to survive extreme negative temperatures during mid-winter and resilient enough to withstand freezing injury of sensitive vegetative plant parts due to late-spring frosts (Kovaleski et al., 2018; Vitasse et al., 2014). Therefore, it is important to understand the genetics contributing to cold hardiness to develop cold hardy and frost-tolerant cultivars. This chapter provides an overview of different pathways involved in grapevine cold hardiness and how these pathways affect the expression of different cold-responsive genes. This knowledge can be useful for grapevine breeding programs aiming to improve abiotic stress tolerance.

Efforts have already been made to develop cold hardy cultivars by hybridizing different *Vitis* species, especially wild species, for the introgression of desirable cold adaptive traits. However, more emphasis should be given to the development of region-specific cultivars as acute changes in environmental conditions and variation in grapevine growing latitudes could affect chilling accumulation (Kovaleski et al., 2018). The chilling requirements and budburst rate often vary across different *Vitis* species, as well as within species (Londo & Johnson, 2014). Further, since cold hardiness within a plant system is a dynamic change (Ferguson et al., 2014; Keller, 2015), machine learning models, such as WAUS.2, and NYUS.1, or artificial intelligence can be utilized to predict the frequency of future freezing events based on the environmental data available for specific regions (Kovaleski et al., 2022).

Advanced genetic mapping approaches are required for the development of molecular markers to locate the genetic regions or QTLs (quantitative trait loci) that contribute to cold hardiness in grapevine. Apart from molecular markers, the genetic variation present in wild grapevine species can be studied using genome-wide association (GWA) to identify other morphological or physiological traits that contribute to mitigating cold stress, such as high trichome density, different leaf structures, etc. Although some genes have been identified as potential candidates involved in low temperature response (Table 1.1), characterization of potential genes imparting a higher degree of cold hardiness is required. Further, extensive research is required to determine how those genes interact with different regulatory networks under different environmental conditions, including low temperature stress. Advanced understanding of epigenetic regulation contributing to cold hardiness could explain the dynamics of acclimation and deacclimation kinetics (De Rosa et al., 2021; Kumar, 2018; Wisniewski et al., 2018). Overall, gaining a thorough understanding of the various pathways involved in cold hardiness will enhance our comprehension of this highly quantitative trait, leading to the development of more robust cultivars.

References

Aazami, M. A., & Mahna, N. (2017). Salicylic acid affects the expression of VvCBF4 gene in grapes subjected to low temperature. *Journal of Genetic Engineering and Biotechnology*, *15*(1), 257–261. https://doi.org/10.1016/j.jgeb.2017.01.005

Agarwal, M., Hao, Y., Kapoor, A., Dong, C.-H., Fujii, H., Zheng, X., & Zhu, J.-K. (2006). A R2R3 Type MYB Transcription Factor Is Involved in the Cold Regulation of CBF Genes and in Acquired Freezing Tolerance. *Journal of Biological Chemistry*, *281*(49), 37636–37645. https://doi.org/10.1074/jbc.M605895200

Ault, T. R., Henebry, G. M., de Beurs, K. M., Schwartz, M. D., Betancourt, J. L., & Moore, D. (2013). The False Spring of 2012, Earliest in North American Record. *Eos, Transactions American Geophysical Union*, *94*(20), 181–182. https://doi.org/10.1002/2013EO200001

Bañuelos, M. L. G., Moreno, L. V., Winzerling, J., Orozco, J. A., & Gardea, A. A. (2008). Winter Metabolism in Deciduous Trees: Mechanisms, Genes and Associated Proteins. *31*, 16.

Bogs, J., Jaffé, F. W., Takos, A. M., Walker, A. R., & Robinson, S. P. (2007). The Grapevine Transcription Factor VvMYBPA1 Regulates Proanthocyanidin Synthesis during Fruit Development. *Plant Physiology*, *143*(3), 1347–1361. https://doi.org/10.1104/pp.106.093203

Boneh, U., Biton, I., Schwartz, A., & Ben-Ari, G. (2012b). Characterization of the ABA signal transduction pathway in *Vitis vinifera*. *Plant Science*, *187*, 89–96. https://doi.org/10.1016/j.plantsci.2012.01.015

Boneh, U., Biton, I., Zheng, C., Schwartz, A., & Ben-Ari, G. (2012a). Characterization of potential ABA receptors in *Vitis vinifera*. *Plant Cell Reports*, *31*(2), 311–321. https://doi.org/10.1007/s00299-011-1166-z

Browse, J., & Xin, Z. (2001). Temperature sensing and cold acclimation. *Current Opinion in Plant Biology*, *4*(3), 241–246. https://doi.org/10.1016/s1369-5266(00)00167-9

Byun, Y.-J., Koo, M.-Y., Joo, H.-J., Ha-Lee, Y.-M., & Lee, D.-H. (2014). Comparative analysis of gene expression under cold acclimation, deacclimation and reacclimation in Arabidopsis. *Physiologia Plantarum*, *152*(2), 256–274. https://doi.org/10.1111/ppl.12163

Carlow, C. E., Faultless, J. T., Lee, C., Siddiqua, M., Edge, A., & Nassuth, A. (2017). Nuclear localization and transactivation by *Vitis* CBF transcription factors are regulated by combinations of conserved amino acid domains. *Plant Physiology and Biochemistry: PPB*, *118*, 306–319. https://doi.org/10.1016/j.plaphy.2017.06.027

Chai, F., Liu, W., Xiang, Y., Meng, X., Sun, X., Cheng, C., Liu, G., Duan, L., Xin, H., & Li, S. (2019). Comparative metabolic profiling of *Vitis amurensis* and *Vitis vinifera* during cold acclimation. *Horticulture Research*, *6*(1), Article 1. https://doi.org/10.1038/s41438-018-0083-5

Cooke, J. E. K., Eriksson, M. E., & Junttila, O. (2012). The dynamic nature of bud dormancy in trees: Environmental control and molecular mechanisms. *Plant, Cell & Environment*, *35*(10), 1707–1728. https://doi.org/10.1111/j.1365-3040.2012.02552.x

Czemmel, S., Stracke, R., Weisshaar, B., Cordon, N., Harris, N. N., Walker, A. R., Robinson, S. P., & Bogs, J. (2009). The Grapevine R2R3-MYB Transcription Factor VvMYBF1 Regulates Flavonol Synthesis in Developing Grape Berries. *Plant Physiology*, *151*(3), 1513–1530. https://doi.org/10.1104/pp.109.142059

Dami, I. (2014). 2014 Grape Winter Damage Survey. *Crop Science*, 11.

Dami, I. (2022). Spring Frost Injury of Grapevines and Protection Methods. OSU Extension Publications. https://extensionpubs.osu.edu/spring-frost-injury-of-grapevines-and-protection-methods/

De Rosa, V., Falchi, R., Moret, E., & Vizzotto, G. (2022). Insight into Carbohydrate Metabolism and Signaling in Grapevine Buds during Dormancy Progression. *Plants*, *11*(8), 1027. https://doi.org/10.3390/plants11081027

De Rosa, V., Vizzotto, G., & Falchi, R. (2021). Cold Hardiness Dynamics and Spring Phenology: Climate-Driven Changes and New Molecular Insights Into Grapevine Adaptive Potential. *Frontiers in Plant Science*, *12*, 644528. https://doi.org/10.3389/fpls.2021.644528

Deluc, L., Bogs, J., Walker, A. R., Ferrier, T., Decendit, A., Merillon, J.-M., Robinson, S. P., & Barrieu, F. (2008). The Transcription Factor VvMYB5b Contributes to the Regulation of Anthocyanin and Proanthocyanidin Biosynthesis in Developing Grape Berries. *Plant Physiology*, *147*(4), 2041–2053. https://doi.org/10.1104/pp.108.118919

Diao, P., Chen, C., Zhang, Y., Meng, Q., Lv, W., & Ma, N. (2020). The role of NAC transcription factor in plant cold response. *Plant Signaling & Behavior*, *15*(9), 1785668. https://doi.org/10.1080/15592324.2020.1785668

Druart, N., Johansson, A., Baba, K., Schrader, J., Sjödin, A., Bhalerao, R. R., Resman, L., Trygg, J., Moritz, T., & Bhalerao, R. P. (2007). Environmental and hormonal regulation of the activity–dormancy cycle in the cambial meristem involves stage-specific modulation of transcriptional and metabolic networks. *The Plant Journal*, *50*(4), 557–573. https://doi.org/10.1111/j.1365-313X.2007.03077.x

Duan, D., Fischer, S., Merz, P., Bogs, J., Riemann, M., & Nick, P. (2016). An ancestral allele of grapevine transcription factor MYB14 promotes plant defence. *Journal of Experimental Botany*, *67*(6), 1795–1804. https://doi.org/10.1093/jxb/erv569

Dubrovina, A. S., Kiselev, K. V., Khristenko, V. S., & Aleynova, O. A. (2015). VaCPK20, a calcium-dependent protein kinase gene of wild grapevine *Vitis amurensis* Rupr., mediates cold and drought stress tolerance. *Journal of Plant Physiology*, *185*, 1–12. https://doi.org/10.1016/j.jplph.2015.05.020

Duchêne, E., Butterlin, G., Dumas, V., & Merdinoglu, D. (2012). Towards the adaptation of grapevine varieties to climate change: QTLs and candidate genes for developmental stages. *Theoretical and Applied Genetics*, *124*(4), 623–635. https://doi.org/10.1007/s00122-011-1734-1

Fennell, A. (2004). Freezing tolerance and injury in grapevines. *Journal of Crop Improvement*, *10*(1/2), 201–235. https://doi.org/10.1300/J411v10n01_09

Ferguson, J. C., Moyer, M., Mills, L., Hoogenboom, G., & Keller, M. (2014). Modeling Dormant Bud Cold Hardiness and Budbreak in Twenty-Three *Vitis* Genotypes Reveals Variation by Region of Origin. *American Journal of Enology and Viticulture*, *65*, 59–71. https://doi.org/10.5344/ajev.2013.13098

Fraire-Velázquez, S., Rodríguez-Guerra, R., & Sánchez-Calderón, L. (2011). Abiotic and biotic stress response crosstalk in plants. *Abiotic Stress Response in Plants—Physiological, Biochemical and Genetic Perspectives*, 3–26.

Grant, T. N. L., Gargrave, J., & Dami, I. E. (2013). Morphological, Physiological, and Biochemical Changes in Vitis Genotypes in Response to Photoperiod Regimes. *American Journal of Enology and Viticulture*, *64*(4), 466–475. https://doi.org/10.5344/ajev.2013.13060

Gu, L., Hanson, P. J., Post, W. M., Kaiser, D. P., Yang, B., Nemani, R., Pallardy, S. G., & Meyers, T. (2008). The 2007 Eastern US Spring Freeze: Increased Cold Damage in a Warming World? *BioScience*, *58*(3), 253–262. https://doi.org/10.1641/B580311

Guy, C. L. (1990). Cold Acclimation and Freezing Stress Tolerance: Role of Protein Metabolism.

Hamman, R. A., Dami, I., Walsh, T. M., & Stushnoff, C. (1996). Seasonal Carbohydrate Changes and Cold Hardiness of Chardonnay and Riesling Grapevines. *American Journal of Enology and Viticulture*, *47*, 31–36. https://doi.org/10.5344/ajev.1996.47.1.31

Hébert-Haché, A., Inglis, D., Kemp, B., & Willwerth, J. J. (2021). Clone and Rootstock Interactions Influence the Cold Hardiness of *Vitis vinifera* cvs. Riesling and Sauvignon blanc. *American Journal of Enology and Viticulture*, *72*(2), 126–136. https://doi.org/10.5344/ajev.2020.20025

Höll, J., Vannozzi, A., Czemmel, S., D'Onofrio, C., Walker, A. R., Rausch, T., Lucchin, M., Boss, P. K., Dry, I. B., & Bogs, J. (2013). The R2R3-MYB Transcription Factors MYB14 and MYB15 Regulate Stilbene Biosynthesis in *Vitis vinifera*. *The Plant Cell*, *25*(10), 4135–4149. https://doi.org/10.1105/tpc.113.117127

Huang, T., Yang, J., Yu, D., Han, X., & Wang, X. (2021). Bioinformatics analysis of WRKY transcription factors in grape and their potential roles prediction in sugar and abscisic acid signaling pathway. *Journal of Plant Biochemistry and Biotechnology*, *30*(1), 67–80. https://doi.org/10.1007/s13562-020-00571-y

Jiang, H.-Y., Li, W., He, B.-J., Gao, Y.-H., & Lu, J.-X. (2014). Sucrose metabolism in grape (Vitis vinifera L.) branches under low temperature during overwintering covered with soil. *Plant Growth Regulation*, *72*(3), 229–238. https://doi.org/10.1007/s10725-013-9854-z

Jiang, W., & Yu, D. (2009). Arabidopsis WRKY2 transcription factor mediates seed germination and postgermination arrest of development by abscisic acid. *BMC Plant Biology*, *9*. https://doi.org/10.1186/1471-2229-9-96

Jones, K. S., Paroschy, J., Mckersie, B. D., & Bowley, S. R. (1999). Carbohydrate Composition and Freezing Tolerance of Canes and Buds in *Vitis vinifera*. *Journal of Plant Physiology*, *155*(1), 101–106. https://doi.org/10.1016/S0176-1617(99)80146-1

Kartschall, T., Wodinski, M., von Bloh, W., Oesterle, H., Rachimow, C., & Hoppmann, D. (2015). Changes in phenology and frost risks of *Vitis vinifera* (cv Riesling). *Meteorologische Zeitschrift*, 189–200. https://doi.org/10.1127/metz/2015/0534

Keller. (2015). The Science of Grapevines—2nd Edition. https://shop.elsevier.com/books/the-science-of-grapevines/keller/978-0-12-419987-3

Kovaleski, A. P., & Londo, J. P. (2019). Tempo of gene regulation in wild and cultivated *Vitis* species shows coordination between cold deacclimation and budbreak. *Plant Science*, *287*, 110178. https://doi.org/10.1016/j.plantsci.2019.110178

Kovaleski, A. P., North, M. G., Martinson, T. E., & Londo, J. P. (2022). Development of a new cold hardiness prediction model for grapevine using phased integration of acclimation and deacclimation responses (p. 2022.09.09.507298). *bioRxiv.* https://doi.org/10.1101/2022.09.09.507298

Kovaleski, A. P., Reisch, B. I., & Londo, J. P. (2018). Deacclimation kinetics as a quantitative phenotype for delineating the dormancy transition and thermal efficiency for budbreak in *Vitis* species. *AoB PLANTS*. https://doi.org/10.1093/aobpla/ply066

Kovi, M. R., Ergon, Å., & Rognli, O. A. (2016). Freezing tolerance revisited—Effects of variable temperatures on gene regulation in temperate grasses and legumes. *Current Opinion in Plant Biology*, *33*, 140–146. https://doi.org/10.1016/j.pbi.2016.07.006

Kühn et al. (2009). Photoperiod modifies the diurnal expression profile of VvPHYA and VvPHYB transcripts in field-grown grapevine leaves. https://pubmed.ncbi.nlm.nih.gov/19232775/

Kumar, S. (2018). Epigenetic Memory of Stress Responses in Plants. *Journal of J Phytochemistry & Biochemistry*, *2*, 1–2.

Lang, G. A. (1987). Endo-, Para-and Ecodormancy: Physiological Terminology and Classification for Dormancy Research. *Hortic. Sci.*, *22*, 271–277.

Li, B., & Wang, W. (2021). Salicylic acid induces tolerance of *Vitisriparia×V.labrusca* to chilling stress by altered photosynthetic, antioxidant mechanisms and expression of cold stress responsive genes. *Plant Signaling & Behavior*, *16*(11), 1973711. https://doi.org/10.1080/15592324.2021.1973711

Li, H., Xu, Y., Xiao, Y., Zhu, Z., Xie, X., Zhao, H., & Wang, Y. (2010). Expression and functional analysis of two genes encoding transcription factors, VpWRKY1 and VpWRKY2, isolated from Chinese wild *Vitis pseudoreticulata. Planta*, *232*(6), 1325–1337. https://doi.org/10.1007/s00425-010-1258-y

Licausi, F., Ohme-Takagi, M., & Perata, P. (2013). APETALA2/Ethylene Responsive Factor (AP2/ERF) transcription factors: Mediators of stress responses and developmental programs. *New Phytologist*, *199*(3), 639–649. https://doi.org/10.1111/nph.12291

Londo, J. P., & Johnson, L. M. (2014). Variation in the chilling requirement and budburst rate of wild *Vitis* species. *Environmental and Experimental Botany*, *106*, 138–147. https://doi.org/10.1016/j.envexpbot.2013.12.012

Londo, J. P., Kovaleski, A. P., & Lillis, J. A. (2018). Divergence in the transcriptional landscape between low temperature and freeze shock in cultivated grapevine (*Vitis vinifera*). *Horticulture Research*, *5*.

Loseke, B., Read, P., & Blankenship, E. (2015). Preventing spring freeze injury on grapevines using multiple applications of Amigo Oil and naphthaleneacetic acid. *Scientia Horticulturae*, *193*, 294–300. https://doi.org/10.1016/J.SCIENTA.2015.07.025

Mahajan, S., & Tuteja, N. (2005). Cold, salinity and drought stresses: An overview. *Archives of Biochemistry and Biophysics*, *444*(2), 139–158. https://doi.org/10.1016/j.abb.2005.10.018

Min, Z., Zhao, X., Li, R., Yang, B., Liu, M., & Fang, Y. (2017). Comparative transcriptome analysis provides insight into differentially expressed genes related to bud dormancy in grapevine (*Vitis vinifera*). *Scientia Horticulturae*, *225*, 213–220. https://doi.org/10.1016/j.scienta.2017.06.033

Mizoi, J., Shinozaki, K., & Yamaguchi-Shinozaki, K. (2012). AP2/ERF family transcription factors in plant abiotic stress responses. *Biochimica et Biophysica Acta (BBA) - Gene Regulatory Mechanisms*, *1819*(2), 86–96. https://doi.org/10.1016/j.bbagrm.2011.08.004

Morrison, J. C. (1991). Bud Development in *Vitis vinifera* L. *Botanical Gazette*, *152*(3), 304–315. https://doi.org/10.1086/337894

Mosedale, J. R., Wilson, R. J., & Maclean, I. M. D. (2015). Climate Change and Crop Exposure to Adverse Weather: Changes to Frost Risk and Grapevine Flowering Conditions. *PloS One*, *10*(10), e0141218. https://doi.org/10.1371/journal.pone.0141218

Nick. (2013). Microtubules, signalling and abiotic stress. *Plant J. 2013:75(2)*. https://onlinelibrary.wiley.com/doi/10.1111/tpj.12102

North, M., Workmaster, B., & Atucha, A. (2021). Cold Hardiness of Cold Climate Interspecific Hybrid Grapevines Grown in a Cold Climate Region. *American Journal of Enology and Viticulture*, *72*, 318–327. https://doi.org/10.5344/ajev.2021.21001

Orrantia-Araujo, M. A., Martínez-Téllez, M. Á., Rivera-Domínguez, M., Hernández-Oñate, M. Á., & Vargas-Arispuro, I. (2021). Changes in the Endogenous Content and Gene Expression of Salicylic Acid Correlate with Grapevine Bud Dormancy Release. *Journal of Plant Growth Regulation*, *40*(1), 254–262. https://doi.org/10.1007/s00344-020-10100-9

Pagter, M., & Arora, R. (2013). Winter survival and deacclimation of perennials under warming climate: Physiological perspectives. *Physiologia Plantarum*, *147*(1), 75–87. https://doi.org/10.1111/j.1399-3054.2012.01650.x

Pearce, R. S. (2001). Plant Freezing and Damage. *Annals of Botany*, *87*(4), 417–424. https://doi.org/10.1006/anbo.2000.1352

Pérez, F., & Rubio, S. (2015). Prolonged Exposure of Grapevine Buds to Low Temperatures Increases Dormancy, Cold Hardiness, and the Expression of Vvα-AMYs Genes. https://doi.org/10.1007/978-3-319-14451-1_10

Pierquet, P., & Stushnoff, C. (1980). Relationship Of Low Temperature Exotherms to Cold Injury in *Vitis riparia* Michx. *31*(1), 7.

Poni, S., Sabbatini, P., & Palliotti, A. (2022). Facing Spring Frost Damage in Grapevine: Recent Developments and the Role of Delayed Winter Pruning – A Review. *American Journal of Enology and Viticulture*. https://doi.org/10.5344/ajev.2022.22011

Puranik, S., Sahu, P. P., Srivastava, P. S., & Prasad, M. (2012). NAC proteins: Regulation and role in stress tolerance. *Trends in Plant Science*, *17*(6), 369–381. https://doi.org/10.1016/j.tplants.2012.02.004

Quamme, H. A. (1991). Application of Thermal Analysis to Breeding Fruit Crops for Increased Cold Hardiness. *HortScience*, *26*(5), 513–517. https://doi.org/10.21273/HORTSCI.26.5.513

Rahman, M. A., Moody, M. A., & Nassuth, A. (2014). Grape contains 4 ICE genes whose expression includes alternative polyadenylation, leading to transcripts encoding at least 7 different ICE proteins. *Environmental and Experimental Botany*, *106*, 70–78. https://doi.org/10.1016/j.envexpbot.2014.01.003

Raza, A., Charagh, S., Najafi-Kakavand, S., Abbas, S., Shoaib, Y., Anwar, S., Sharifi, S., Lu, G., & Siddique, K. H. M. (2023). Role of phytohormones in regulating cold stress tolerance: Physiological and molecular approaches for developing cold-smart crop plants. *Plant Stress*, *8*, 100152. https://doi.org/10.1016/j.stress.2023.100152

Ren, C., Fan, P., Li, S., & Liang, Z. (2023). Advances in understanding cold tolerance in grapevine. *Plant Physiology*, *192*(3), 1733–1746. https://doi.org/10.1093/plphys/kiad092

Ren, C., Kuang, Y., Lin, Y., Guo, Y., Li, H., Fan, P., Li, S., & Liang, Z. (2022). Overexpression of grape ABA receptor gene VaPYL4 enhances tolerance to multiple abiotic stresses in Arabidopsis. *BMC Plant Biology*, *22*(1), 271. https://doi.org/10.1186/s12870-022-03663-0

Rubio, S., Dantas, D., Bressan-Smith, R., & Pérez, F. (2016). Relationship Between Endodormancy and Cold Hardiness in Grapevine Buds. *Journal of Plant Growth Regulation*, *35*, 266–275. https://doi.org/10.1007/s00344-015-9531-8

Rubio, S., Noriega, X., & Perez, F. (2019). Abscisic acid (ABA) and low temperatures synergistically increase the expression of CBF/DREB1 transcription factors and cold-hardiness in grapevine dormant buds. *Annals of Botany*, *123*(4), 681–689. https://doi.org/10.1093/aob/mcy201

Salzman et al. (1996). Programmed accumulation of LEA-like proteins during desiccation and cold acclimation of overwintering grape buds—*Plant, Cell & Environment—Wiley Online Library*. https://onlinelibrary.wiley.com/doi/10.1111/j.1365-3040.1996.tb00406.x

Santos, J., Fraga, H., Malheiro, A., Moutinho-Pereira, J., Dinis, L., Correia, C., Moriondo, M., Leolini, L., Dibari, C., Costafreda-Aumedes, S., Kartschall, T., Menz, C., Molitor, D., Junk, J., Beyer, M., & Schultz, H. (2020). A Review of the Potential Climate Change Impacts and Adaptation Options for European Viticulture. *Applied Sciences-Basel*, *10*(9). https://doi.org/10.3390/app10093092

Scurlock, D. (2014). Ohio Grape-Wine Electronic Newsletter. 16.

Sengupta, S., Mukherjee, S., Basak, P., & Majumder, A. L. (2015). Significance of galactinol and raffinose family oligosaccharide synthesis in plants. *Frontiers in Plant Science*, *6*. https://www.frontiersin.org/articles/10.3389/fpls.2015.00656

Steponkus, P. L. (1984). Role of the Plasma Membrane in Freezing Injury and Cold Acclimation. *Annual Review of Plant Physiology*, *35*(1), 543–584. https://doi.org/10.1146/annurev.pp.35.060184.002551

Sun, X., Tomas Matus, J., Wong, D. C. J., Wang, Z., Chai, F., Zhang, L., Fang, T., Zhao, L., Wang, Y., Han, Y., Wang, Q., Li, S., Liang, Z., & Xin, H. (2018). The GARP/MYB-related grape transcription factor AQUILO improves cold tolerance and promotes the accumulation of raffinose family oligosaccharides. *Journal of Experimental Botany*, *69*(7), 1749–1764. https://doi.org/10.1093/jxb/ery020

Sun, Z., Li, S., Chen, W., Zhang, J., Zhang, L., Sun, W., & Wang, Z. (2021). Plant Dehydrins: Expression, Regulatory Networks, and Protective Roles in Plants Challenged by Abiotic Stress. *International Journal of Molecular Sciences*, *22*(23), 12619. https://doi.org/10.3390/ijms222312619

Thomashow, M. F. (1999). Plant Cold Acclimation: Freezing Tolerance Genes and Regulatory Mechanisms. *Annual Review of Plant Physiology and Plant Molecular Biology*, *50*, 571–599. https://doi.org/10.1146/annurev.arplant.50.1.571

USDA. (2022). NonCitrus Fruits and Nuts 2021 Summary (p. 88). https://downloads.usda.library.cornell.edu/usda-esmis/files/zs25x846c/4q77gv96p/8336j672v/ncit0522.txt

van Leeuwen, C., & Darriet, P. (2016). The Impact of Climate Change on Viticulture and Wine Quality. *Journal of Wine Economics*, *11*, 150–167. https://doi.org/10.1017/jwe.2015.21

Vergara, R., Noriega, X., Aravena, K., Prieto, H., & Pérez, F. (2017). ABA Represses the Expression of Cell Cycle Genes and May Modulate the Development of Endodormancy in Grapevine Buds. *Frontiers in Plant Science*, *8*, null. https://doi.org/10.3389/fpls.2017.00812

Vitasse, Y., Lenz, A., & Körner, C. (2014). The interaction between freezing tolerance and phenology in temperate deciduous trees. *Frontiers in Plant Science*, *5*. https://www.frontiersin.org/articles/10.3389/fpls.2014.00541

Wang, L., Zhu, W., Fang, L., Sun, X., Su, L., Liang, Z., Wang, N., Londo, J. P., Li, S., & Xin, H. (2014). Genome-wide identification of WRKY family genes and their response to cold stress in *Vitis vinifera*. *BMC Plant Biology*, *14*. https://doi.org/10.1186/1471-2229-14-103

Wang, Y., Xin, H., Fan, P., Zhang, J., Liu, Y., Dong, Y., Wang, Z., Yang, Y., Zhang, Q., Ming, R., Zhong, G.-Y., Li, S., & Liang, Z. (2021). The genome of Shanputao (*Vitis amurensis*) provides a new insight into cold tolerance of grapevine. *The Plant Journal: For Cell and Molecular Biology*, *105*(6), 1495–1506. https://doi.org/10.1111/tpj.15127

Wang, Z., Wong, D. C. J., Wang, Y., Xu, G., Ren, C., Liu, Y., Kuang, Y., Fan, P., Li, S., Xin, H., & Liang, Z. (2021). GRAS-domain transcription factor PAT1 regulates jasmonic acid biosynthesis in grape cold stress response. *Plant Physiology*, *186*(3), 1660–1678. https://doi.org/10.1093/plphys/kiab142

Wisniewski, M., Nassuth, A., & Arora, R. (2018). Cold Hardiness in Trees: A Mini-Review. *Frontiers in Plant Science*, *9*. https://www.frontiersin.org/article/10.3389/fpls.2018.01394

Wisniewski, M., Nassuth, A., Teulieres, C., Marque, C., Rowland, J., Cao, P. B., & Brown, A. (2014). Genomics of Cold Hardiness in Woody Plants. *Critical Reviews in Plant Sciences*, *33*(2–3), 92–124. https://doi.org/10.1080/07352689.2014.870408

Wu, J., Zhang, Y., Yin, L., Qu, J., & Lu, J. (2014). Linkage of cold acclimation and disease resistance through plant–pathogen interaction pathway in *Vitis amurensis* grapevine. *Functional & Integrative Genomics*, *14*(4), 741–755. https://doi.org/10.1007/s10142-014-0392-1

Wu, N., Li, J., Zheng, Q., Wu, J., & Xu, W. (2023). Characterization of the core region of grape VvHOS1 promoter activity and its upstream regulatory proteins. *Environmental and Experimental Botany*, *207*. https://doi.org/10.1016/j.envexpbot.2022.105199

Xin, Z., & Browse, J. (2000). Cold comfort farm: The acclimation of plants to freezing temperatures. *Plant, Cell & Environment*, *23*(9), 893–902. https://doi.org/10.1046/j.1365-3040.2000.00611.x

Xu, W., Zhang, N., Jiao, Y., Li, R., Xiao, D., & Wang, Z. (2014). The grapevine basic helix-loop-helix (bHLH) transcription factor positively modulates CBF-pathway and confers tolerance to cold-stress in *Arabidopsis*. *Molecular Biology Reports*, *41*(8), 5329–5342. https://doi.org/10.1007/s11033-014-3404-2

Yang, Y., He, M., Zhu, Z., Li, S., Xu, Y., Zhang, C., Singer, S. D., & Wang, Y. (2012). Identification of the dehydrin gene family from grapevine species and analysis of their responsiveness to various forms of abiotic and biotic stress. *BMC Plant Biology*, *12*, 140. https://doi.org/10.1186/1471-2229-12-140

Yao, W., Wang, L., Wang, J., Ma, F., Yang, Y., Wang, C., Tong, W., Zhang, J., Xu, Y., Wang, X., Zhang, C., & Wang, Y. (2017). VpPUB24, a novel gene from Chinese grapevine, *Vitis pseudoreticulata*, targets VpICE1 to enhance cold tolerance. *Journal of Experimental Botany*, *68*(11), 2933–2949. https://doi.org/10.1093/jxb/erx136

Zabadal, T., Dami, I., & Goffinet, M. (2007). Winter Injury to Grapevines and Methods of Protection.

Zhang et al. (2019). Overexpression of VaWRKY12, a transcription factor from *Vitis amurensis* with increased nuclear localization under low temperature, enhances cold tolerance of plants. *Plant Mol Biol. 2019:100(1–2):95–110.* https://doi.org/10.1007/s11103-019-00846-6

Zhang, H., Hu, Y., Gu, B., Cui, X., & Zhang, J. (2022). VaMYB44 transcription factor from Chinese wild *Vitis amurensis* negatively regulates cold tolerance in transgenic Arabidopsis thaliana and V. vinifera. *Plant Cell Reports*, *41*(8), 1673–1691. https://doi.org/10.1007/s00299-022-02883-w

Zhang, R., Wang, Y., Li, S., Yang, L., & Liang, Z. (2021). ABA signaling pathway genes and function during abiotic stress and berry ripening in Vitis vinifera. *Gene*, *769*. https://doi.org/10.1016/j.gene.2020.145226

Zhang, Y., & Dami, I. (2012). Improving Freezing Tolerance of "Chambourcin" Grapevines with Exogenous Abscisic Acid. *HortScience: A Publication of the*

American Society for Horticultural Science, *47*, 1750–1757. https://doi.org/10.21273/HORTSCI.47.12.1750

Zhao, N., Lin, H., Lan, S., Jia, Q., Chen, X., Guo, H., & Chen, F. (2016). VvMJE1 of the grapevine (*Vitis vinifera*) VvMES methylesterase family encodes for methyl jasmonate esterase and has a role in stress response. *Plant Physiology and Biochemistry*, *102*, 125–132. https://doi.org/10.1016/j.plaphy.2016.02.027

Zheng, C., Acheampong, A. K., Shi, Z., Mugzech, A., Halaly-Basha, T., Shaya, F., Sun, Y., Colova, V., Mosquna, A., Ophir, R., Galbraith, D. W., & Or, E. (2018). Abscisic acid catabolism enhances dormancy release of grapevine buds. *Plant, Cell & Environment*, *41*(10), 2490–2503. https://doi.org/10.1111/pce.13371

Zheng, C., Acheampong, A., Shi, Z., Halaly, T., Kamiya, Y., Ophir, R., Galbraith, D., & Or, E. (2018). Distinct gibberellin functions during and after grapevine bud dormancy release. *Journal of Experimental Botany*, *69*(7), 1635–1648. https://doi.org/10.1093/jxb/ery022

Zhu, Z., Quan, R., Chen, G., Yu, G., Li, X., Han, Z., Xu, W., Li, G., Shi, J., & Li, B. (2022). An R2R3-MYB transcription factor VyMYB24, isolated from wild grape *Vitis yanshanesis* J. X. Chen., regulates the plant development and confers the tolerance to drought. *Frontiers in Plant Science*, *13*. https://doi.org/10.3389/fpls.2022.966641

Table

Table 1.1 *Vitis* genes involved in cold hardiness.

Gene Name	Species	Reference
CALCIUM-DEPENDENT PROTEIN KINASE 20 (CPK20)	*Vitis amurensis*	Dunrovina et al. (2015)
CYCLIC NON-GATED ION CHANNEL 15 (CNGC15)	*Vitis spp.*	Kovaleski and Londo (2019)
FATTY ACID DESATURASE 5 (FAD5)	*Vitis spp.*	Kovaleski and Londo (2019)
BASIC HELIX-LOOP-HELIX (bHLHs)	*Vitis amurensis and Vitis vinifera*	Xu et al. (2014)
INDUCER OF CBF EXPRESSION 1 (ICE 1)	*Vitis amurensis*	Rahman et al. (2014)
PLANT U-BOX PROTEIN (PUB24)	*Vitis pseudoreticulata*	Yao et al. (2017)
HIGH EXPRESSION OF OSMOTICALLY RESPONSIVE GENE 1 (HOS1)	*Vitis spp.*	Wu et al. (2023)
DEHYDRINS 1 and 2 (DHN1 and DHN2)	*V. vinifera* and *V. yeshanensis*	Yang et al. (2012)
RAFFINOSE SYNTHASE (VvRS)	*Interspecific hybrid UD 31-103*	De Rosa et al. (2022)
HEXOSE TRANSPORTERS 1 and 5 (VvHT1 and VvHT5)	*Interspecific hybrid UD 31-103*	De Rosa et al. (2022)
MATURATION, STRESS, ABA (VvMSA)	*Interspecific hybrid UD 31-103*	De Rosa et al. (2022)
PHOSPHOGLYCERATE KINASE (PGK)	*Vitis amurensis*	Wang et al. (2021)
9-CIS EPOXYCAROTENOID DIOXYGENASE 3 (NCED3)	*Vitis vinifera*	Londo et al. (2018)

Continued…

(Table 1.1 continued)

PYROBACTIN-RESISTANCE LIKE 1,4,5 and 13 (VaPYL1, VaPYL4, VaPYL5, and VaPYL13)	*Vitis amurensis*	Ren et al. (2022)
PROTEIN PHOSPHATASE TYPE 2C (VvPP2C1, VvPP2C14, VvPP2C59, VvPP2C60, and VvPP2C66)	*Vitis vinifera*	Boneh et al. (2012a), Zhang et al. (2021)
SNF-1 RELATED PROTEIN KINASE 2.2 (SnRK2.2)	*Vitis vinifera*	Boneh et al. (2012b)
AREBs/ABFs	*Vitis vinifera*	Londo et al. (2018)
WRKYs	*Vitis vinifera*	Londo et al. (2018)
VvWrKY24	*Vitis vinifera*	Wang et al. (2014)
VpWRKY2	*Vitis pseudireticulata*	Li et al. (2010)
VaWRKY12	*Vitis amurensis*	Zhang et al. (2019)
WRKY70	*Vitis vinifera*	Orranta-Araujo et al. (2021)
MYB24	*Vitis vinifera*	Zhu et al. (2022)

Chapter 2 - Comparison of Frost Tolerance and Transcriptional Response Related to Low temperature stresses in Young Shoots of *Vitis labrusca* and *Vitis vinifera*

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Abstract

Subfreezing temperatures during spring can damage young shoots and developing inflorescences of grapevine, impacting crop yields. During mid-winter, dormant buds of native American grapevine species, such as *Vitis labrusca*, are more cold hardy than *Vitis vinifera*, however, it is unknown if young shoots of *V. labrusca* emerging during spring are more frost tolerant than *V. vinifera*. In this study, we aimed to find if dormant buds of *V. labrusca* acc. 'GREM4' were more cold hardy and if young shoots were more frost-tolerant than *V. vinifera* cv. 'Cabernet Sauvignon'. Additionally, we investigated the transcriptional responses of 'GREM4' and 'Cabernet Sauvignon' shoots under chill (4°C) and freeze (-2°C) stress. Cold hardiness was determined in buds using differential thermal analysis (DTA). Young shoots of 'GREM4' and 'Cabernet Sauvignon' were assessed for their frost tolerance under low temperatures ranging between -2°C and -4°C. DTA results demonstrate that bud cold hardiness of 'GREM4' was significantly higher than 'Cabernet Sauvignon'. Further, 'GREM4' shoots were also more frost-tolerant (47.5%) than 'Cabernet Sauvignon' (22.5%). Transcriptomic analysis identified genes encoding *extensin* proteins and cell-wall-associated *receptor kinases* were highly upregulated in 'GREM4' young shoots exposed to low temperature conditions and, thus, might be involved in changing the mechanical properties of the cell wall to avoid frost injury. Genes encoding *3-ketoacylCoA synthase*, an enzyme involved in the biosynthesis of very-long-chain fatty acids (VLCFAs), were also differentially expressed between 'GREM4' and 'Cabernet Sauvignon'. These VLCFAs are further utilized in the formation of cutin waxes, which can prevent tissue injury due to external stresses. Abscisic acid (ABA)

degrading enzyme, *ABA 8'-hydroxylase 3*, was highly upregulated in 'GREM4' shoots under both chill and freeze stress, implicating a decrease in ABA accumulation during low temperature stress in 'GREM4'. Overall, we found higher frost tolerance in young shoots of 'GREM4' as compared to 'Cabernet Sauvignon' and our transcriptomic findings suggest that cell wall modifications or the synthesis of VLCFAs might contribute to this higher frost tolerance.

Keywords: *Vitis labrusca*; *Vitis vinifera*; Frost tolerance; Comparative transcriptomics; Cell-wall modification

Introduction

Grapevine (*Vitis vinifera* L.) is one of the most important cultivated fruit crops grown worldwide (FAOSTAT, 2023). In the US, the annual production of grapes is around 6 million tons, generating $5.5 billion (USDA, 2022). Temperature is one of the critical factors that determine the phenological growth of grapevine (van Leeuwen & Darriet, 2016). Low temperatures can pose a threat to grapevine yield and fruit quality by damaging the most sensitive buds and emerging young shoots during mid-winter and late spring, respectively (Carmona et al., 2008; De Rosa et al., 2022; Lamichhane, 2021; Lenz et al., 2016; Vitasse et al., 2014; Zabadal et al., 2007).

Wild North American grapevine species, such as *Vitis labrusca*, are more cold hardy and are adapted to low temperature conditions during winter (Londo & Kovaleski, 2017). These species have been utilized in grapevine breeding programs for the development of cold climate interspecific hybrid grapevine cultivars (North et al., 2021). However, some of these northern grapevine species have faster deacclimation rates, which could result in the exposure of sensitive young shoots to spring frost events (Londo & Kovaleski, 2017). Although dormant buds of *Vitis labrusca* can survive under sub-zero temperatures (-26℃ to -28℃), the survivability of emerged young shoots encountering late-spring frost conditions is still unknown.

Studies in different plant species, including grapevine, have revealed that cold response induces calcium signaling and activates different transcription factors, especially the *Inducer of CBF* (*ICE*) genes (Londo et al., 2018; Rahman et al., 2014; Xiao et al., 2006, 2008). These *ICE* genes trigger the expression of *cold-binding*

factor/dehydration-responsive element-binding (CBF/DREB) transcription factors, which further activate common cold response (*COR*) genes. *COR* genes further regulate different physiological changes, such as membrane fluidity, sugar metabolism, and accumulation of osmoprotectants, thus, avoiding tissue injury (Londo et al., 2018; Mahajan & Tuteja, 2005; Xin et al., 2013; Xu et al., 2014). Additionally, hormonal changes, such as abscisic acid (ABA) and ethylene, play a synergistic role in regulating gene expression and triggering these physiological changes such as increasing sugar levels and decreasing water content to delay budburst (Kovaleski & Londo, 2019; Rubio et al., 2019; Rubio & Pérez, 2019; Vergara et al., 2017). Most studies conducted to determine the effects of cold temperatures on grapevine leaves have focused on non-freezing temperatures (4°C). However, these responses to above-zero temperatures may differ from changes observed under sub-zero temperature conditions. A study conducted using different *V. vinifera* cultivars ('Reisling', 'Cabernet Franc', 'Chardonnay', 'Sangiovese', and 'Tocai Fruliano') showed differences in transcriptomic responses associated with chill (4°C), freeze (-3°C), and acclimated-freeze (4°C to -3°C) stress conditions (Londo et al., 2018). These different gene expression patterns were related to ethylene signaling, ABA signaling, sugar metabolic pathways, and other transcription factor families, such as *APETALA2/Ethylene Responsive Factor* (*AP2/ERF*), *WRKY*, and *NAC*. While this study only evaluated the young leaf (one-leaf unfolded stage) transcriptome of cold sensitive *V. vinifera* cultivars, there has yet to be a study to determine the transcriptional responses of cold hardy grapevine species to below freezing temperatures.

This study aimed to determine the difference in bud cold hardiness, frost tolerance, and low temperature-related transcriptomic responses between young shoots of cold hardy *Vitis labrusca* and cold sensitive *Vitis vinifera*. Based on previous reports of higher bud cold hardiness in *Vitis labrusca*, we hypothesize young shoots of *Vitis labrusca* will also have higher frost tolerance than *Vitis vinifera* (Londo & Kovaleski, 2017; Zabadal et al., 2007). We hypothesize the difference in frost tolerance is due to differential expression of genes related to the CBF-transcription factor dependent pathway, sugar metabolism, and ABA-signaling pathway in the shoot tissue, based on previous findings (De Rosa et al., 2022; Kovaleski & Londo, 2019; Londo et al., 2018; Mahajan & Tuteja, 2005; Rahman et al., 2023; Rubio et al., 2019; Sengupta et al., 2015; Sun et al., 2021; Wisniewski et al., 2014; Zhang & Dami, 2012). Therefore, the objectives of this study were to 1) determine the bud cold hardiness of different *V. labrusca* accessions and *V. vinifera* cultivars, 2) determine the frost tolerance of *V. labrusca* acc. 'GREM4' and *V. vinifera* cv. 'Cabernet Sauvignon' young shoots under sub-zero temperatures, 3) determine the transcriptomic responses of 'GREM4' and 'Cabernet Sauvignon' to freeze (-2°C) and chill (4°C) stress compared to room temperature (control), and 4) compare shoot transcriptomes between 'GREM4' and 'Cabernet Sauvignon' to identify genes and pathways involved in chill or freeze stress tolerance in 'GREM4'. To our knowledge, this is the first study to compare the shoot transcriptomes of two different grapevine species for identification of candidate genes imparting higher frost tolerance in grapevine.

Materials and Methods

Plant material

V. labrusca accessions, 'GREM4' (PI-588583) and 'Dunkel#1' (PI-588194), and *V. vinifera* cultivars, 'Cabernet Sauvignon' and 'PN40024', were grown in 20-liter pots in the Howlett greenhouse complex at The Ohio State University. Four to five vines with two or three canes, were available for each of these accessions or cultivars. During the growing season, these four-year-old vines were regularly irrigated with water and fertilized biweekly using Jack's Professional® water-soluble fertilizer (20-10-20 general purpose; JR Peters Inc., Allentown, PA) at a concentration of 200 ppm for nitrogen. The vines were maintained at temperatures ranging between 16℃ and 20℃, with 16hr light:8hr dark conditions with supplemental light. For this study, vines were gradually cold-acclimated by lowering the temperature from 16℃ starting November 7, 2022 to ~4℃ during the first week of December. The cold-acclimated vines were finally transferred to a cold room (4℃) on December 9, 2022, to fulfill the chilling requirement and ensure synchronous budburst. Dormant one-year old canes were taken from these vines in December, 2022 and used to determine bud cold hardiness, while other cuttings from the dormant canes of the same vines were collected in May, 2023, to carry out the frost test assays and transcriptomic analyses.

Bud cold hardiness determination

Cold hardiness was measured by differential thermal analysis (Wolf & Cook, 1994) on December 21, 2022. It is a method to determine the critical temperatures (low temperature exotherms or LTE) a tissue can survive by measuring the latent heat energy released from water upon freezing (Figure 2.1, Pierquet & Stushnoff, 1980). Based on the availability of buds, we selected two *Vitis labrusca* accessions, 'GREM4' and 'Dunkel#1', and two *Vitis vinifera* cultivars ('Cabernet Sauvignon' and 'PN40024') to assess the cold hardiness of dormant buds. These vines were cold-acclimated and were provided ~700 chilling hours (Dokoozlian, 1999; Dokoozlian et al., 1995). For assessing cold hardiness, buds from each of the *V. labrusca* accessions and *V. vinifera* cultivars were collected from different canes at node positions 3-8. Twenty excised buds were then divided into four replicates, each replicate consisting of 5 buds, and were placed on a thermoelectric module (TEM). The TEM was placed in a Tenney environmental chamber (Thermal Products Solutions™, New Columbia, PA). The temperature in the Tenney environmental chamber was gradually decreased from 18.5℃ to -40℃ at a constant rate of 4℃/hr. The median LTE for each replicate was considered as the LT50 or the lethal temperature that kills 50% of the primary buds.

The data were tested for normality and variance using the Shapiro-Wilk test and Levene's test, respectively, with the rstatix R package v0.7.2 (Kassambara, 2023). Mean LT50 values of the *V. labrusca* accessions and *V. vinifera* cultivars were compared using one-way ANOVA, followed by post-hoc analysis using the Tukey's Honestly Significant Difference (HSD) test using the agricolae package v1.3-7 (de Mendiburu, 2023) to

determine significant differences for cold hardiness levels at $p \leq 0.05$. All these statistical analyses were performed using R (v4.3.1; R Core Team, 2023a).

Growing young shoots

After about 3700 chilling hours (Dokoozlian, 1999; Dokoozlian et al., 1995), dormant 'GREM4' and 'Cabernet Sauvignon' cuttings were taken out of the cold room in May, 2023. Seventy single-bud cuttings of 'GREM4' and 'Cabernet Sauvignon' each were planted in foam-containing Oasis wedge-shaped trays in a completely randomized design. These cuttings were allowed to grow in a growth chamber provided with 14hr light:10hr dark conditions, 24℃ constant temperature, and 80% relative humidity. The trays were half-filled with water every two to three days. After attaining the modified Eichhorn-Lorenz (EL) 7-9 stage (one-three unfolded leaves) (Dry & Coombe, 2004), a portion of these cuttings from both 'GREM4' and 'Cabernet Sauvignon' were used for frost tolerance determination, while the rest of the cuttings were subjected to chill and freeze conditions to collect young shoot tissue for RNA isolations and RNA sequencing (Figure 2.2).

Frost tolerance determination

Frost tolerance was evaluated in young shoots of *V. labrusca* acc. 'GREM4' and *V. vinifera* cv. 'Cabernet Sauvignon' that reached the modified EL 7-9 stage. Four frost tests were conducted in total, with each test consisting of 10 young shoots from both 'GREM4'

and ‘Cabernet Sauvignon’ (4 frost tests × 10 young shoots per accession/cultivar = total 40 shoots per accession/cultivar; Figure 2.3). All the 20 young shoots (10 ‘GREM4’ and 10 ‘Cabernet Sauvignon’) used per frost test were placed in a completely randomized design in a foam-containing Oasis wedge-shaped tray, half filled with water. To avoid supercooling and induce ice nucleation, the leaves were sprayed with distilled water. Trays were then covered with a plastic dome-shaped cover to achieve nearly 100% relative humidity and to avoid exposing the young shoots to the strong air flow within the environmental chamber. The Tenney environmental chamber was programmed to decrease the temperature from 18.5°C to -14.5°C at a constant rate of 2°C/hr for 16.5 hours. Since the trays were covered with plastic cover, the actual lowest temperature received (30 minutes or more) by the young shoots was between -2°C to -4°C as recorded by an ibutton thermochron temperature sensor-logger (Embedded Data Systems, Lawrenceburg, KY). Following the frost tests, the trays were taken out of the environmental chamber and placed at room temperature (21°C) to allow the young shoots to recover. After 24 to 48 hours at room temperature, tissue injury was visually assessed as number of live and dead shoots. Frost tolerance was expressed as the percentage of live young shoots, or survivability, out of the total shoots tested.

Data recorded for the survivability of cuttings was analyzed using R (v4.3.1; R Core Team, 2023a). The normality of data was evaluated using the Shapiro-Wilk test and Levene’s test was used to determine the variance using the rstatix R package v0.7.2 (Kassambara, 2023). The survivability percentage of young shoots between ‘GREM4’ and ‘Cabernet Sauvignon’ was compared using Welch’s *t-test* (stats package v4.3.1; R

Core Team, 2023b) and box-plots were prepared using ggplot2 package v3.4.4 (Wickham, 2016). Even though the cuttings used in the study were in developmental stages between modified EL 7 and 9, some of the cuttings were closer to the modified EL 7 stage (one leaf unfolded), while others were closer to the modified EL 9 stage (two-three leaves unfolded). Therefore, we conducted a two-way analysis of variance (ANOVA) using the stats package (v4.3.1) to determine whether variation in survivability (%) was attributed to the different species (i.e., 'GREM4' and 'Cabernet Sauvignon') or the phenological stages of the cuttings (EL 7, EL 7-9, and EL 9).

Tissue collection for RNA-Seq

Once they attained the modified EL 7-9 stage, young shoot cuttings (leaves and stems) of both 'GREM4' and 'Cabernet Sauvignon' were subjected to either: a) Freeze stress (-2°C), b) Chill stress (4°C), or c) Control conditions (room temperature 21°C). For each temperature treatment, 8-10 young shoot cuttings of 'GREM4' and 'Cabernet Sauvignon' were arranged in a tray using a completely randomized design. RNA was isolated from four biological replicates (young shoots) of 'GREM4' and 'Cabernet Sauvignon' for each treatment and the control (Figure 2.4). Number of biological replicates was determined by estimating the sample size using RNASeqPower package v1.42.0 with parameters 'sequencing depth = 12.98 for 'GREM4' and 11.21 for 'Cabernet Sauvignon'' for desired effect sizes (1.5, 2, 4, and 5), levels of significance (0.05, 0.01, and 0.001), and power (0.8 and 0.9) (Therneau, Hart, & Kocher, 2023).

For freeze stress, 10 young shoots of 'GREM4' and 'Cabernet Sauvignon' were subjected to artificial freezing conditions providing a minimum temperature of -2°C using a Tenney environmental chamber (Thermal Products Solutions™, New Columbia, PA). The temperature of -2°C was chosen because it is considered as a critical temperature to cause 50% damage in grapevine shoots at the one-leaf stage, as reported in *V. vinifera* cv. 'Pinot Noir' (Sugar et al., 2003). The tray was covered with a plastic dome-shaped cover to prevent vegetative tissues from air movement within the environmental chamber. The environmental chamber was programmed to gradually decrease the temperature from 18.5°C to -9.5°C at a constant rate of 2°C/hr. Since the tray was covered with a plastic cover, the actual lowest temperature experienced by the young shoots was -2°C for 31 minutes, which was recorded by an ibutton thermochron temperature sensor-logger (Embedded Data Systems, Lawrenceburg, KY), that was placed in the middle of the tray. The temperature data recorded per minute during the freeze run was visualized using the software 1-Wire Viewer (Embedded Data Systems, Lawrenceburg, KY). The freezing test took a total of 14 hours to conduct. Young shoot tissues (leaves and shoot apical meristems) were collected from four biological replicates of 'GREM4' and 'Cabernet Sauvignon' in 50 mL centrifuge tubes for isolating RNA. The tubes were immediately flash-frozen in liquid nitrogen and were stored at -80°C until used for RNA isolation.

For chill stress, a tray containing 10 young shoots of 'GREM4' and 'Cabernet Sauvignon' (completely randomized design) was placed in a refrigerator and exposed to a temperature of 4°C for 14 hours, which was recorded by an ibutton thermochron temperature sensor-logger placed in the middle of the tray. After 14 hours of chilling

stress, young shoot tissues (leaves and shoot apical meristems) were collected from four cuttings in 50 mL centrifuge tubes, flash-frozen, and then stored at -80℃.

During the freeze and chill stress treatments, the young shoots of ‘GREM4’ and ‘Cabernet Sauvignon’ received no light for 14 hours in the environmental chamber and the refrigerator. Therefore, to compensate for any circadian rhythm changes due to light exposure, eight young shoots of ‘GREM4’ and ‘Cabernet Sauvignon’ were placed at room temperature covered with black polythene for 14 hours (control). Tissues (leaves and shoot apical meristems) from four cuttings of ‘GREM4’ and ‘Cabernet Sauvignon’ were then collected in 50 mL centrifuge tubes and stored at -80℃ for RNA isolation. Shoot transcriptomes of these collected samples served as the control to compare changes in gene expression under chill and freeze stress in ‘GREM4’ and ‘Cabernet Sauvignon’.

RNA isolation and Sequencing

For each temperature treatment (freeze or chill) and controls, four biological replicates, i.e., young shoot tissues (leaves and apical meristems) of ‘GREM4’ and ‘Cabernet Sauvignon’ were collected for RNA isolation (4 freeze + 4 chill + 4 control = 12 shoots per accession/cultivar; see above section). RNA was isolated from 24 samples using protocol B of Spectrum™ Plant Total RNA kit (Sigma-Aldrich®, Inc., St. Louis, MO) following the manufacturer’s instructions. The quality and quantity of extracted RNA was measured using a Thermo Scientific™ NanoDrop™ One Microvolume UV-Vis Spectrophotometer, a Thermo Scientific™ Qubit™ RNA HS Assay Kit, and formaldehyde gel electrophoresis. All 24 RNA samples were sent to Novogene

Corporation Inc. (Sacramento, CA) for RNA sequencing. RNA-seq libraries were prepared for each of the 24 samples and they were sequenced using Illumina NovaSeq 6000 paired-end RNA sequencing (150 bp, 20M reads).

Comparative transcriptomic analysis

Transcriptomic analysis was performed using a pipeline developed by Dixon & Gschwend (under review; Figure 2.5). The specific code used for transcriptomic analysis can be found on GitHub at https://github.com/GurkiratSingh25/Transcriptomic-responses-related-to-low-temperature-stress-in-grapevine. In brief, RNA-seq reads were assessed for quality control via FastQC v0.11.8 (Babraham Bioinformatics, n.d.) and MultiQC v1.15 (Ewels et al., 2016) before and after removal of adapters via Trimmomatic v0.38 using parameters "ILLUMINACLIP:Novogene-PE.fa:2:30:10 SLIDINGWINDOW:4:5 LEADING:5 TRAILING:5 MINLEN:36" (Bolger et al., 2014). RNA-Seq reads were aligned to their respective genomes, i.e., *Vitis labrusca* acc. 'GREM4' (Li & Gschwend, 2023) and *Vitis vinifera* cv. 'Cabernet Sauvignon' (Chin et al., 2016; Li & Gschwend, 2023) using STAR v2.7.9a with parameters '--runMode genomeGenerate' and '--genomeSAindexNbases 13' (Dobin et al., 2013). A count matrix of RNA-seq reads was prepared using CoCo to account for multimapped reads (Deschamps-Francoeur et al., 2019).

DESeq2 v1.42.0 (Love et al., 2014) was used for the identification of differentially expressed genes (DEGs) for each low temperature treatment (chill or freeze) compared to controls (room temperature) for both 'GREM4' and 'Cabernet

Sauvignon' independently, as well as combined for interaction analysis between the species and the treatments. The parameters set to identify DEGs for all intraspecies comparisons were $p \leq 0.05$, p-adjusted ≤ 0.05, and $|log2FoldChange| \geq 2$. For treatment comparisons within 'GREM4' or 'Cabernet Sauvignon', effect sizes were used to estimate the power of each gene comparison using package pwr v1.3-0 with the function 'pwr.t.test()' (n = 4, significance level = 0.05; Champely, 2020). The overall power to detect true DEGs for a range of effect sizes (2, 3, 4, and 5) was estimated using RNASeqPower package v1.42.0 with parameters 'sequencing depth = 12.98 for 'GREM4' and 11.21 for 'Cabernet Sauvignon'', 'cv = 0.5' and 'alpha = 0.05' (Therneau, Hart, & Kocher, 2023).

For inter-species comparisons, orthologous genes were first identified between 'GREM4' and 'Cabernet Sauvignon' using OrthoFinder v2.2.5 (Emms & Kelly, 2019), DIAMOND (Buchfink et al., 2021) and custom scripts (Dixon & Gschwend, under review; https://github.com/GurkiratSingh25/Transcriptomic-responses-related-to-low-temperature-stress-in-grapevine). Differences in basal gene expression was assessed by comparing the transcriptomic profiles of 'GREM4' and 'Cabernet Sauvignon' control samples ($|log2foldchange| \geq 2$, p- adjusted ≤ 0.05). Shoot transcriptomes of both 'GREM4' and 'Cabernet Sauvignon' were independently analyzed for differential gene expression under chill or freeze stress compared with controls and further those DEGs were compared between the two species. DEGs were then evaluated by considering the interaction between species ('GREM4' compared to 'Cabernet Sauvignon') and the treatments (chill or freeze compared to control) (Figure 2.6). For example, to identify

DEGs between 'GREM4' and 'Cabernet Sauvignon' under chill (4°C) stress, we first compared the chill-stressed samples to the control samples for 'GREM4' and 'Cabernet Sauvignon' separately and evaluated the log2FoldChange values. These log2FoldChange values were further compared between 'GREM4' and 'Cabernet Sauvignon' by calculating the change in log2FoldChange ($|\Delta \text{log2FoldChange}| \geq 2$) at a p-adjusted value of ≤ 0.05.

Volcano plots were generated for all within species and inter-species comparisons using the EnhancedVolcano R package v1.16.0 (Blighe et al., 2023). Functions were inferred for a subset of DEGs by identifying homologous genes via NCBI nucleotide Basic Local Alignment Search Tool (BLAST) by aligning their coding (CDS) sequences with that of the *Vitis* L. *spp.* (taxid:3603) and *Arabidopsis thaliana* (taxid: 3702) non-redundant nucleotide databases, optimized for highly similar sequences (megablast) or somewhat similar sequences (blastn) (NCBI Resource Coordinators, 2016). Further, functional enrichment analyses were conducted using the Kyoto Encyclopedia of Genes and Genomes (KEGG) and the Over-Representation Analysis (ORA) method (Bu et al., 2021; Kanehisa & Goto, 2000; Wu et al., 2021). ORA was used to determine the Gene Ontology (GO) term enrichments in a set of DEGs via ‘enricher’ with a post-hoc ‘gsfilter’ (DOSE) using the clusterProfiler R package v4.6.2 (Yu et al., 2015). KEGG pathway enrichment analysis of the DEGs was carried out via the KEGG Orthology-Based Annotation System-intelligent (KOBAS-i) using default parameters (Bu et al., 2021).

Results

Bud cold hardiness determination

Bud cold hardiness was determined for two *V. labrusca* accessions and two *V. vinifera* cultivars. The lower the LT50 value, the higher the bud cold hardiness level of an accession or a cultivar. DTA results showed that both *V. labrusca* accessions, 'GREM4' and 'Dunkel#1', had higher bud cold hardiness than that of *V. vinifera* cvs. 'Cabernet Sauvignon' and 'PN40024' (Figure 2.7). The most significant difference in mean LT50 was observed between 'Cabernet Sauvignon' (-15.62℃) and 'GREM4' (-25.14℃; one-way ANOVA $p = 0.003$). Therefore, young shoots of 'GREM4' and 'Cabernet Sauvignon' were used to assess frost tolerance and determine transcriptional changes in young shoot tissues under low temperature stresses (chill and freeze).

Data related to bud cold hardiness was normally distributed and had equal variance between *V. labrusca* accessions and *V. vinifera* cultivars (Table 2.1, Figure 2.8).

Frost tolerance determination

Shoot injury was induced in 'GREM4' and 'Cabernet Sauvignon' at sub-freezing (-2℃ to -4℃) temperatures (Figure 2.9). The mean survivability percentage of 'GREM4' shoots was 47.5%, whereas only 22.5% of 'Cabernet Sauvignon' shoots were alive under frost conditions ($p = 0.03$; Figure 2.10). The survivability percentages of all frost tests were normally distributed and had equal variance (Table 2.1, Figure 2.11). A two-way

ANOVA was performed to determine if the difference in survivability was due to species (‘GREM4’ and ‘Cabernet Sauvignon’) or due to slight differences in the developmental stages of the shoots (EL 7 vs EL 9). The difference in survivability was attributed to species ($p = 0.01$) and not the stages of development ($p = 0.65$). These results demonstrate that ‘GREM4’ young shoots had higher frost tolerance than ‘Cabernet Sauvignon’ at the modified EL 7-9 developmental stage.

Transcriptomic responses related to chill and freeze stress within ‘GREM4’ and ‘Cabernet Sauvignon'

Overall power for different treatment comparisons within 'GREM4' and 'Cabernet Sauvignon' was estimated by averaging the effect sizes of all DEGs within each comparison. The average effect size was approximately 5, resulting in estimated powers of 0.98 and 0.97 for detecting differential gene expression with $|\log2FoldChange| \geq 2$ in 'GREM4' and 'Cabernet Sauvignon', respectively. The sample size required to achieve 0.90 power at a 0.05 significance level was estimated to be less than 4 for both 'GREM4' and 'Cabernet Sauvignon', justifying the use of four biological replicates (shoot tissues) per temperature treatment for pairwise comparisons.

A. ‘GREM4’ transcriptomic response to chill (4°C) and freeze (-2°C) temperatures

The transcriptomic changes contributing to ‘GREM4’’s response to chill and freeze stress were determined through comparative transcriptomics with the room temperature ‘GREM4’ shoot transcriptome (control). 300 genes were differentially expressed between

the chill-treated and control tissues, out of which 170 were upregulated under chill stress, while 130 were downregulated (Figure 2.12). The top five upregulated genes with the highest log2FoldChange include genes involved in cell-wall sensing and its modification, such as, *pollen-specific leucine-rich repeat extensin-like protein* 3, cell-wall associated *receptor* kinases. On the other hand, genes involved in transmembrane transport, such as *organic cation/carnitine transporter 1*, and protein NRT1/ PTR FAMILY 7.1 were downregulated under chill stress (Table 2.2). The only significant pathway enrichment shown by KEGG analysis (combined up- and downregulated genes) was 'Circadian rhythm-plant' (7 implicated DEGs), while no significant Gene Ontology (GO) terms were shown via Over-Representation Analysis (ORA).

On the other hand, a smaller number of DEGs (171) were detected between the freeze stress and control shoot transcriptomes, 104 of which were upregulated and 67 were downregulated (Figure 2.13). Upregulated genes with highest log2FoldChange include cell-wall associated *receptor kinases*, *calcium binding protein CML31*, and *3-ketoacyl-CoA synthase 15* (*KCS15*). Additionally, five genes encoding different *Ethylene responsive factors* (*ERF*) transcription factors were also highly upregulated having a log2FoldChange of 5 or higher. Meanwhile, genes encoding *chlorophyll a-b binding proteins* and those involved in sugar transportation, such as, *sugar transporter ERD6-like5*, and *cell wall/vacuolar inhibitor of fructosidase 2* (*CIF2*) were among downregulated genes having highest log2FoldChange (Table 2.3). KEGG analysis of all DEGs under freeze stress showed enrichment of 'Photosynthesis-antenna proteins' (8 implicated DEGs). Gene ontology (GO) term enrichments were found via ORA only for

downregulated genes and were related to a) Photosynthetic/Light harvesting, b) Proteolysis, and c) Transmembrane transporter activity.

91 genes (66 upregulated, 25 downregulated) were differentially expressed under both chill and freeze stress (Figure 2.14). Upregulated 'GREM4' conserved genes include transmembrane *receptor kinases*, which are involved in cell wall integrity (CWI) sensing (Herger et al., 2019), and a gene encoding *3-ketoacyl CoA synthase 15* (*KCS15*), which is a key enzyme involved in biosynthesis of very-long-chain fatty acids (VLCFAs). There were genes uniquely differentially expressed only under either chill or freeze stress in 'GREM4', with 209 (104 upregulated, 105 downregulated) unique to chill and 80 (38 upregulated, 42 downregulated) unique to freeze stress.

B. 'Cabernet Sauvignon' transcriptomic response to chill (4°C) and freeze (-2°C) temperatures

The transcriptomic changes contributing to 'Cabernet Sauvignon' shoots' response to chill and freezing temperatures were also determined through comparative transcriptomics. The total number of DEGs was higher in 'Cabernet Sauvignon' compared to 'GREM4'. 804 DEGs (567 upregulated, 237 downregulated) were found between chill and control in 'Cabernet Sauvignon' (Figure 2.15). On combining all DEGs (up- and downregulated), KEGG analysis showed five pathways enriched between chill and the control condition – 'circadian rhythm – plant' (12 DEGs), 'diterpenoid biosynthesis' (6 DEGs), 'biosynthesis of secondary metabolites' (57 genes), 'alpha-linolenic acid metabolism' (8 DEGs), and 'plant-pathogen interaction' (16 DEGs). Genes

with high expression include two cold responsive *galactinol synthase 2* genes, and a *dehydration-responsive element binding protein 1E-like (DREB 1E-like)/cold binding factor 5 (CBF5)*; however, genes involved in fatty acid biosynthesis (*KCS2*, *KCS6*) and fatty acid binding were among top 20 highly downregulated genes (Table 2.4).

On the other hand, 291 DEGs (196 upregulated, 95 downregulated) were found between freeze and control (Figure 2.16). *CBF6* and *CBF2* were among top 20 highly upregulated genes with a log2FoldChange higher than 6. Some of the other highly upregulated genes were orthologous to DEGs found in 'GREM4' under freeze stress such as *transcription factor MYB118*, *ERF017*, and *calcium binding protein CML31*, while genes encoding different *chlorophyll a-b binding proteins* were downregulated in both 'GREM4' and 'Cabernet Sauvignon' (Table 2.3 and 2.5). DEGs between the freeze stress and control shoots were enriched in four KEGG pathways – 'Photosynthesis-antenna proteins' (9 DEGs), 'circadian rhythm – plant' (6 DEGs), 'thiamine metabolism' (4 DEGs), and 'starch and sucrose metabolism' (6 DEGs). Similar to 'GREM4', no GO term enrichments were shown for upregulated genes both for chill and freeze-treated young shoots, however, the downregulated genes under freeze stress were enriched in one GO-term related to 'Photosynthesis'.

185 genes (141 upregulated, 44 downregulated) were differentially expressed under both chill and freeze stress in 'Cabernet Sauvignon' (Figure 2.14). Conserved upregulated genes include *Ethylene responsive factors* (*ERF017* and *ERF109*), *chaperone protein dnaJ C76*, and *Zinc finger protein CONSTANS-LIKE 7*. On the other hand, genes encoding *3-ketoacyl CoA synthase 2 (KCS2)*, and *oligopeptide transporter 4* were among

the downregulated genes with the highest log2FoldChange under both chill and freeze conditions (Table 2.4 and 2.5). 619 genes (426 upregulated, 193 downregulated) were uniquely differentially expressed under chill stress, while 106 DEGs (55 upregulated, 51 downregulated) were unique to freeze stress in 'Cabernet Sauvignon'.

Inter-species transcriptomic comparisons for chill (4°C) and freeze (-2°C) stress

Differentially expressed genes found in each of the treatment comparisons (chill or freeze compared to control) were compared between 'GREM4' and 'Cabernet Sauvignon'. Only 57 orthologous genes overlapped between 'GREM4' and 'Cabernet Sauvignon' when comparing chill (4°C) stress DEGs. The majority of the DEGs were specifically found in just one of the two species (174 DEGs in 'GREM4' and 584 DEGs in 'Cabernet Sauvignon'; Figure 2.14). Shared DEGs between 'GREM4' and 'Cabernet Sauvignon' include four *Zinc finger proteins*, a *galactinol synthase 2* (*GolS2*), and *Ethylene-responsive factor ERF017* being upregulated, while *basic helix-loop-helix* 153 (*bHLH153*) and water-transporting *aquaporin nodulin 26-line intrinsic protein* (*NIP*) were downregulated in both species. Among 174 uniquely differentially expressed genes in 'GREM4', genes encoding transcription factors, such as, *NAC domain-containing protein* 68 and *ERF054-like*, as well as fatty acid hydroxylating *cytochrome P450 94A1-like* were upregulated, while a gene encoding pectin-degrading enzyme, *pectate lyase 22* was downregulated under chill stress. On the other hand, cold stress induced genes, such as *CBF5* and *GolS2* were among highly upregulated genes, while genes encoding *KCS6*

(VLCFAs biosynthesis), *cellulose synthase like protein G3* (cellulose synthesis), and *laccase-1* (lignin biosynthesis) were downregulated in 'Cabernet Sauvignon'.

To understand the transcript expression involved in a response to frost, we compared the unique DEGs found only in 'GREM4' and 'Cabernet Sauvignon' (Figure 2.14). 68 unique DEGs (32 upregulated, 36 downregulated) were found in 'GREM4' under freeze stress as compared to control, which may contribute to increased frost tolerance. These include copies of *ERF5* and *ERF105*, as well as *KCS15* (involved in wax biosynthesis). Genes involved in sugar metabolism were also differentially expressed, including *beta-amylase 3* which was highly upregulated under freeze stress, while *cell wall/ vacuolar inhibitor of fructosidase 2* and *sugar transporter ERD6-like 5* were among the downregulated genes in 'GREM4' (Table 2.3). On the other hand, *Cold binding factors, CBF2* and *CBF4* were among the highly upregulated unique genes found in 'Cabernet Sauvignon'. The higher number of uniquely expressed genes than the shared genes implies that both species respond differently to low temperature stresses.

27 DEGs were shared between 'GREM4' and 'Cabernet Sauvignon' under freeze stress. These include upregulated *Ethylene-responsive factors* (*ERF017*, *ERF5* and *ERF105*), calcium-binding *calmodulin-like proteins* (*CML31* and *CML46*), and *zinc finger proteins*. Downregulated genes shared between two species include *chlorophyll a-b binding factors* which play a major role in photosynthesis (Keller, 2015; Møller et al., 2007).

To ultimately determine the DEGs that were significantly different between 'GREM4' and 'Cabernet Sauvignon' that may contribute to the increased low

temperature stress tolerance of ‘GREM4’ young shoots, 23,609 orthologous genes were first identified between the two species (out of the 37,443 and 36,668 total annotated genes in the ‘GREM4’ and ‘Cabernet Sauvignon’ genomes, respectively) using OrthoFinder v2.2.5 and DIAMOND. The expression of orthologous gene transcripts was compared between ‘GREM4’ and ‘Cabernet Sauvignon’ for each stress treatment (chill and freeze) using an interaction analysis.

From a chill-stress related interaction analysis [‘GREM4’ (chill vs control) compared to ‘Cabernet Sauvignon’ (chill vs control)], a total of 33 DEGs were identified, with 8 being upregulated and 25 being downregulated in ‘GREM4’ (Figure 2.17). Genes involved in cell wall lignification or modification, such as *laccase-1* (*LAC-1*) and *COBRA-like protein 4* were highly upregulated in ‘GREM4’. Along with these, a gene encoding abscisic acid (ABA) degrading enzyme, *ABA-8’ hydroxylase 3*, was also highly upregulated in ‘GREM4’, while another gene encoding gibberellin (GA) catabolizing enzyme, *Gibberellin 2-beta-dioxygenase 2* (*GA2ox2*), was downregulated (Table 2.6). On the other hand, downregulated genes in ‘GREM4’ (a.k.a. upregulated in ‘Cabernet Sauvignon’) include *disease resistance proteins*, and transcription factors, such as *MYB1-like*, and *basic helix-loop-helix* 162 (*bHLH162*). KEGG analysis showed that the *disease resistance proteins* were related to ‘plant-pathogen interactions’, suggesting that these genes might have a role in abiotic stress tolerance, as well as biotic stress.

Interestingly, only 5 DEGs (2 upregulated, 3 downregulated) were identified via the freeze stress-related interaction analysis [‘GREM4’ (freeze vs control) compared to ‘Cabernet Sauvignon’ (freeze vs control)] (Figure 2.18). Similar to the chill-stress-related

interaction analysis, a gene encoding *ABA acid 8' hydroxylase 3* was upregulated in 'GREM4', while genes involved in disease resistance were upregulated in 'Cabernet Sauvignon' (Table 2.7).

Along with interaction analysis, the expression of orthologous gene transcripts was compared between 'GREM4' and 'Cabernet Sauvignon' control samples to determine constitutive differential gene expression between the two species at room temperature. 2,118 DEGs (8.97% of orthologous genes) were found between control samples of 'GREM4' and 'Cabernet Sauvignon', out of which 1,071 were upregulated and 1,047 were downregulated in 'GREM4' (Figure 2.19). For upregulated genes in 'GREM4', ORA showed significant GO term enrichments related to 'Polysaccharide binding' (20 DEGs), while downregulated enrichments were related to 'carboxylase activity' and 'serine-type carboxypeptidase activity'. 20 genes associated with 'Polysaccharide binding' ORA GO term enrichment encode for wall-associated *receptor kinases* and *leaf rust resistance kinases*. KEGG analysis, on the other hand, showed enriched pathways – 'plant pathogen interaction' (27 upregulated and 21 downregulated in 'GREM4' compared to 'Cabernet Sauvignon'), and 'sesquiterpenoid and triterpenoid biosynthesis' (9 DEGs downregulated in 'GREM4'), demonstrating fundamental differences in the constitutive expression of genes involved in these pathways between both species.

Discussion

Frost tolerance difference between grapevine species at the same stage of development

Sub-zero temperatures during or after budburst are detrimental to grapevine development having negative impacts on bud and shoot survival as well as on yield and wine quality (van Leeuwen & Darriet, 2016). Although wild grapevine species have been utilized for the development of more cold hardy hybrid cultivars, many are also considered low-chill and fast-bursting species (Londo & Johnson, 2014). Thus, the fast-emerging young shoots are exposed to spring frost events. In this study, we found that 'GREM4' not only exhibited higher bud cold hardiness than 'Cabernet Sauvignon', but the shoots were also more tolerant to frost conditions. To our knowledge, this is the first study to demonstrate that a wild grapevine species (i.e., *V. labrusca* acc. 'GREM4') that has a low chilling hour requirement and rapid budburst, was relatively resilient to frost stress could still survive late spring frost events.

Both molecular and physical factors could contribute to 'GREM4''s frost tolerance. For example, morphological traits, such as leaf structure and higher trichome density could play a role in avoiding frost injury in *V. labrusca*. Dixon & Gschwend (under review) reported higher trichome density in 'GREM4' leaves as compared to *V. vinifera* cv. 'PN40024'. These trichomes could prevent the direct contact of water with the leaf surface and thus prevent tissue dehydration due to ice formation. Further, trichome densities may differ between different grapevine accessions as well as cultivars. Therefore, the role of these morphological traits in imparting tolerance against different

abiotic stresses can be investigated by exploring the physical and genetic diversity of grapevine.

Cell wall modification and wax biosynthesis might contribute to imparting low temperature stress tolerance in 'GREM4'

As hypothesized, pairwise comparisons between treatments in 'GREM4' showed that genes involved in the CBF-dependent pathway and ABA-regulatory pathway were differentially expressed. This includes the upregulation of different *ERFs*, *ABA 8'-hydroxylase 3*, *Galactinol synthase*, and other transcription factors. Interestingly, we found that genes involved in cell wall signaling and modification were highly upregulated, suggesting these pathways may play a key role in mitigating low-temperature stress in 'GREM4'.

'GREM4' shoots showed conserved expression of 91 genes (66 upregulated and 25 downregulated) under chill and freeze stress compared to controls. A subset of these upregulated DEGs with the highest log2FoldChange were involved in maintaining cell wall integrity (CWI). These include *pollen-specific leucine rich repeat extensin-like 3 (LRX-like 3)*, *receptor-like protein kinase At3g21340 (RLK)*, *wall-associated receptor kinase-like 20*, and *probable serine/threonine protein kinase PBL11*. ORA GO-term enrichments for the control transcriptome comparison between the two species showed that additional wall-associated *protein kinases* (11 out of total 20 found related to 'polysaccharide binding') were already upregulated in 'GREM4' compared to 'Cabernet Sauvignon' under room temperature conditions. These plasma membrane-localized

transmembrane receptor kinases are involved in CWI sensing as they can bind to cell wall components, which can further change intracellular processes (Decreux & Messiaen, 2005; Herger et al., 2019).

LRXs are chimeric extensin proteins that regulate calcium (Ca^{2+}) dynamics and cell wall homeostasis. These proteins provide a direct physical link transferring the information from the cell to the plasma membrane by interacting with receptor kinases (*WAKs*). The extensin domain of *LRXs*, characterized by serine-proline repeats, is an essential component of the cell wall that can modify cell wall properties to avoid pathogen invasion (Bradley et al., 1992; Brady & Fry, 1997; Castilleux et al., 2018; Herger et al., 2019; Liu et al., 2016). This is achieved by cross-linking between extensin proteins and pectin, which is one of the major cell wall components. Further, in the presence of calcium, *wall-associated receptor kinases* (*WAKs*) may covalently bind to pectin and increase the cell wall stiffening. Overall, this change in cell-wall properties might play an important role in avoiding frost injury in grapevine.

Additionally, we found that *3-ketoacyl CoA synthase 15 (KCS15)* was one of the top 20 most highly expressed genes in chill and freeze-exposed 'GREM4' shoots. However, a homolog of this gene, *KCS2*, was one of the most downregulated genes in chill and freeze-stressed 'Cabernet Sauvignon' shoots. *KCSs* are key enzymes involved in the biosynthesis of very-long chain fatty acids (VFCLAs). These fatty acids are involved in the formation of plant cuticles and cutin waxes in the epidermal cells, providing resistance from external stresses. Studies on different crop species, including grapevine, have demonstrated the role of *KCS* genes in imparting tolerance against salt, drought, and

cold stress (Guo et al., 2016; Lewandowska et al., 2020; Liu et al., 2023; Wang et al., 2020; Yang et al., 2020; Zheng et al., 2023). Therefore, these genes might be one of the candidate genes involved in frost tolerance. Another gene encoding fatty acid monooxygenase, *Cytochrome P450 94A1-like*, was found upregulated only in 'GREM4' chill stressed shoots. This enzyme hydroxylases fatty acids that can further be utilized either for energy production within the cell or in the biosynthesis of cutin and suberin, thus involved in plant defense (Benveniste et al., 2005; Kolattukudy, 1980).

In contrast, 'Cabernet Sauvignon' showed different responses to low temperatures. Results from the intra-species comparisons (chill or freeze compared to control) showed conserved higher expression of genes encoding *ERF017-like*, *ERF109*, *Zinc finger protein CONSTANS-LIKE-7*, and *Chaperone protein dnaJ C76, chloroplastic*. Along with importing chloroplast proteins (Flores-Pérez & Jarvis, 2013), studies have reported increased expression of genes encoding chaperone proteins under cold conditions to improve low temperature tolerance (Anderson et al., 1994; Krishna et al., 1995; Mahajan & Tuteja, 2005). Chill-exposed 'Cabernet Sauvignon' shoots showed higher expression of *CBF5*, while freeze-stress increased the expression of *CBF6*, *CBF4* and *CBF2*. *CBFs* bind to the promoter region of other cold-responsive genes and, thus, are one of the most studied genes involved in low temperature stress tolerance (De Rosa et al., 2021). Expression of *VvCBF2*, *VvCBF4*, and *VvCBF6* have been found to increase in dormant buds of *Vitis vinifera* cv. 'Thompson Seedless' under cold and ABA treatment (Rubio et al., 2019). Further, two homologous *galactinol synthase* (*GolS*) genes were upregulated under chill stress. *Galactinol synthase 2* (*GolS2*) is the rate-limiting enzyme

regulating the synthesis of raffinose family oligosaccharides (RFOs), a pathway reported to induce under low temperature conditions (Chai et al., 2019; Londo et al., 2018). However, a study in *Arabidopsis thaliana* showed that overexpression of *GolS* or higher accumulation of raffinose was not necessary for improving tolerance against low temperatures (Zuther et al., 2004). Under chill stress, it was also reported that genes encoding *cellulose synthase-like protein G3* (*CSLG3*) and *laccase-1* (*LAC1*) were downregulated in 'Cabernet Sauvignon'. Conversely, *LAC1* was among the upregulated genes with the highest log2FoldChange in 'GREM4' under chill stress. *LACs* play a predominant role in plant growth and development, particularly providing mechanical strength to the cells by regulating lignin polymerization (Bai et al., 2023). The mechanical strength can be further enhanced by crosslinking between lignin and cellulose (Tronchet et al., 2009; Wang et al., 2022), which may contribute to avoiding frost injury in the tissues.

Interaction analysis showed upregulation of genes encoding *disease resistance proteins*, and other transcription factors, such as *MYB1-like* and *bHLH162* in 'Cabernet Sauvignon' shoots under low temperature stress. Various *MYBs* and *bHLHs* have been identified in grapevine and some of these also play a role in regulating low temperature tolerance (Ren et al., 2023). Some of the genes were shared between both species under freeze and chill stress. In particular, *ERFs* and *CMLs*, which were highly upregulated in both species under freeze stress, have been found to regulate downstream genes involved in different pathways, including cold tolerance, in various crop species (Kovaleski &

Londo, 2019; La Verde et al., 2018; Mahajan & Tuteja, 2005; Polisensky & Braam, 1996; Sun et al., 2016, 2019; Wang et al., 2022; Wu et al., 2022; Zhu et al., 2013).

This study shows that despite higher expression of low temperature responsive genes, the frost tolerance of 'Cabernet Sauvignon' shoots was lower compared to those of 'GREM4'. 'GREM4' shoots showed higher expression of genes encoding cell-wall-associated *receptor kinases* and proteins involved in cell wall modifications. On the other hand, *KCS* genes were differentially expressed between two species. Therefore, genes involved in cell wall modification, CWI signaling, and very long chain fatty acid (VLCFAs) biosynthesis might be involved in enhancing frost tolerance of 'GREM4'. Further research on the role of these different receptor kinases and *KCS* genes is required to understand their role in the frost tolerance mechanism in grapevine. It would be interesting to study if species-specific genes or other paralogous genes play an important role in imparting frost tolerance.

Abscisic acid degradation may occur in 'GREM4' young shoots under low temperature stress

The gene encoding *ABA 8'-hydroxylase 3* showed conserved higher expression in 'GREM4' shoots under chill as well as freeze stress, whereas no significant change in gene expression was observed in 'Cabernet Sauvignon'. *ABA 8'-hydroxylase 3* is a key regulatory enzyme involved in ABA catabolism (Cutler & Krochko, 1999; Nambara et al., 2010; Zheng et al., 2018). Higher expression of *ABA 8'-hydroxylase 3* has been associated with breaking bud dormancy in grapevine or enhancing seed germination in

other crops such as *Arabidopsis* and barley (Gubler et al., 2008; Okamoto et al., 2006; Rubio & Pérez, 2019; Vergara et al., 2017; Zheng et al., 2018). Our study showed that *ABA 8'-hydroxylase 3* was highly expressed in *V. labrusca* acc. 'GREM4' which could be a species-specific response related to frost tolerance. From this, it can be assumed that ABA gets degraded during low temperature stress in young 'GREM4' shoots, which are more frost tolerant than 'Cabernet Sauvignon' shoots. This indicates that higher frost tolerance of 'GREM4' might not be due to higher ABA accumulation, but there might be other factors involved in avoiding frost injury. It is also possible that this degradation of ABA could be required to initiate plant growth and development. Higher expression of genes such as *Expansin-A7-like* (involved in cell wall loosening and cell expansion) (Santo et al., 2013) can be correlated to ABA catabolism in 'GREM4', especially under chill conditions. This was further supported by the downregulation of gibberellin (GA) catabolizing enzyme *GA2ox2*. GA is involved in plant growth and development that is negatively regulated by ABA (He et al., 2019). Therefore, degradation of ABA by *ABA 8' hydroxylase 3* might have increased the availability of biologically active GA, thus, promoting growth in 'GREM4', however, ABA might not be involved in imparting frost tolerance.

Alteration in sugar metabolism and transport could improve low temperature stress tolerance in 'GREM4' shoots

Under freeze (-2°C) stress, we reported downregulation of *chlorophyll a-b binding proteins*, *sugar transporter early dehydration responsive 6-like 5 (ERD6-like 5)*, and *cell*

wall/vacuolar inhibitor of fructosidase 2 (CIF2). On the other hand, starch-degrading enzyme *β-amylase 3* was highly upregulated in 'GREM4' shoots. Low temperatures affect photosynthetic activity by disrupting the proteins involved in light-harvesting complex II (*LHCII*) (Saibo et al., 2009; Thomashow, 2001). To compensate for the decreased photosynthetic efficiency, soluble sugars need to be accumulated within the cells either by halting their transportation to other tissues or by increasing the accumulation of osmotically active sugars, such as glucose and fructose (Druart et al., 2007; Keller, 2015). Induced under low temperatures, amylase genes play an important role in the accumulation of soluble sugars by degrading starch (Pérez & Rubio, 2015). Besides, low transcript levels of *AtERD6* were found in *Arabidopsis* under cold stress and when externally supplied with sugars, suggesting their role in glucose accumulation under starvation (Poschet et al., 2011). *CIFs* inhibit the activity of *cell wall invertases* (*CWIs*) and *vacuolar invertases* (*VIs*) preventing the irreversible conversion of sucrose to glucose and fructose (Koch, 2004; Sturm, 1999; Wan et al., 2018). Similar to our results, Xu et al. (2017) showed that silencing a *CIF* gene can improve cold tolerance in tomato. As reported in dormant grapevine buds, higher glucose accumulation in the cells under low temperature conditions could help decrease the freezing point of water (Fennell, 2004; Guy, 1990; Hamman et al., 1996; Jones et al., 1999; Keller, 2015). It is possible that a similar mechanism related to sugar accumulation might be involved in osmoprotection in young emerging grapevine shoots, thus preventing their frost injury.

Conclusion

This study was conducted to compare the frost tolerance of young shoots of cold hardy *V. labrusca* acc. 'GREM4' and cold sensitive *V. vinifera* cv. 'Cabernet Sauvignon'. Frost tolerance assay shows that 'GREM4' shoots (47.5%) had a significantly higher survivability rate under sub-zero temperatures (between -2℃ to -4℃) as compared to those of 'Cabernet Sauvignon' (22.5%). Transcriptional responses showed that genes encoding *extensin* proteins, and *cell wall receptor-like kinases* were among highly upregulated genes in 'GREM4', suggesting the role of the cell wall acting as the first line of defense in protecting the intracellular components from frost injury. Additionally, glucose accumulation within cells might play a role as osmoprotectants to improve the low temperature stress tolerance, whereas wax-synthesizing *KCS* genes might be involved in avoiding frost injury in 'GREM4' shoots. 'Cabernet Sauvignon', on the other hand, showed different transcriptional responses related to low temperature conditions. Genes encoding *CBFs*, chaperones, and *GolS* were upregulated in 'Cabernet Sauvignon', but the frost injury was still higher in these young shoots. Genes involved in maintaining cell wall integrity and plasma membrane modifications are candidates for imparting higher frost tolerance in *V. labrusca*. Another interesting finding was the conserved high upregulation of ABA-degrading enzyme, *Abscisic acid 8' hydroxylase* 3, in 'GREM4' under chill and freeze stress. Further investigation into the functions of these genes and their metabolic pathways is required to understand the mechanism of this polygenic trait.

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Additional Information

Additional information on custom codes and raw data files for transcriptomic comparisons can be found on GitHub at https://github.com/GurkiratSingh25/Transcriptomic-responses-related-to-low-temperature-stress-in-grapevine.

References

Anderson, J. V., Li, Q. B., Haskell, D. W., & Guy, C. L. (1994). Structural organization of the spinach endoplasmic reticulum-luminal 70-kilodalton heat-shock cognate gene and expression of 70-kilodalton heat-shock genes during cold acclimation. *Plant Physiology*, *104*(4), 1359–1370. https://doi.org/10.1104/pp.104.4.1359

Babraham Bioinformatics—FastQC A Quality Control tool for High Throughput Sequence Data. (n.d.). Retrieved February 26, 2024, from https://www.bioinformatics.babraham.ac.uk/projects/fastqc/

Bai, Y., Ali, S., Liu, S., Zhou, J., & Tang, Y. (2023). Characterization of Plant Laccase Genes and Their Functions. *Gene*, *852*, 147060. https://doi.org/10.1016/j.gene.2022.147060

Benveniste, I., Bronner, R., Wang, Y., Compagnon, V., Michler, P., Schreiber, L., Salaün, J.-P., Durst, F., & Pinot, F. (2005). CYP94A1, a Plant Cytochrome P450-catalyzing Fatty Acid Omega-hydroxylase, is Selectively Induced by Chemical Stress in *Vicia sativa* Seedlings. *Planta*, *221*(6), 881–890. https://doi.org/10.1007/s00425-005-1503-y

Blighe et al. (2023). EnhancedVolcano: Publication-ready volcano plots with enhanced colouring and labeling. Bioconductor version: Release (3.16) doi: 10.18129/B9.bioc.EnhancedVolcano. (1.16.0) [Computer software].

Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics (Oxford, England)*, *30*(15), 2114–2120. https://doi.org/10.1093/bioinformatics/btu170

Bradley et al. (1992). Elicitor- and wound-induced oxidative cross-linking of a proline-rich plant cell wall protein: A novel, rapid defense response—*ScienceDirect*. https://www.sciencedirect.com/science/article/abs/pii/009286749290530P

Brady, J. D., & Fry, S. C. (1997). Formation of Di-Isodityrosine and Loss of Isodityrosine in the Cell Walls of Tomato Cell-Suspension Cultures Treated with Fungal Elicitors or H2O2. *Plant Physiology*, *115*(1), 87–92. https://doi.org/10.1104/pp.115.1.87

Bu et al. (2021). *KOBAS-i:* Intelligent prioritization and exploratory visualization of biological functions for gene enrichment analysis | *Nucleic Acids Research* | *Oxford Academic*. https://academic.oup.com/nar/article/49/W1/W317/6292104

Buchfink, B., Reuter, K., & Drost, H.-G. (2021). Sensitive protein alignments at tree-of-life scale using DIAMOND. *Nature Methods*, *18*(4), 366–368. https://doi.org/10.1038/s41592-021-01101-x

Carmona, M., Chaib, J., Martinez-Zapater, J., & Thomas, M. (2008). A molecular genetic perspective of reproductive development in grapevine. *Journal of Experimental Botany*, *59*(10), 2579–2596. https://doi.org/10.1093/jxb/ern160

Castilleux, R., Plancot, B., Ropitaux, M., Carreras, A., Leprince, J., Boulogne, I., Follet-Gueye, M.-L., Popper, Z. A., Driouich, A., & Vicré, M. (2018). Cell wall extensins in root–microbe interactions and root secretions. *Journal of Experimental Botany*, *69*(18), 4235–4247. https://doi.org/10.1093/jxb/ery238

Chai, F., Liu, W., Xiang, Y., Meng, X., Sun, X., Cheng, C., Liu, G., Duan, L., Xin, H., & Li, S. (2019). Comparative metabolic profiling of *Vitis amurensis* and *Vitis vinifera* during cold acclimation. *Horticulture Research*, *6*(1), Article 1. https://doi.org/10.1038/s41438-018-0083-5

Champely, S. (2020). *pwr: Basic Functions for Power Analysis* (Version 1.3-0) [R package]. https://github.com/heliosdrm/pwr

Chin, C.-S., Peluso, P., Sedlazeck, F. J., Nattestad, M., Concepcion, G. T., Clum, A., Dunn, C., O'Malley, R., Figueroa-Balderas, R., Morales-Cruz, A., Cramer, G. R., Delledonne, M., Luo, C., Ecker, J. R., Cantu, D., Rank, D. R., & Schatz, M. C. (2016). Phased diploid genome assembly with single-molecule real-time sequencing. *Nature Methods*, *13*(12), 1050–1054. https://doi.org/10.1038/nmeth.4035

Coombe, B. g. (1995). Growth Stages of the Grapevine: Adoption of a system for identifying grapevine growth stages. *Australian Journal of Grape and Wine Research*, *1*(2), 104–110. https://doi.org/10.1111/j.1755-0238.1995.tb00086.x

Cutler, A. J., & Krochko, J. E. (1999). Formation and breakdown of ABA. *Trends in Plant Science*, *4*(12), 472–478. https://doi.org/10.1016/s1360-1385(99)01497-1

Dal Santo, S., Vannozzi, A., Tornielli, G. B., Fasoli, M., Venturini, L., Pezzotti, M., & Zenoni, S. (2013). Genome-wide analysis of the expansin gene superfamily reveals grapevine-specific structural and functional characteristics. *PloS One*, *8*(4), e62206. https://doi.org/10.1371/journal.pone.0062206

Dami, I. (2022). Spring Frost Injury of Grapevines and Protection Methods. OSU Extension Publications. https://extensionpubs.osu.edu/spring-frost-injury-of-grapevines-and-protection-methods/

de Mendiburu, F. (2023). agricolae: Statistical Procedures for Agricultural Research. R package version 1.3-7. https://CRAN.R-project.org/package=agricolae

De Rosa, V., Falchi, R., Peressotti, A., & Vizzotto, G. (2022). Expression patterns of DEMETER-like DNA demethylases homologs hint at potential involvement in grapevine dormancy release. *BIO Web of Conferences*, *44*, 04001. https://doi.org/10.1051/bioconf/20224404001

Decreux, A., & Messiaen, J. (2005). Wall-associated kinase WAK1 interacts with cell wall pectins in a calcium-induced conformation. *Plant & Cell Physiology*, *46*(2), 268–278. https://doi.org/10.1093/pcp/pci026

Deschamps-Francoeur, G., Boivin, V., Abou Elela, S., & Scott, M. S. (2019). CoCo: RNA-seq read assignment correction for nested genes and multimapped reads. *Bioinformatics (Oxford, England)*, *35*(23), 5039–5047. https://doi.org/10.1093/bioinformatics/btz433

Dixon & Gschwend. (2023). (Under Review). Trichomes and Unique Gene Expression Confer Insect Herbivory Resistance in Vitis labrusca Grapevines. *BMC Plant Biology.*

Dobin, A., Davis, C. A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., Batut, P., Chaisson, M., & Gingeras, T. R. (2013). STAR: Ultrafast universal RNA-seq aligner. *Bioinformatics (Oxford, England)*, *29*(1), 15–21. https://doi.org/10.1093/bioinformatics/bts635

Dokoozlian, N. K. (1999). Chilling Temperature and Duration Interact on the Budbreak of `Perlette' Grapevine Cuttings. *HortScience*, *34*(6), 1–3. https://doi.org/10.21273/HORTSCI.34.6.1

Dokoozlian, N. K., Williams, L. E., & Neja, R. A. (1995). Chilling Exposure and Hydrogen Cyanamide Interact in Breaking Dormancy of Grape Buds. *HortScience*, *30*(6), 1244–1247. https://doi.org/10.21273/HORTSCI.30.6.1244

Druart, N., Johansson, A., Baba, K., Schrader, J., Sjödin, A., Bhalerao, R. R., Resman, L., Trygg, J., Moritz, T., & Bhalerao, R. P. (2007). Environmental and hormonal regulation of the activity–dormancy cycle in the cambial meristem involves stage-specific modulation of transcriptional and metabolic networks. *The Plant Journal*, *50*(4), 557–573. https://doi.org/10.1111/j.1365-313X.2007.03077.x

Emms, D. M., & Kelly, S. (2019). OrthoFinder: Phylogenetic orthology inference for comparative genomics. *Genome Biology*, *20*(1), 1–14. https://doi.org/10.1186/S13059-019-1832-Y/FIGURES/5

Ewels, P., Magnusson, M., Lundin, S., & Käller, M. (2016). MultiQC: Summarize analysis results for multiple tools and samples in a single report. *Bioinformatics (Oxford, England)*, *32*(19), 3047–3048. https://doi.org/10.1093/bioinformatics/btw354

FAOSTAT. (2023). Food and Agriculture Organization of the United Nations *(FAO). FAOSTAT [Internet].* Rome; 2023. Available from: Http://www.fao.org/faostat/en/#data/QC.

Fennell, A. (2004). Freezing tolerance and injury in grapevines. *Journal of Crop Improvement*, *10*(1/2), 201–235. https://doi.org/10.1300/J411v10n01_09

Flores-Pérez, Ú., & Jarvis, P. (2013). Molecular chaperone involvement in chloroplast protein import. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, *1833*(2), 332–340. https://doi.org/10.1016/j.bbamcr.2012.03.019

Gubler, F., Hughes, T., Waterhouse, P., & Jacobsen, J. (2008). Regulation of dormancy in barley by blue light and after-ripening: Effects on abscisic acid and gibberellin metabolism. *Plant Physiology*, *147*(2), 886–896. https://doi.org/10.1104/pp.107.115469

Guo, H.-S., Zhang, Y.-M., Sun, X.-Q., Li, M.-M., Hang, Y.-Y., & Xue, J.-Y. (2016). Evolution of the KCS gene family in plants: The history of gene duplication, sub/neofunctionalization and redundancy. *Molecular Genetics and Genomics*, *291*(2), 739–752. https://doi.org/10.1007/s00438-015-1142-3

Guy, C. L. (1990). Cold Acclimation and Freezing Stress Tolerance: Role of Protein Metabolism.

Hamman, R. A., Dami, I., Walsh, T. M., & Stushnoff, C. (1996). Seasonal Carbohydrate Changes and Cold Hardiness of Chardonnay and Riesling Grapevines. *American Journal of Enology and Viticulture*, *47*, 31–36. https://doi.org/10.5344/ajev.1996.47.1.31

He, H., Liang, G., Lu, S., Wang, P., Liu, T., Ma, Z., Zuo, C., Sun, X., Chen, B., & Mao, J. (2019). Genome-Wide Identification and Expression Analysis of GA2ox, GA3ox, and GA20ox Are Related to Gibberellin Oxidase Genes in Grape (*Vitis vinifera* L.). *Genes*, *10*(9), 680. https://doi.org/10.3390/genes10090680

Herger, A., Dünser, K., Kleine-Vehn, J., & Ringli, C. (2019). Leucine-Rich Repeat Extensin Proteins and Their Role in Cell Wall Sensing. *Current Biology: CB*, *29*(17), R851–R858. https://doi.org/10.1016/j.cub.2019.07.039

Jones, K. S., Paroschy, J., Mckersie, B. D., & Bowley, S. R. (1999). Carbohydrate Composition and Freezing Tolerance of Canes and Buds in *Vitis vinifera*. *Journal of Plant Physiology*, *155*(1), 101–106. https://doi.org/10.1016/S0176-1617(99)80146-1

Kanehisa, M., & Goto, S. (2000). KEGG: Kyoto encyclopedia of genes and genomes. *Nucleic Acids Research*, *28*(1), 27–30. https://doi.org/10.1093/nar/28.1.27

Kassambara A (2023). *rstatix: Pipe-Friendly Framework for Basic Statistical Tests*. R package version 0.7.2, https://rpkgs.datanovia.com/rstatix/

Keller. (2015). The Science of Grapevines—2nd Edition. https://shop.elsevier.com/books/the-science-of-grapevines/keller/978-0-12-419987-3

Koch, K. (2004). Sucrose metabolism: Regulatory mechanisms and pivotal roles in sugar sensing and plant development. *Current Opinion in Plant Biology*, *7*(3), 235–246. https://doi.org/10.1016/j.pbi.2004.03.014

Kolattukudy, P. E. (1980). Cutin, Suberin, and Waxes. In P. K. Stumpf (Ed.), *Lipids: Structure and Function* (pp. 571–645). Academic Press. https://doi.org/10.1016/B978-0-12-675404-9.50024-2

Kovaleski, A. P., & Londo, J. P. (2019). Tempo of gene regulation in wild and cultivated *Vitis* species shows coordination between cold deacclimation and budbreak. *Plant Science*, *287*, 110178. https://doi.org/10.1016/j.plantsci.2019.110178

Krishna, P., Sacco, M., Cherutti, J. F., & Hill, S. (1995). Cold-Induced Accumulation of hsp90 Transcripts in *Brassica napus*. *Plant Physiology*, *107*(3), 915–923. https://doi.org/10.1104/pp.107.3.915

La Verde, V., Dominici, P., & Astegno, A. (2018). Towards Understanding Plant Calcium Signaling through Calmodulin-Like Proteins: A Biochemical and Structural Perspective. *International Journal of Molecular Sciences*, *19*(5), 1331. https://doi.org/10.3390/ijms19051331

Lamichhane, J. R. (2021). Rising risks of late-spring frosts in a changing climate. *Nature Climate Change*, *11*(7), Article 7. https://doi.org/10.1038/s41558-021-01090-x

Lenz, A., Hoch, G., Korner, C., & Vitasse, Y. (2016). Convergence of leaf-out towards minimum risk of freezing damage in temperate trees. *Functional Ecology*, *30*(9), 1480–1490. https://doi.org/10.1111/1365-2435.12623

Lewandowska, M., Keyl, A., & Feussner, I. (2020). Wax biosynthesis in response to danger: Its regulation upon abiotic and biotic stress. *New Phytologist*, *227*(3), 698–713. https://doi.org/10.1111/nph.16571

Li, B., & Gschwend, A. R. (2023). Vitis labrusca genome assembly reveals diversification between wild and cultivated grapevine genomes. *Frontiers in Plant Science*, *14*. https://www.frontiersin.org/journals/plant-science/articles/10.3389/fpls.2023.1234130

Liu, B., Sun, Y., Li, X., Guo, D., Zhao, L., Ma, C., Wang, L., & Wang, S. (2023). β-ketoacyl-CoA synthase improves the drought tolerance of root restricted grown grapevines by regulating the cuticular wax biosynthesis. *Scientia Horticulturae*, *307*, 111494. https://doi.org/10.1016/j.scienta.2022.111494

Liu et al. (2016). Bioinformatic Identification and Analysis of Extensins in the Plant Kingdom | *Plos ONE*. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0150177

Londo, J., & Johnson, L. M. (2014). Variation in the chilling requirement and budburst rate of wild *Vitis* species. *Environmental and Experimental Botany*, *106*, 138–147. https://doi.org/10.1016/J.ENVEXPBOT.2013.12.012

Londo, J., & Kovaleski, A. (2017). Characterization of Wild North American Grapevine Cold Hardiness Using Differential Thermal Analysis. *American Journal of Enology and Viticulture*, *68*(2), 203–212.

Londo, J. P., Kovaleski, A. P., & Lillis, J. A. (2018). Divergence in the transcriptional landscape between low temperature and freeze shock in cultivated grapevine (*Vitis vinifera*). *Horticulture Research*, *5*.

Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology*, *15*(12), 550. https://doi.org/10.1186/s13059-014-0550-8

Mahajan, S., & Tuteja, N. (2005). Cold, salinity and drought stresses: An overview. *Archives of Biochemistry and Biophysics*, *444*(2), 139–158. https://doi.org/10.1016/j.abb.2005.10.018

Møller, I. M., Jensen, P. E., & Hansson, A. (2007). Oxidative modifications to cellular components in plants. *Annual Review of Plant Biology*, *58*, 459–481. https://doi.org/10.1146/annurev.arplant.58.032806.103946

Nambara, E., Okamoto, M., Tatematsu, K., Yano, R., Seo, M., & Kamiya, Y. (2010). Abscisic acid and the control of seed dormancy and germination. *Seed Science Research*, *20*(2), 55–67.

NCBI Resource Coordinators. (2016). Database resources of the National Center for Biotechnology Information. *Nucleic Acids Research*, *44*(D1), D7-19. https://doi.org/10.1093/nar/gkv1290

North, M., Workmaster, B., & Atucha, A. (2021). Cold Hardiness of Cold Climate Interspecific Hybrid Grapevines Grown in a Cold Climate Region. *American Journal of Enology and Viticulture*, *72*, 318–327. https://doi.org/10.5344/ajev.2021.21001

Okamoto, M., Kuwahara, A., Seo, M., Kushiro, T., Asami, T., Hirai, N., Kamiya, Y., Koshiba, T., & Nambara, E. (2006). CYP707A1 and CYP707A2, which encode abscisic acid 8'-hydroxylases, are indispensable for proper control of seed dormancy and germination in *Arabidopsis*. *Plant Physiology*, *141*(1), 97–107. https://doi.org/10.1104/pp.106.079475

Pérez, F., & Rubio, S. (2015). Prolonged Exposure of Grapevine Buds to Low Temperatures Increases Dormancy, Cold Hardiness, and the Expression of Vvα-AMYs Genes. https://doi.org/10.1007/978-3-319-14451-1_10

Pierquet, P., & Stushnoff, C. (1980). Relationship of Low Temperature Exotherms to Cold Injury in *Vitis riparia* Michx. *31*(1), 7.

Polisensky, D. H., & Braam, J. (1996). Cold-shock regulation of the Arabidopsis TCH genes and the effects of modulating intracellular calcium levels. *Plant Physiology*, *111*(4), 1271–1279. https://doi.org/10.1104/pp.111.4.1271

Poschet, G., Hannich, B., Raab, S., Jungkunz, I., Klemens, P. A. W., Krueger, S., Wic, S., Neuhaus, H. E., & Büttner, M. (2011). A Novel *Arabidopsis* Vacuolar Glucose Exporter Is Involved in Cellular Sugar Homeostasis and Affects the Composition of Seed Storage Compounds. *Plant Physiology*, *157*(4), 1664–1676. https://doi.org/10.1104/pp.111.186825

R Core Team. (2023a). *R: A language and environment for statistical computing* (Version 4.3.1) [Software]. R Foundation for Statistical Computing. https://www.R-project.org/

R Core Team (2023b). *stats: The R Stats Package*. R package version 4.3.1. https://cran.r-project.org/package=stats

Rahman, M. A., Moody, M. A., & Nassuth, A. (2014). Grape contains 4 ICE genes whose expression includes alternative polyadenylation, leading to transcripts encoding at least 7 different ICE proteins. *Environmental and Experimental Botany*, *106*, 70–78. https://doi.org/10.1016/j.envexpbot.2014.01.003

Ren, C., Fan, P., Li, S., & Liang, Z. (2023). Advances in understanding cold tolerance in grapevine. *Plant Physiology*, 192(3), 1733–1746. https://doi.org/10.1093/plphys/kiad092

Rubio, S., Noriega, X., & Perez, F. (2019). Abscisic acid (ABA) and low temperatures synergistically increase the expression of CBF/DREB1 transcription factors and cold-hardiness in grapevine dormant buds. *Annals of Botany*, *123*(4), 681–689. https://doi.org/10.1093/aob/mcy201

Rubio, S., & Pérez, F. J. (2019). ABA and its signaling pathway are involved in the cold acclimation and deacclimation of grapevine buds. *Scientia Horticulturae*, *256*, 108565. https://doi.org/10.1016/j.scienta.2019.108565

Saibo et al. (2009). Transcription factors and regulation of photosynthetic and related metabolism under environmental stresses. *Annals of Botany*. *Oxford Academic*. https://academic.oup.com/aob/article/103/4/609/164513

Sturm, A. (1999). Invertases. Primary Structures, Functions, and Roles in Plant Development and Sucrose Partitioning. *Plant Physiology*, *121*(1), 1–8. https://doi.org/10.1104/pp.121.1.1

Sugar, D., Gold, R., Lombard, P., & Gardea, A. (2003). Strategies for Frost Protection. E. W. Hellman (Ed.), *Oregon Viticulture*, 213–217. Oregon State University,

Corvallis. Retrieved from https://osupress.oregonstate.edu/book/oregon-viticulture

Sun, X., Zhang, L., Wong, D. C. J., Wang, Y., Zhu, Z., Xu, G., Wang, Q., Li, S., Liang, Z., & Xin, H. (2019). The ethylene response factor VaERF092 from Amur grape regulates the transcription factor VaWRKY33, improving cold tolerance. *Plant Journal*, *99*(5), 988–1002. https://doi.org/10.1111/tpj.14378

Sun, X., Zhao, T., Gan, S., Ren, X., Fang, L., Karungo, S. K., Wang, Y., Chen, L., Li, S., & Xin, H. (2016). Ethylene positively regulates cold tolerance in grapevine by modulating the expression of ETHYLENE RESPONSE FACTOR 057. *Scientific Reports*, *6*(1), 24066. https://doi.org/10.1038/srep24066

Therneau, T., Hart, S., & Kocher, J.P. (2023). *Calculating sample size estimates for RNA Seq studies*. R package version 1.42.0.

Thomashow, M. F. (2001). So what's new in the field of plant cold acclimation? Lots! *Plant Physiology*, *125*(1), 89–93. https://doi.org/10.1104/pp.125.1.89

Tronchet, M., Balagué, C., Kroj, T., Jouanin, L., & Roby, D. (2009). Cinnamyl alcohol dehydrogenases-C and D, key enzymes in lignin biosynthesis, play an essential role in disease resistance in *Arabidopsis*. *Molecular Plant Pathology*, *11*(1), 83–92. https://doi.org/10.1111/j.1364-3703.2009.00578.x

USDA. (2022). NonCitrus Fruits and Nuts 2021 Summary (p. 88). https://downloads.usda.library.cornell.edu/usda-esmis/files/zs25x846c/4q77gv96p/8336j672v/ncit0522.txt

van Leeuwen, C., & Darriet, P. (2016). The Impact of Climate Change on Viticulture and Wine Quality. *Journal of Wine Economics*, *11*, 150–167. https://doi.org/10.1017/jwe.2015.21

Vergara, R., Noriega, X., Aravena, K., Prieto, H., & Pérez, F. (2017). ABA Represses the Expression of Cell Cycle Genes and May Modulate the Development of Endodormancy in Grapevine Buds. *Frontiers in Plant Science*, *8*, null. https://doi.org/10.3389/fpls.2017.00812

Vitasse, Y., Lenz, A., & Körner, C. (2014). The interaction between freezing tolerance and phenology in temperate deciduous trees. *Frontiers in Plant Science*, *5*. https://www.frontiersin.org/articles/10.3389/fpls.2014.00541

Wan, H., Wu, L., Yang, Y., Zhou, G., & Ruan, Y.-L. (2018). Evolution of Sucrose Metabolism: The Dichotomy of Invertases and Beyond. *Trends in Plant Science*, *23*(2), 163–177. https://doi.org/10.1016/j.tplants.2017.11.001

Wang, J., Ye, H., Zhou, H., Chen, P., Liu, H., Xi, R., Wang, G., Hou, N., & Zhao, P. (2022). Genome-wide association analysis of 101 accessions dissects the genetic

basis of shell thickness for genetic improvement in Persian walnut (*Juglans regia* L.). *BMC Plant Biology*, *22*, 436. https://doi.org/10.1186/s12870-022-03824-1

Wang, L., Zhang, X., Qu, Z., Guo, W., van Nocker, S., & Zhang, C. (2022). Grapevine *VviERF105* promotes tolerance to abiotic stress and is degraded by the E3 ubiquitin ligase VviPUB19. *Environmental and Experimental Botany*, *201*, 105001. https://doi.org/10.1016/j.envexpbot.2022.105001

Wang, N., Zheng, Y., Xin, H., Fang, L., & Li, S. (2013). Comprehensive analysis of NAC domain transcription factor gene family in *Vitis vinifera*. *Plant Cell Reports*, *32*(1), 61–75. https://doi.org/10.1007/s00299-012-1340-y

Wang, Q., Du, X., Zhou, Y., Xie, L., Bie, S., Tu, L., Zhang, N., Yang, X., Xiao, S., & Zhang, X. (2020). The β-ketoacyl-CoA synthase KCS13 regulates the cold response in cotton by modulating lipid and oxylipin biosynthesis. *Journal of Experimental Botany*, *71*(18), 5615–5630. https://doi.org/10.1093/jxb/eraa254

Wickham H (2016). *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York. ISBN 978-3-319-24277-4

Wolf, T. K., & Cook, M. K. (1994). Cold Hardiness of Dormant Buds of Grape Cultivars: Comparison of Thermal Analysis and Field Survival. *HortScience*, *29*(12), 1453–1455. https://doi.org/10.21273/HORTSCI.29.12.1453

Wu, T., Hu, E., Xu, S., Chen, M., Guo, P., Dai, Z., Feng, T., Zhou, L., Tang, W., Zhan, L., Fu, X., Liu, S., Bo, X., & Yu, G. (2021). clusterProfiler 4.0: A universal enrichment tool for interpreting omics data. *Innovation (Cambridge (Mass.))*, *2*(3), 100141. https://doi.org/10.1016/j.xinn.2021.100141

Wu, Y., Li, X., Zhang, J., Zhao, H., Tan, S., Xu, W., Pan, J., Yang, F., & Pi, E. (2022). ERF subfamily transcription factors and their function in plant responses to abiotic stresses. *Frontiers in Plant Science*, *13*, 1042084. https://doi.org/10.3389/fpls.2022.1042084

Xiao, H., Siddiqua, M., Braybrook, S., & Nassuth, A. (2006). Three grape CBF/DREB1 genes respond to low temperature, drought and abscisic acid. *Plant Cell and Environment*, *29*(7), 1410–1421. https://doi.org/10.1111/j.1365-3040.2006.01524.x

Xiao, H., Tattersall, E., Siddiqua, M., Cramer, G., & Nassuth, A. (2008). CBF4 is a unique member of the CBF transcription factor family of *Vitis vinifera* and *Vitis riparia*. *Plant Cell and Environment*, *31*(1), 1–10. https://doi.org/10.1111/j.1365-3040.2007.01741.x

Xin, H., Zhu, W., Wang, L., Xiang, Y., Fang, L., Li, J., Sun, X., Wang, N., Londo, J. P., & Li, S. (2013). Genome Wide Transcriptional Profile Analysis of *Vitis amurensis*

and *Vitis vinifera* in Response to Cold Stress. *PloS One*, *8*(3). https://doi.org/10.1371/journal.pone.0058740

Xu, W., Li, R., Zhang, N., Ma, F., Jiao, Y., & Wang, Z. (2014). Transcriptome profiling of *Vitis amurensis*, an extremely cold-tolerant Chinese wild *Vitis* species, reveals candidate genes and events that potentially connected to cold stress. *Plant Molecular Biology*, *86*(4–5), 527–541. https://doi.org/10.1007/s11103-014-0245-2

Xu, X., Hu, Q., Yang, W., & Jin, Y. (2017). The roles of cell wall invertase inhibitor in regulating chilling tolerance in tomato. *BMC Plant Biology*, *17*(1), 195. https://doi.org/10.1186/s12870-017-1145-9

Yang, Z., Yang, X., Dong, S., Ge, Y., Zhang, X., Zhao, X., & Han, N. (2020). Overexpression of β-Ketoacyl-CoA Synthase From *Vitis vinifera* L. Improves Salt Tolerance in Arabidopsis thaliana. *Frontiers in Plant Science*, *11*, 564385. https://doi.org/10.3389/fpls.2020.564385

Yu, G., Wang, L.-G., Yan, G.-R., & He, Q.-Y. (2015). DOSE: An R/Bioconductor package for disease ontology semantic and enrichment analysis. *Bioinformatics (Oxford, England)*, *31*(4), 608–609. https://doi.org/10.1093/bioinformatics/btu684

Zabadal, T., Dami, I., & Goffinet, M. (2007). Winter Injury to Grapevines and Methods of Protection.

Zheng, C., Acheampong, A. K., Shi, Z., Mugzech, A., Halaly-Basha, T., Shaya, F., Sun, Y., Colova, V., Mosquna, A., Ophir, R., Galbraith, D. W., & Or, E. (2018). Abscisic acid catabolism enhances dormancy release of grapevine buds. *Plant, Cell & Environment*, *41*(10), 2490–2503. https://doi.org/10.1111/pce.13371

Zheng, H., Liang, Y., Hong, B., Xu, Y., Ren, M., Wang, Y., Huang, L., Yang, L., & Tao, J. (2023). Genome-Scale Analysis of the Grapevine KCS Genes Reveals Its Potential Role in Male Sterility. *International Journal of Molecular Sciences*, *24*(7), 6510. https://doi.org/10.3390/ijms24076510

Zhu, Z., Shi, J., Xu, W., Li, H., He, M., Xu, Y., Xu, T., Yang, Y., Cao, J., & Wang, Y. (2013). Three ERF transcription factors from Chinese wild grapevine *Vitis pseudoreticulata* participate in different biotic and abiotic stress-responsive pathways. *Journal of Plant Physiology*, *170*(10), 923–933.

Zuther, E., Büchel, K., Hundertmark, M., Stitt, M., Hincha, D. K., & Heyer, A. G. (2004). The role of raffinose in the cold acclimation response of *Arabidopsis thaliana*. *FEBS Letters*, *576*(1–2), 169–173. https://doi.org/10.1016/j.febslet.2004.09.006

Tables

Table 2.1 Data Analysis for normality and variance.

Bud Cold Hardiness			
Accession or Cultivar	**LT50[1]**	**Normality[2]**	**Variance[2]**
'Cabernet Sauvignon'	-15.62 ± 1.42	0.37	0.40
'PN40024'	-18.53 ± 1.42	0.37	
'GREM4'	-25.14 ± 1.42	0.74	
'Dunkel #1'	-23.03 ± 1.64	0.50	
Frost Tolerance Assay			
Accession or Cultivar	**Survivability (%)[1]**	**Normality[2]**	**Variance[2]**
'GREM4'	47.5 ± 8.54	0.85	0.49
'Cabernet Sauvignon'	22.5 ± 6.29	0.41	

[1] LT50: Lethal Temperature for 50% bud injury denoting bud cold hardiness level of *V. labrusca* accessions ('GREM4' and 'Dunkel #1') and *V. vinifera* cvs. ('PN40024' and 'Cabernet Sauvignon'). The values denote means ± standard errors.
[2] The values denote the p-values calculated from the Shapiro-Wilk normality test and Levene's test for homogeneity of variance.

Table 2.2 Subset of 'GREM4' DEGs with highest log2FoldChange under chill stress.

A) Upregulated genes **'GREM4' ID**	**Gene description***	**log2FoldChange**
augustus-3-processed-gene-55.6†	*Pollen-specific leucine-rich repeat extensin-like protein 3*	8.47
augustus-3-processed-gene-81.7†	*Pollen-specific leucine-rich repeat extensin-like protein 3*	7.56
maker-6-snap-gene-50.48†	*Receptor-like protein kinase At3g21340*	7.31
maker-8-augustus-gene-151.47†	*Wall-associated receptor kinase-like 20*	7.11
maker-1-augustus-gene-115.27†	*Probable serine/threonine-protein kinase PBL11*	7.07
augustus-11-processed-gene-6.0†	*Ethylene-responsive transcription factor ERF017-like*	7.02
augustus-4-processed-gene-22.9	*Ethylene-responsive transcription factor ERF017-like*	6.41
maker-6-snap-gene-0.35	*NAC domain-containing protein 68*	6.25
maker-18-snap-gene-151.51†	*Abscisic acid 8'-hydroxylase 3*	6.24
maker-2-snap-gene-38.46	*Expansin-A7-like*	6.11
maker-9-augustus-gene-139.44†	*3-ketoacyl-CoA synthase 15*	5.96
maker-6-augustus-gene-82.38	*Zinc finger protein CONSTANS-LIKE 7*	5.83

Continued…

* Predicted functions based on homology identified via NCBI BLAST.
† Denotes shared DEGs between chill and freeze stress in 'GREM4' while the rest are unique to each response. All DEGs were selected at p-adjusted ≤0.05.

(Table 2.2 continued)

B) Downregulated genes 'GREM'4 ID	**Gene description***	**log2FoldChange**
maker-17-snap-gene-105.40†	*Organic cation/carnitine transporter 1*	-6.76
maker-13-augustus-gene-114.26	*Probable 2-oxoglutarate-dependent dioxygenase SLC1*	-6.53
maker-1-snap-gene-68.25	*Fasciclin-like arabinogalactan protein 21*	-5.58
maker-10-snap-gene-121.49†	*Twinkle homolog protein, chloroplastic/mitochondrial*	-5.02
augustus-1-processed-gene-97.4	*UDP-glycosyltransferase 74B1*	-4.11
maker-6-snap-gene-15.34†	*Protein NRT1/ PTR FAMILY 7.1*	-4.03
maker-19-snap-gene-85.51†	*Endoglucanase 11*	-3.81

Table 2.3 Subset of 'GREM4' DEGs with highest log2FoldChange under freeze stress.

A) Upregulated genes **'GREM4' ID**	**Gene Description***	**log2FoldChange**
maker-8-augustus-gene-151.47†	*Wall-associated receptor kinase-like 20*	7.12
maker-6-snap-gene-50.48†	*Receptor-like protein kinase Ag3g21340*	6.80
augustus-11-processed-gene-6.0†	*Ethylene-responsive transcription factor ERF017-like*	6.76
maker-1-snap-gene-202.55	*Probable calcium-binding protein CML31*	6.54
maker-6-augustus-gene-3.42	*Beta-amyrin 28-monooxygenase-like*	6.44
augustus-3-processed-gene-55.6†	*Pollen-specific leucine-rich repeat extensin-like protein 3*	6.35
maker-1-augustus-gene-115.27†	*Probable serine/threonine-protein kinase PBL11*	6.10
maker-14-snap-gene-275.45	*Transcription factor MYB118*	5.94
maker-16-augustus-gene-84.38	*Ethylene-responsive transcription factor 5*	5.71
snap-16-processed-gene-82.20	*Ethylene-responsive transcription factor ERF105-like*	5.70
maker-9-augustus-gene-139.44†	*3-ketoacyl-CoA synthase 15*	5.62
maker-16-augustus-gene-83.18	*Ethylene-responsive transcription factor ERF105*	5.57
augustus-3-processed-gene-81.7†	*Pollen-specific leucine-rich repeat extensin-like protein 3*	5.55
snap-Un-processed-gene-36522	*Ethylene-responsive transcription factor 5*	5.49

Continued…

***** Predicted functions based on homology identified via NCBI BLAST.
† Denotes shared DEGs between chill and freeze stress in 'GREM4' while the rest are unique to each response. All DEGs were selected at p-adjusted ≤0.05.

(Table 2.3 Continued)

B) Downregulated genes 'GREM4' ID	**Gene Description***	**log2FoldChange**
snap-15-processed-gene-92.17	*Transcription factor bHLH91-like*	-5.79
maker-14-snap-gene-44.57	*Sugar transporter ERD6-like 5*	-5.61
maker-19-snap-gene-85.51†	*Endoglucanase 11*	-5.58
augustus-1-processed-gene-67.2	*Fasciclin-like arabinogalactan protein 21*	-5.37
maker-17-snap-gene-105.40†	*Organic cation/carnitine transporter 1*	-4.63
augustus-10-processed-gene-70.4	*Chlorophyll a-b binding protein of LHCII type 1-like*	-4.49
maker-6-snap-gene-15.34†	*Protein NRT1/ PTR FAMILY 7.1*	-4.10
augustus-4-processed-gene-233.2	*cell wall / vacuolar inhibitor of fructosidase 2*	-4.03
maker-Un-augustus-gene-142.28	*Chlorophyll a-b binding protein 13, chloroplastic-like*	-3.99
augustus-10-processed-gene-70.10	*Chlorophyll a-b binding protein of LHCII type 1-like*	-3.88
maker-10-snap-gene-121.49†	*Twinkle homolog protein, chloroplastic/mitochondrial*	-3.72

Table 2.4 Subset of 'Cabernet Sauvignon' DEGs with highest log2FoldChange under chill stress.

A) Upregulated genes 'Cabernet Sauvignon' ID	**Gene description**[†]	**log2FoldChange**
VvCabSauv08_P0084F.ver 1.0.g468220*	*Zinc finger protein CONSTANS-LIKE 7*	10.22
VvCabSauv08_P0010F.ver 1.0.g327050	*Rd22-b protein (RD22-B)*	9.58
VvCabSauv08_P0032F.ver 1.0.g395680	*Delta(24)-sterol reductase*	9.19
VvCabSauv08_P0010F.ver 1.0.g323490*	*Ethylene-responsive transcription factor ERF017*	8.76
VvCabSauv08_P0155F.ver 1.0.g537850*	*Chaperone protein dnaJ C76, chloroplastic*	8.20
VvCabSauv08_P0854F.ver 1.0.g621770	*Putative 12-oxophytodienoate reductase 11*	7.99
VvCabSauv08_P0018F.ver 1.0.g349120	*Galactinol synthase 2*	7.67
VvCabSauv08_P0018F.ver 1.0.g349150	*Galactinol synthase 2*	7.52
VvCabSauv08_P0419F.ver 1.0.g607260	*DREB1E-like or CBF5*	7.50
VvCabSauv08_P0041F.ver 1.0.g412800*	*Ethylene-responsive transcription factor ERF109*	7.36

Continued…

[†] Predicted functions based on homology identified via NCBI BLAST.
***** Denotes shared DEGs between chill and freeze stress in 'Cabernet Sauvignon' while the rest are unique to each response. All DEGs were selected at p-adjusted ≤0.05.

(Table 2.4 continued)

B) Downregulated genes 'Cabernet Sauvignon' ID	**Gene description**[†]	**log2FoldChange**
VvCabSauv08_P0005F.ver 1.0.g295410	*Probable 2-oxoglutarate-dependent dioxygenase SLC1*	-5.94
VvCabSauv08_P0020F.ver 1.0.g357010	*Auxin-responsive protein SAUR71*	-5.73
VvCabSauv08_P0008F.ver 1.0.g314090	*GDSL esterase/lipase At1g09390*	-5.69
VvCabSauv08_P0227F.ver 1.0.g573750*	*Organic cation/carnitine transporter 1*	-4.98
VvCabSauv08_P0008F.ver 1.0.g318700*	*3-ketoacyl-CoA synthase 2*	-4.78
VvCabSauv08_P0454F.ver 1.0.g610600	*Rust resistance kinase Lr10*	-4.08
VvCabSauv08_P0075F.ver 1.0.g457160	*3-ketoacyl-CoA synthase 6*	-3.96
VvCabSauv08_P0123F.ver 1.0.g512120	*Transcription factor MYB4*	-3.93
VvCabSauv08_P0003F.ver 1.0.g289400*	*Oligopeptide transporter 4*	-3.64
VvCabSauv08_P0171F.ver 1.0.g548900	*Endoglucanase 11*	-3.53

Table 2.5 Subset of 'Cabernet Sauvignon' DEGs with highest log2FoldChange under freeze stress.

A) Upregulated genes 'Cabernet Sauvignon' ID	**Gene description**[†]	**log2FoldChange**
VvCabSauv08_P0010F.ver 1.0.g323490*	*Ethylene-responsive transcription factor ERF017*	9.60
VvCabSauv08_P0065F.ver 1.0.g445320	*Ethylene-responsive transcription factor ERF017-like*	9.36
VvCabSauv08_P0002F.ver 1.0.g280590	*Dehydration-responsive element-binding protein 1E-like or CBF6*	9.32
VvCabSauv08_P0084F.ver 1.0.g468220*	*Zinc finger protein CONSTANS-LIKE 7*	7.56
VvCabSauv08_P0020F.ver 1.0.g355610	*Probable calcium-binding protein CML31*	7.52
VvCabSauv08_P0007F.ver 1.0.g312200	*Ethylene-responsive transcription factor ERF109*	7.41
VvCabSauv08_P0020F.ver 1.0.g355660	*Probable calcium-binding protein CML31*	7.10
VvCabSauv08_P0041F.ver 1.0.g412800*	*Ethylene-responsive transcription factor ERF109*	7.02
VvCabSauv08_P0026F.ver 1.0.g377560	*Transcription factor MYB118*	6.95
VvCabSauv08_P0116F.ver 1.0.g506300	*Alpha-1,3-arabinosyltransferase XAT3-like*	6.35
VvCabSauv08_P0029F.ver 1.0.g387900	*Dehydration-responsive element-binding protein 1F or CBF2*	6.24
VvCabSauv08_P0155F.ver 1.0.g537850*	*Chaperone protein dnaJ C76, chloroplastic*	6.24

Continued…

[†] Predicted functions based on homology identified via NCBI BLAST.
* Denotes shared DEGs between chill and freeze stress in 'Cabernet Sauvignon' while the rest are unique to each response. All DEGs were selected at p-adjusted ≤0.05.

(Table 2.5 continued)

B) Downregulated genes 'Cabernet Sauvignon' ID	**Gene description**[†]	**log2FoldChange**
VvCabSauv08_P0227F.ver 1.0.g573750*	*Organic cation/carnitine transporter 1*	-5.98
VvCabSauv08_P0028F.ver 1.0.g383670	*Chlorophyll a-b binding protein 13, chloroplastic-like*	-3.79
VvCabSauv08_P0008F.ver 1.0.g318700*	*3-ketoacyl-CoA synthase 2*	-3.79
VvCabSauv08_P0003F.ver 1.0.g289400*	*Oligopeptide transporter 4*	-3.75
VvCabSauv08_P0133F.ver 1.0.g522550	*Chlorophyll a-b binding protein of LHCII type 1*	-3.52
VvCabSauv08_P0037F.ver 1.0.g405490	*Chlorophyll a-b binding protein of LHCII type 1*	-3.19
VvCabSauv08_P0022F.ver 1.0.g363260	*Chlorophyll a-b binding protein 4, chloroplastic*	-2.92

Table 2.6 Subset of DEGs in ‘GREM4’ (chill vs control) compared to 'Cabernet Sauvignon' (chill vs control).

A) Upregulated genes*		
‘GREM4’ ID	**Gene Description†**	**Δlog2FoldChange**
augustus-8-processed-gene-46.1	*Pentatricopeptide repeat-containing protein At5g06540*	4.13
maker-18-snap-gene-151.51	*Abscisic acid 8'-hydroxylase 3*	5.29
maker-13-snap-gene-23.37	*COBRA-like protein 4*	2.30
augustus-14-processed-gene-269.9	*Uridine kinase-like protein 5*	3.51
maker-15-augustus-gene-58.34	*Endoglucanase 3-like*	3.96
maker-4-snap-gene-28.33	*Beta-amyrin synthase*	3.00
maker-17-augustus-gene-24.28	*Laccase-1*	5.06

B) Downregulated genes*		
‘GREM4’ ID	**Gene Description†**	**Δlog2FoldChange**
maker-3-snap-gene-22.105	*Disease resistance protein RPP13-like*	-4.78
maker-3-snap-gene-23.48	*Putative disease resistance protein At1g50180*	-5.09
maker-10-snap-gene-121.49	*Twinkle homolog protein, chloroplastic/mitochondrial*	-4.72
maker-12-augustus-gene-200.37	*MLO-like protein 4*	-3.64
maker-10-snap-gene-3.48	*LysM domain receptor-like kinase 3*	-3.29
maker-14-augustus-gene-169.37	*Transcription factor MYB1-like*	-2.82
maker-12-augustus-gene-40.41	*bHLH162*	-4.00
maker-10-augustus-gene-1.40	*Gibberellin 2-beta-dioxygenase 2 (GA2ox2)*	-2.92
maker-14-augustus-gene-93.25	*Pathogenesis-related protein 4*	-3.39

***** Genes upregulated in ‘GREM4’ were downregulated in ‘Cabernet Sauvignon’. All DEGs were selected at p-adjusted ≤0.05.

† Predicted functions based on homology identified via NCBI BLAST.

Table 2.7 DEGs in 'GREM4' (freeze vs control) compared to 'Cabernet Sauvignon' (freeze vs control).

A) Upregulated genes*		
'GREM4' ID	**Gene description†**	**Δlog2FoldChange**
augustus-18-processed-gene-23.11	*Probable receptor-like protein kinase At1g11050*	2.58
maker-18-snap-gene-151.51	*Abscisic acid 8'-hydroxylase 3*	4.07

B) Downregulated genes*		
'GREM4' ID	**Gene description†**	**Δlog2FoldChange**
maker-3-snap-gene-23.48	*Putative disease resistance protein At1g50180*	-3.24
maker-10-snap-gene-121.49	*Twinkle homolog protein, chloroplastic/mitochondrial*	-3.38
maker-12-augustus-gene-200.37	*MLO-like protein 4*	-3.75

***** Genes upregulated in 'GREM4' were downregulated in 'Cabernet Sauvignon'. All DEGs were selected at p-adjusted ≤0.05.

† Predicted functions based on homology identified via NCBI BLAST.

Figures

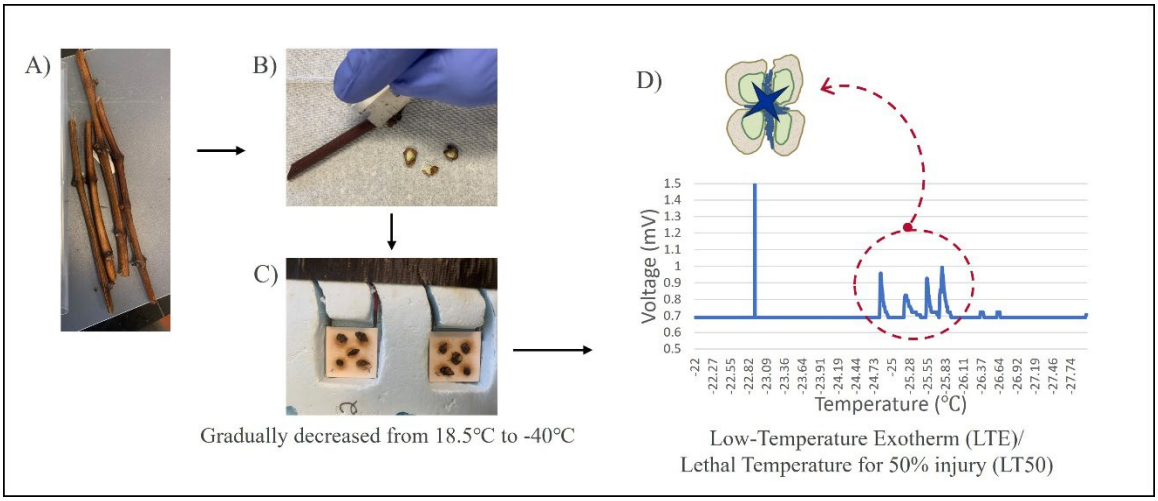

Figure 2.1 Overview of Differential Thermal Analysis (DTA) to measure bud cold hardiness. A) Dormant grapevine canes were collected from mature vines in the cold room, B) Buds were excised from dormant canes, C) Buds were placed on thermoelectric modules. The thermoelectric modules were placed in the environmental chamber which was programmed to gradually decrease the temperature from 18.5°C to -40°C at a constant rate of 4°C/hr. D) Peaks on the line graph represent the low temperature exotherm (LTE) or the freezing point of intracellular water which can cause crystallization and injury to bud cells as illustrated in the image.

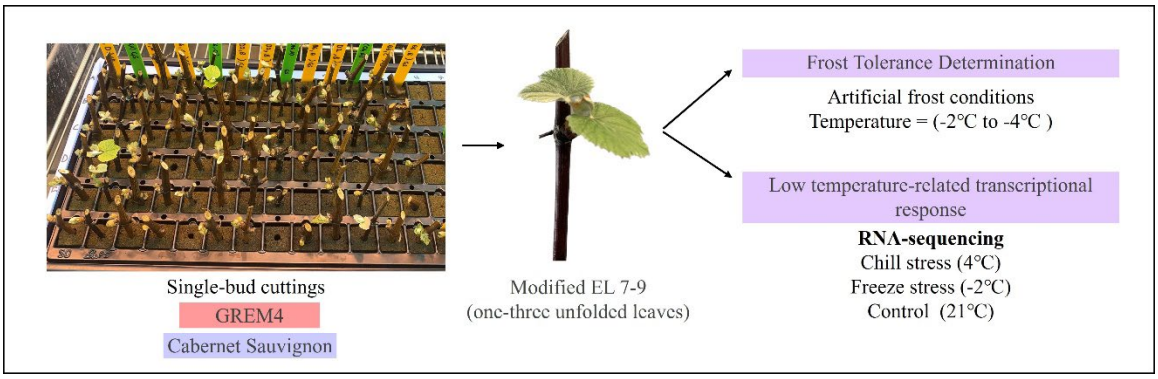

Figure 2.2 Overview of shoots used in project experiments. Dormant single-bud cuttings of both 'GREM4' and 'Cabernet Sauvignon' were allowed to grow in a growth chamber. On reaching the Modified EL 7-9 stage (one to three unfolded leaf stage), some of the shoots were used for frost tolerance determination, while others were exposed to chill (4°C), freeze (-2°C), or room (control) temperatures and then used for RNA-sequencing.

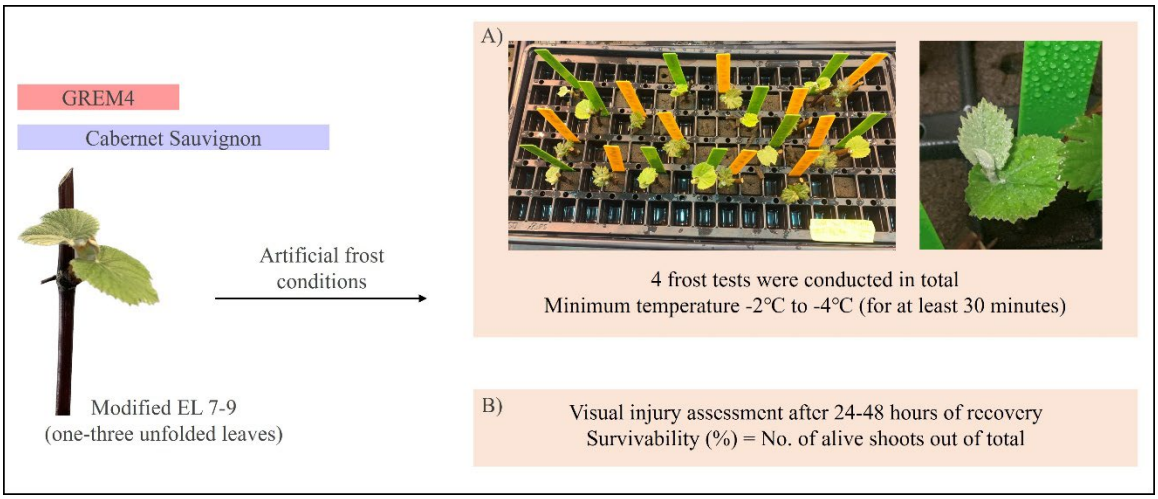

Figure 2.3 Frost tolerance determination experimental design.

A) Young shoots of 'GREM4' and 'Cabernet Sauvignon' at the Modified EL 7-9 stage were subjected to artificial frost conditions (lowest temperature ranging between -2°C to -4°C). B) After 24-48 hours of recovery at room temperature, survivability of shoots was measured by counting the number of alive shoots out of total shoots tested per accession/cultivar.

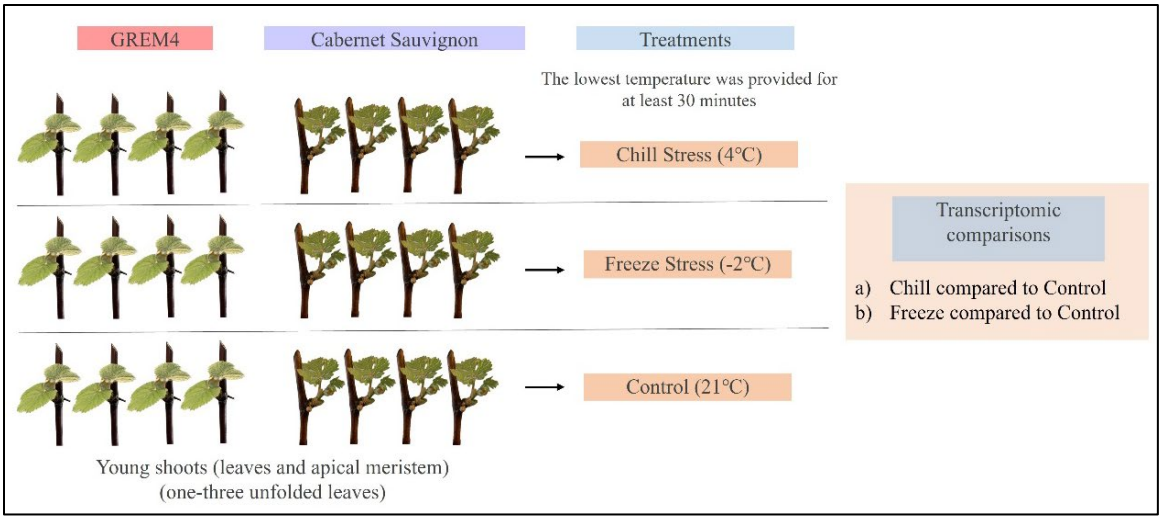

Figure 2.4 Experimental design for tissue collection to conduct RNA-sequencing.

Young shoots (leaves and apical meristem) of 'GREM4' and 'Cabernet Sauvignon' were subjected to chill (4°C), freeze (-2°C), and control (21°C) conditions. For each temperature treatment, RNA was isolated from four 'GREM4' and 'Cabernet Sauvignon' shoots for RNA-sequencing.

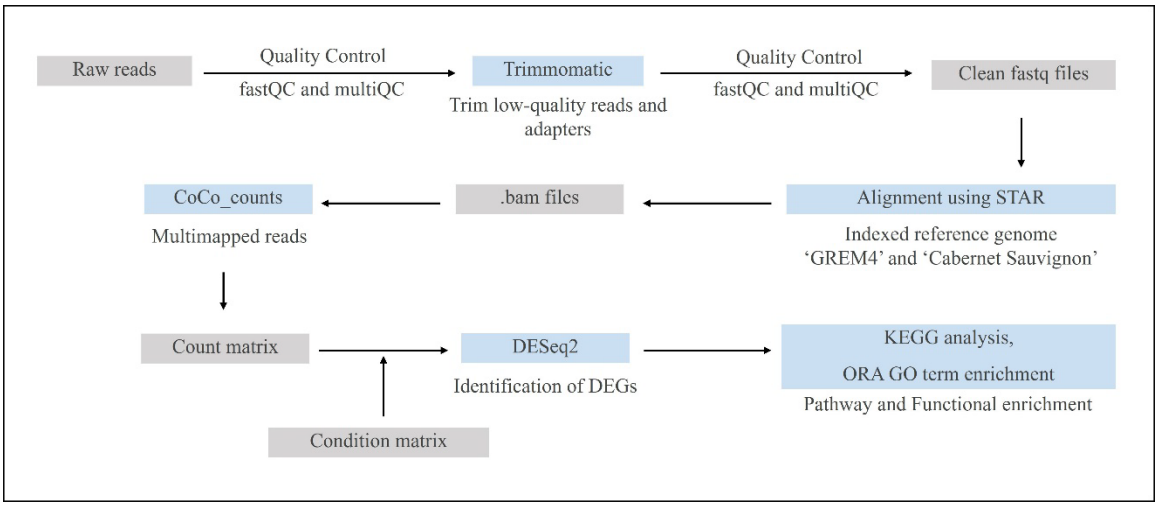

Figure 2.5 Bioinformatic workflow for identification of differential gene expression between temperatures within 'GREM4' and 'Cabernet Sauvignon'.

Blue boxes denote programs and grey boxes denote the input files used. The program description is mentioned below the boxes.

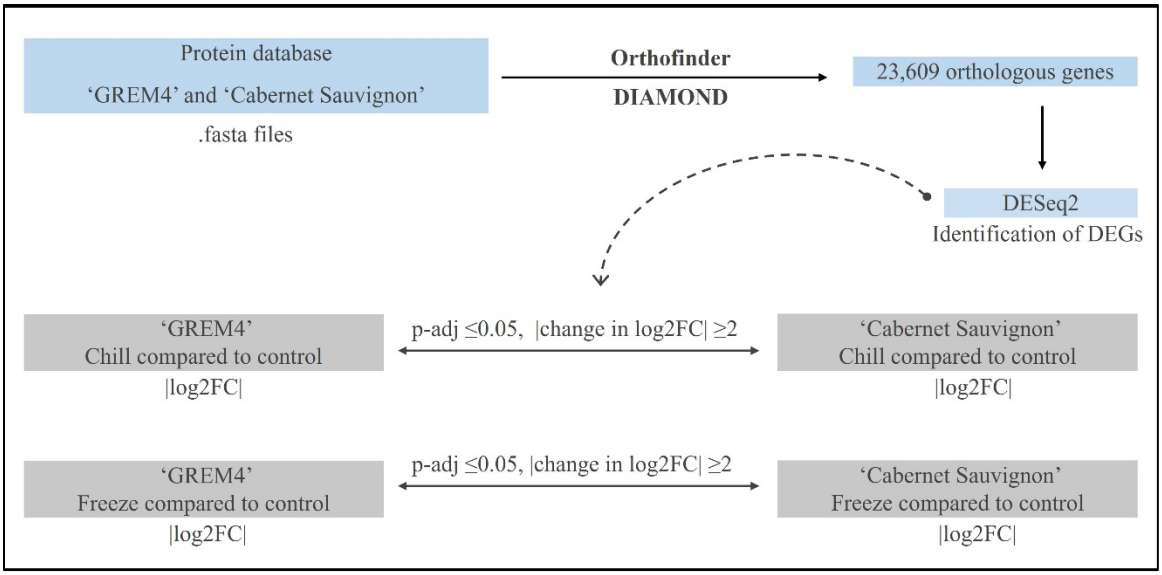

Figure 2.6 Bioinformatic workflow to identify orthologous genes between 'GREM4' and 'Cabernet Sauvignon' and identify differentially expressed genes.

Blue boxes denote the workflow of creating list of orthologous genes, grey boxes denote the comparisons for interaction analysis.

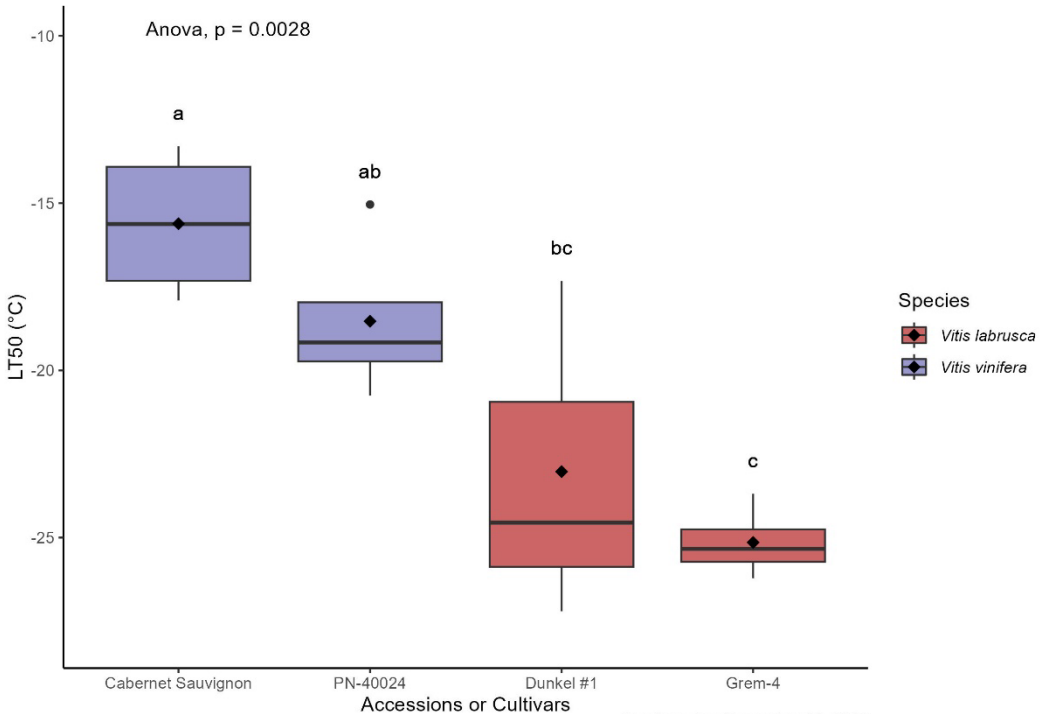

Figure 2.7 Bud cold hardiness of *V. labrusca* accessions and *V. vinifera* cultivars as determined by differential thermal analysis (DTA).

A box-plot graph showing the range of bud cold hardiness, measured as LT50 (lethal temperature for 50% injury) per *V. labrusca* accession and *V. vinifera* cultivar. Diamonds denote mean LT50, while the horizontal line represents median LT50 for each accession or cultivar. The box represents interquartile range, while whiskers depict the range of the data. Outliers, if present, are denoted by individual points outside the whisker. Distinct letters represent significant differences in bud cold hardiness determined using Tukey's Honestly Significant Difference (HSD) test with a significance level of $p \leq 0.05$.

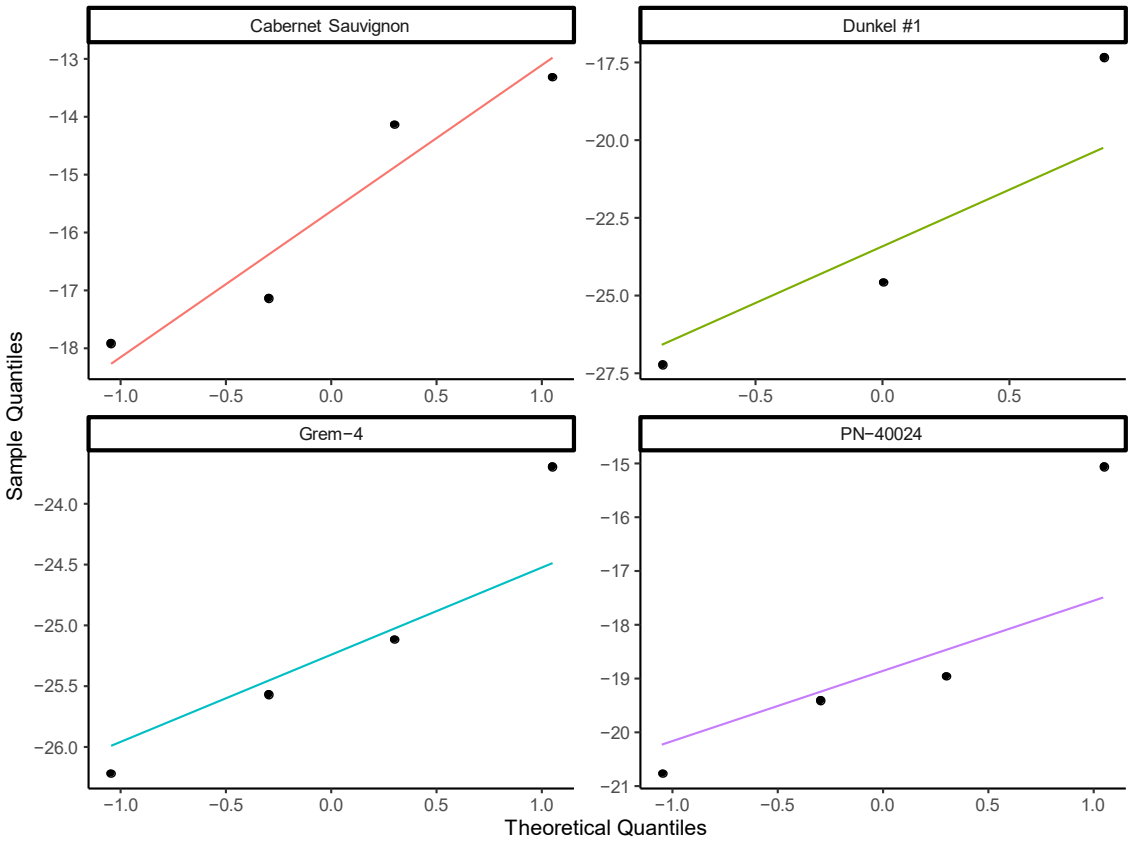

Figure 2.8 Quantile-quantile plots showing the distribution of bud LT50 (lethal temperature for 50% injury) values for *V. labrusca* accessions and *V. vinifera* cultivars. The x-axis denotes the theoretical quantiles, while the y-axis denotes sample quantile values for LT50.

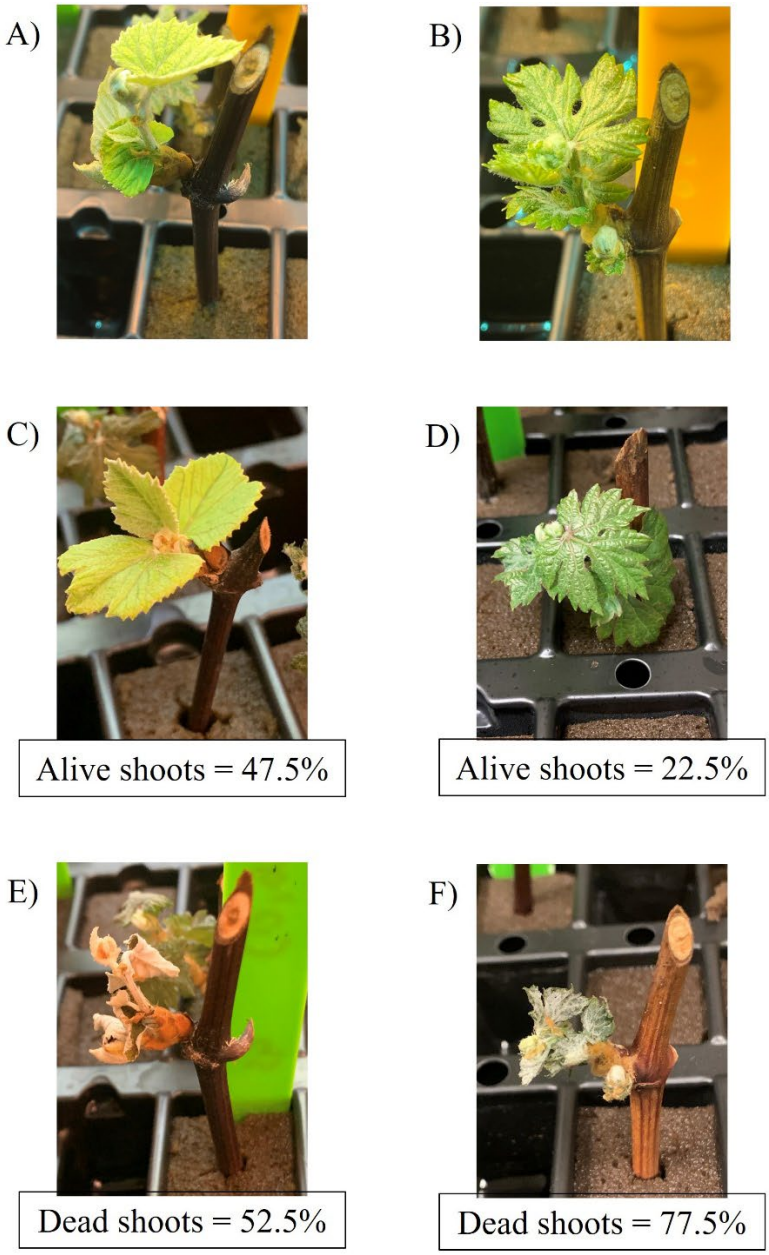

Figure 2.9 Frost injury in young shoots of 'GREM4' and 'Cabernet Sauvignon'. A) 'GREM4' and B) 'Cabernet Sauvignon' young shoots before frost test assay; C) 'GREM4' and D) 'Cabernet Sauvignon' live shoots after recovery from the frost test; E) 'GREM4' and F) 'Cabernet Sauvignon' dead shoots as assessed after 24-48 hours of recovery. The percentage denotes the number of live or dead shoots out of the total shoots tested in all frost tests.

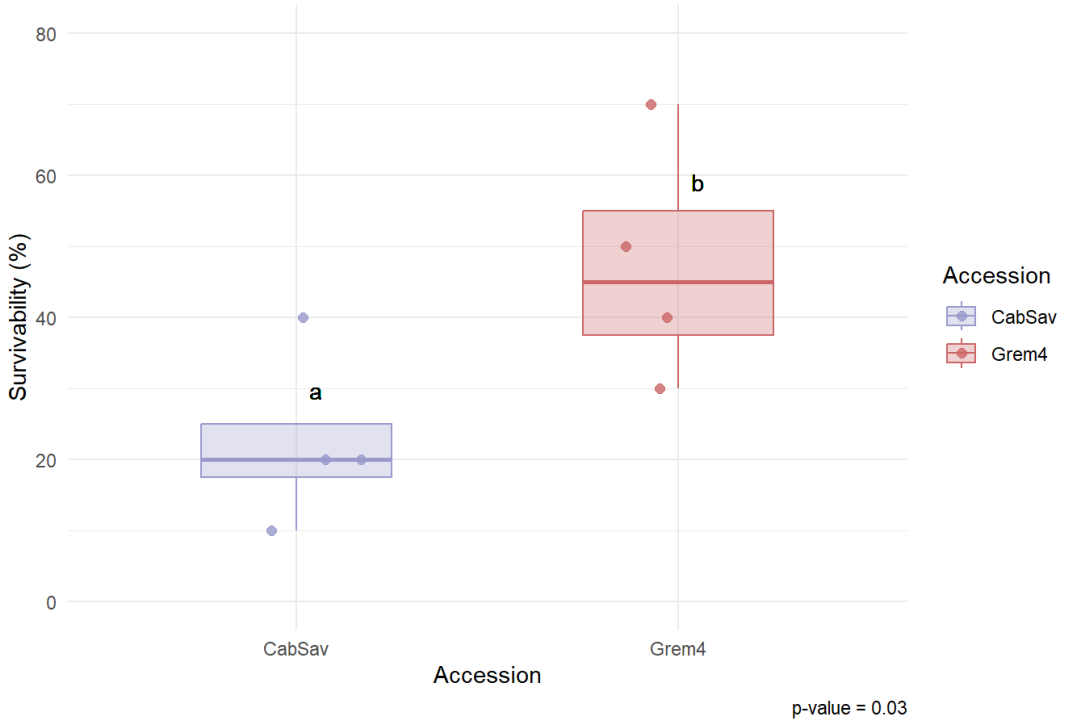

Figure 2.10 Survivability of young shoots of 'GREM4' and 'Cabernet Sauvignon' under sub-zero temperatures (-2°C to -4°C).

Four frost tests (replications) were conducted, each consisting of 10 shoots of 'GREM4' and 'Cabernet Sauvignon'. The boxplots show the interquartile range while whiskers denote total range of survivability percentage of shoots, and the dots represent the survivability rate per frost test replication. The line in the middle of a boxplot represents median survivability (%). The letters above the boxplots denote the significance ($p \leq 0.05$). Outliers, if present, are denoted by individual points outside the whisker.

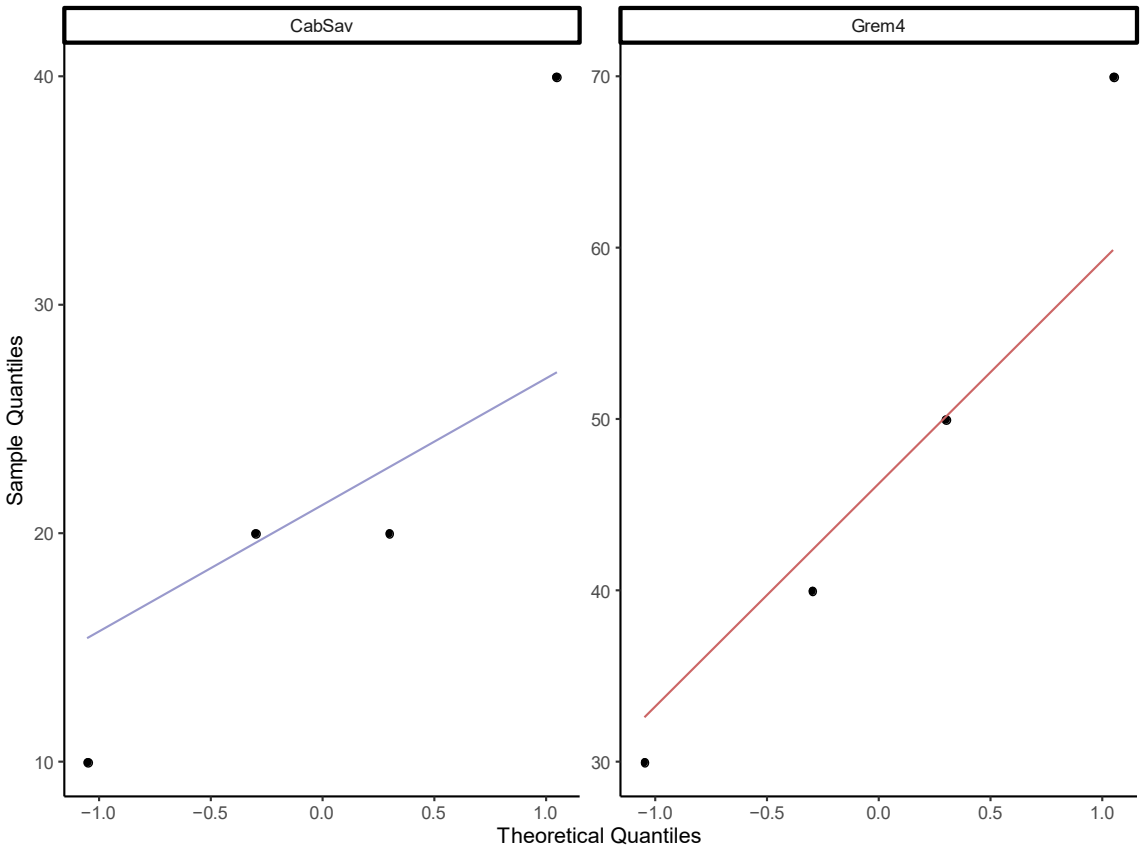

Figure 2.11 Quantile-quantile plots showing distribution of survivability percentage data for ‘GREM4’ and ‘Cabernet Sauvignon’ shoots. The x-axis denotes the theoretical quantiles, while y-axis denotes sample quantile values for survivability percentage.

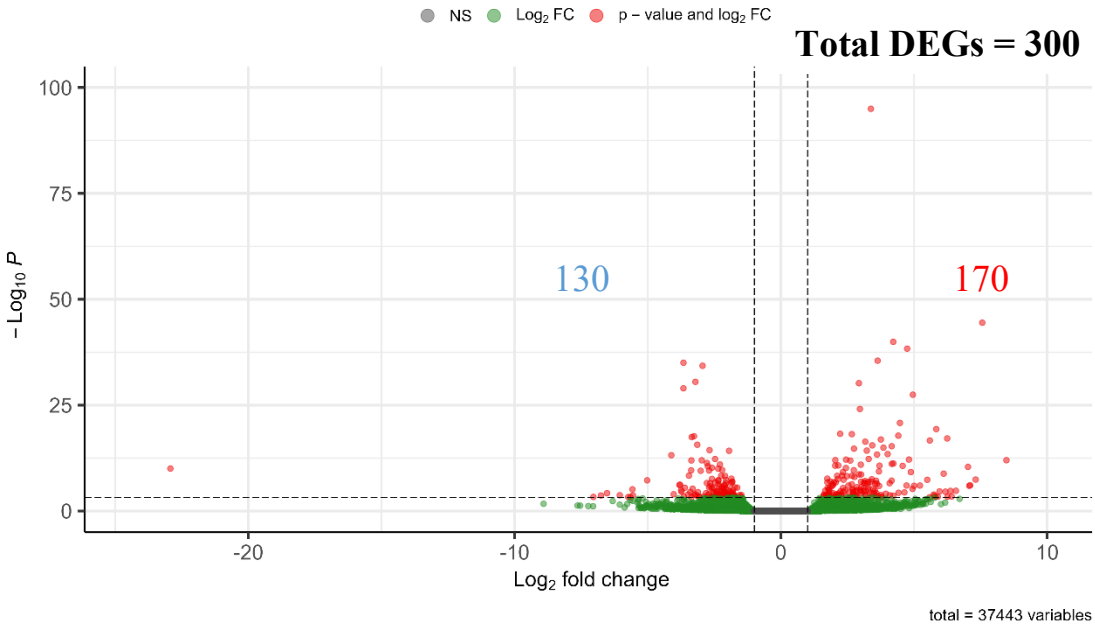

Figure 2.12 Enhanced Volcano plots showing the number of differentially expressed genes (DEGs) between 'GREM4' chill vs control shoot transcriptome.

Red dots denote genes showing a significant change in expression, while green dots denote genes with no significant change in expression. The parameters to identify significant DEGs were p-value ≤0.05, p-adj ≤0.05, and |log2FoldChange| ≥ 2.

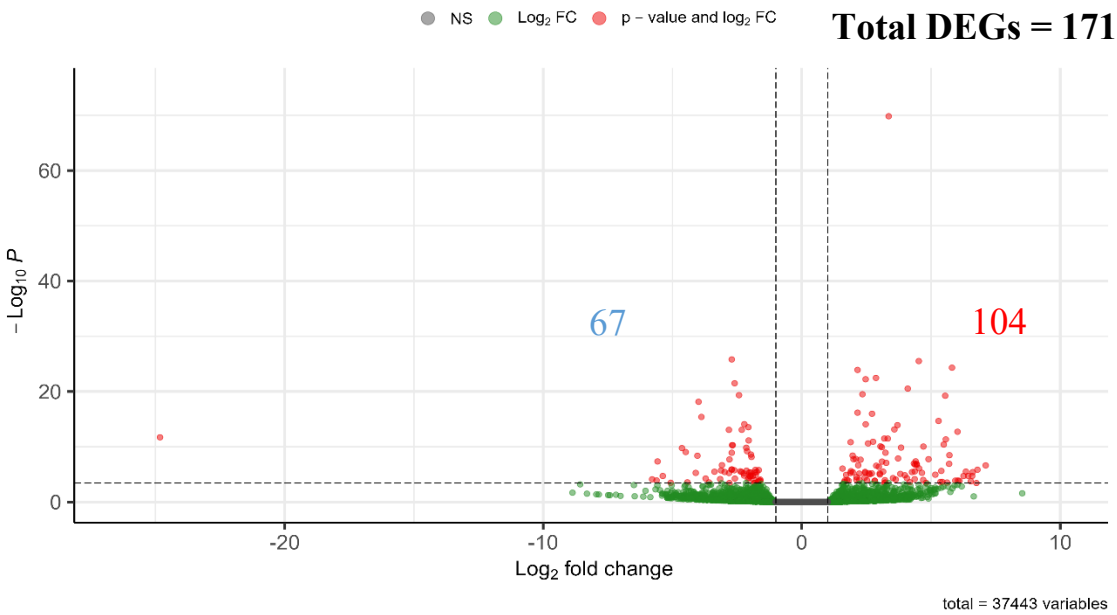

Figure 2.13 Enhanced Volcano plots showing the number of differentially expressed genes (DEGs) between 'GREM4' freeze vs control shoot transcriptome.

Red dots denote genes showing a significant change in expression, while green dots denote genes with no significant change in expression. The parameters to identify significant DEGs were p-value ≤0.05, p-adj ≤0.05, and |log2FoldChange| ≥ 2.

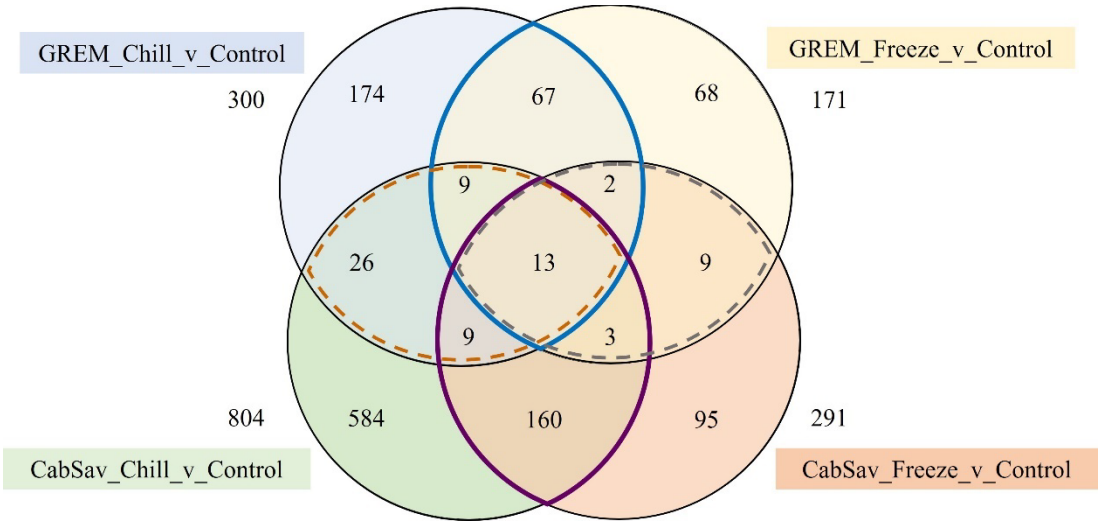

Figure 2.14 Venn diagram depicting the number of DEGs unique or shared under chill and freeze stress among species.

The numbers outside the Venn diagram indicate the total differentially expressed genes (DEGs) found in each of the comparisons made. The number of genes enclosed in a solid blue outline and a solid purple outline denote the shared DEGs between chill and freeze stress in 'GREM4' and 'Cabernet Sauvignon', respectively. Genes within the brown dashed line are shared between 'GREM4' and 'Cabernet Sauvignon' under chill stress, while the dashed grey outline region shows the number of DEGs shared between 'GREM4' and 'Cabernet Sauvignon' under freeze stress.

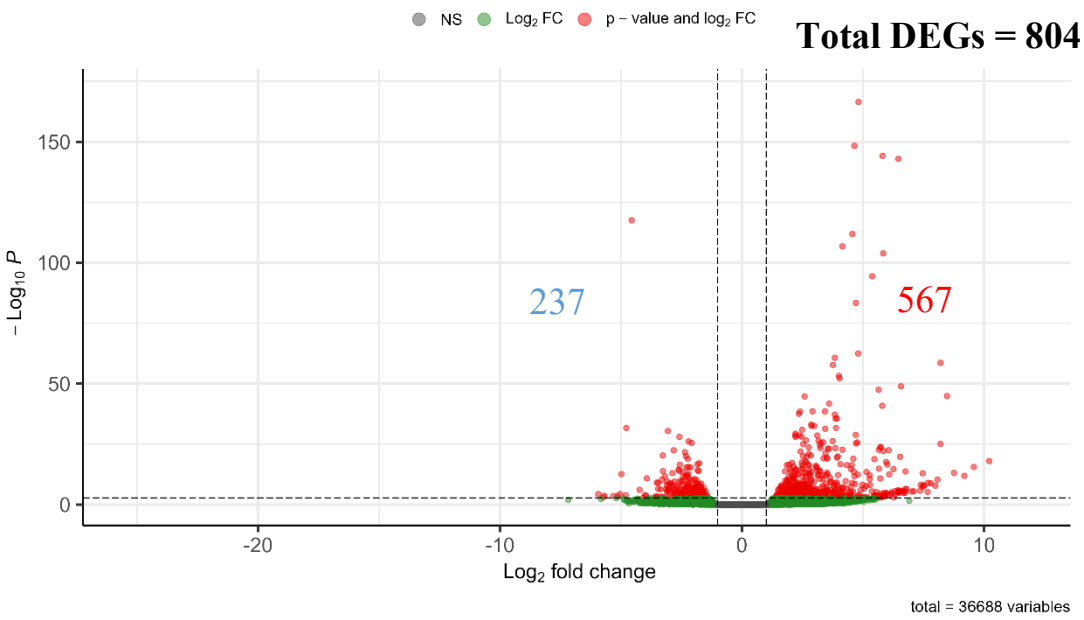

Figure 2.15 Enhanced Volcano plots showing the number of DEGs between 'Cabernet Sauvignon' chill vs control shoot transcriptome.

Red dots denote genes showing a significant change in expression, while green dots denote genes with no significant change in expression. The parameters to identify significant DEGs were p-value ≤0.05, p-adj ≤0.05, and |log2FoldChange| ≥ 2.

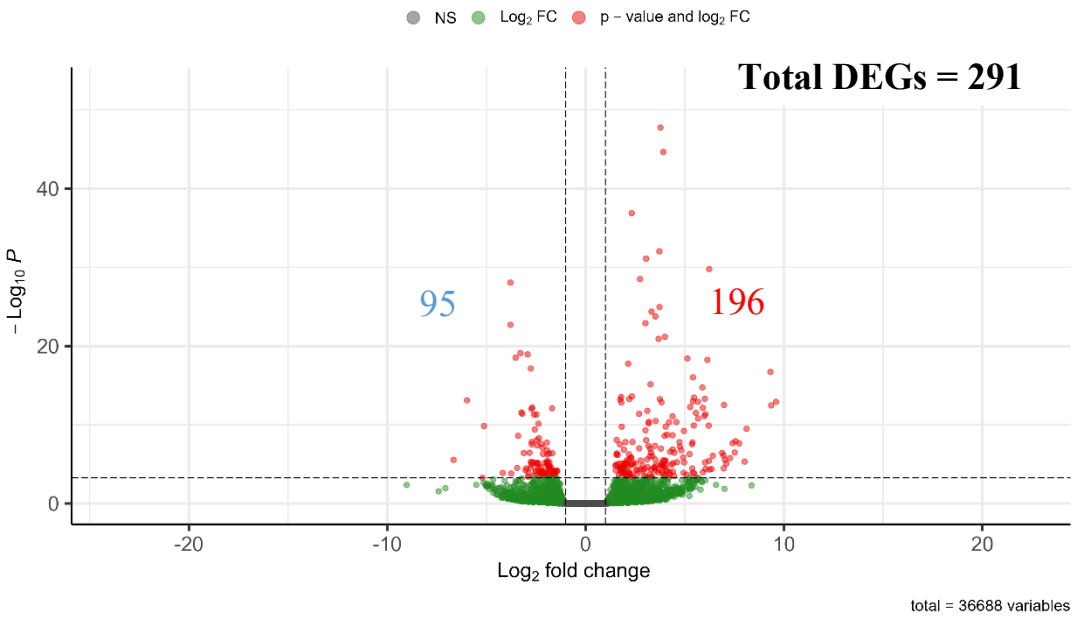

Figure 2.16 Enhanced Volcano plots showing the number of DEGs between 'Cabernet Sauvignon' freeze vs control shoot transcriptome.

Red dots denote genes showing a significant change in expression, while green dots denote genes with no significant change in expression. The parameters to identify significant DEGs were p-value ≤0.05, p-adj ≤0.05, and |log2FoldChange| ≥ 2.

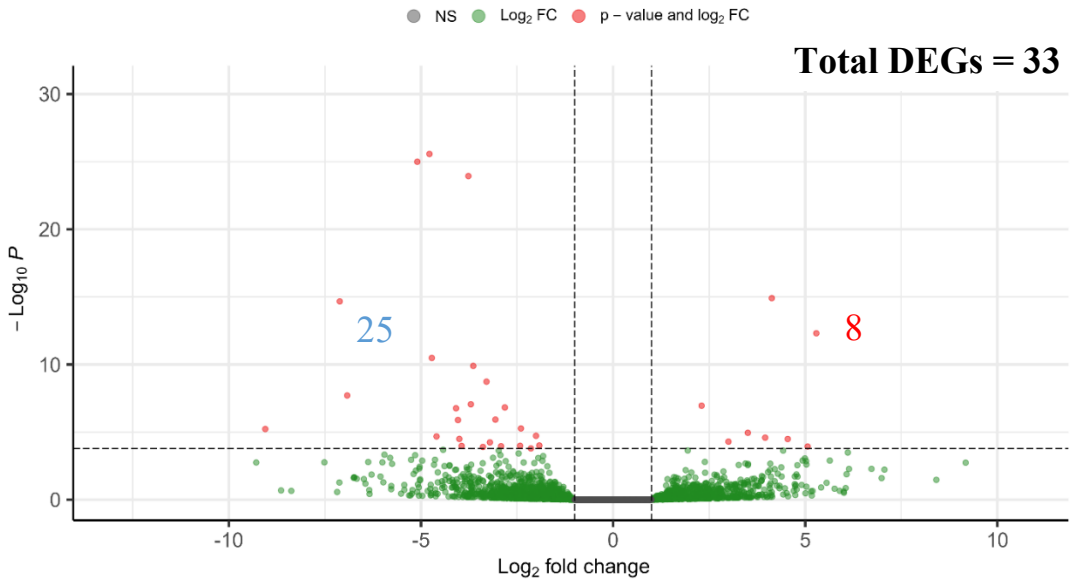

Figure 2.17 Enhanced Volcano plots showing the number of differentially expressed genes (DEGs) for interaction between 'GREM4' chill and 'Cabernet Sauvignon' chill.

Red dots denote genes showing a significant change in expression, while green dots denote genes with no significant change in expression. A total of 33 DEGs (8 upregulated, 25 downregulated in 'GREM4') were identified between the two species. The parameters to identify significant DEGs were set to p-value ≤0.05, p-adj ≤0.05, and |log2FoldChange| ≥ 2.

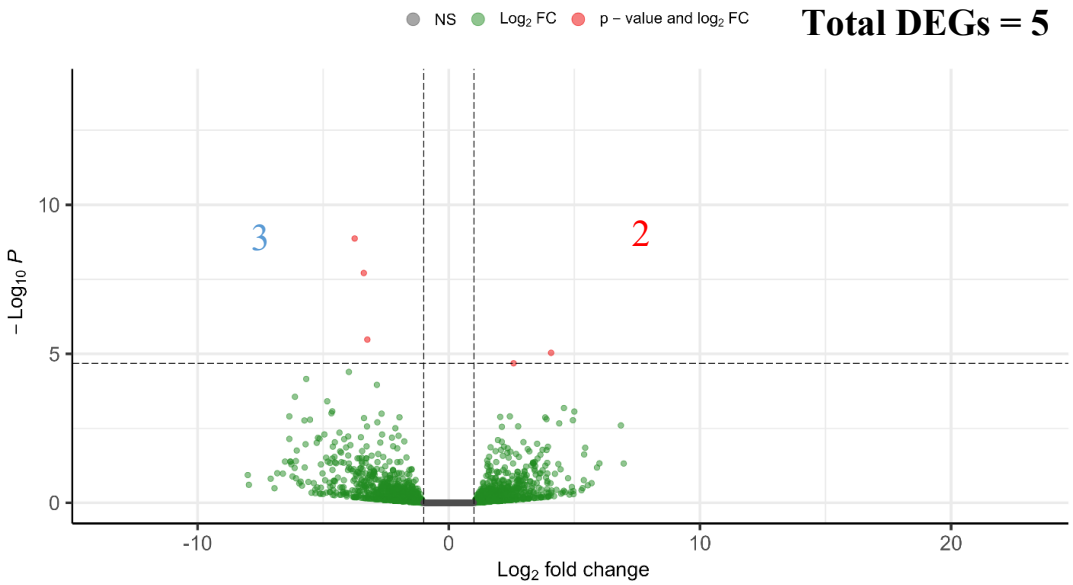

Figure 2.18 Enhanced Volcano plots showing number of differentially expressed genes (DEGs) for interaction between 'GREM4' freeze and 'Cabernet Sauvignon' freeze.

Red dots denote genes showing a significant change in expression, while green dots denote genes with no significant change in expression. A total of 5 DEGs (2 upregulated, 3 downregulated in 'GREM4') were identified between the two species. The parameters to identify significant DEGs were set to p-value ≤0.05, p-adj ≤0.05, and |log2FoldChange| ≥ 2.

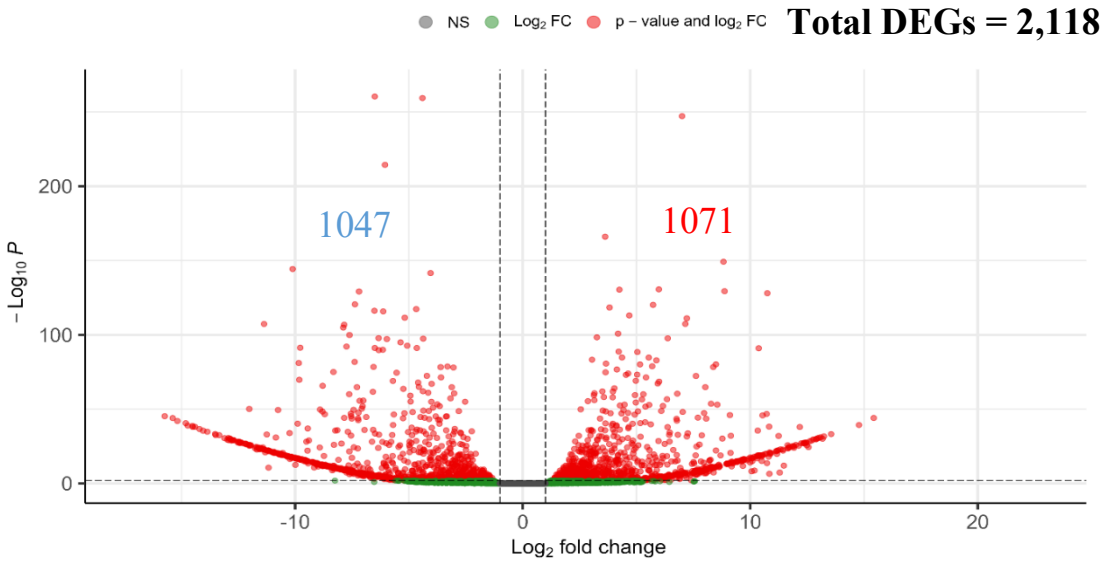

Figure 2.19 Enhanced Volcano plots showing the number of DEGs between 'GREM4' control and 'Cabernet Sauvignon' control shoot transcriptome.

A total of 2,118 DEGs were identified, out of which 1,071 were upregulated and 1,047 were downregulated in 'GREM4'. Red dots denote genes showing a significant change in expression, while green dots denote genes with no significant change in expression. The parameters to identify significant DEGs were set to p-value ≤0.05, p-adj ≤0.05, and |log2FoldChange| ≥ 2.

Chapter 3 - Effect of Foliar Application of Potassium Fertilizer on Yield, Fruit Quality, and Cold Hardiness of *Vitis spp.* 'Chambourcin'

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Abstract

Temperate regions experiencing sub-zero temperatures negatively impact grapevine yield. Potassium has been claimed as a cryoprotectant to improve cold hardiness in grapevine. This study investigated the effect of foliar application of liquid potassium-based fertilizer, ReaX^{TM}, on cold-hardiness of grapevine *Vitis spp.* 'Chambourcin' along with its effect on yield and fruit quality. The vines were sprayed four to five times between the fruit set and veraison stage at a concentration of 1.5% (v/v) for two consecutive seasons. Petioles were analyzed for nutrients, clusters for yield and fruit quality, and cold hardiness was determined by differential thermal analysis and bud injury assessment. Potassium deficiency was observed in all vines, but K-treated vines exhibited significantly higher sodium levels. Berry potassium levels and total soluble solids were generally higher in K-treated vines, however, there was no significant effect on yield and other fruit quality traits. Significant differences in cold hardiness levels were observed in both dormant seasons. Foliar application of potassium is a promising cultural practice to increase cold hardiness, but further studies are needed to understand the limits of its effectiveness.

Keywords: Bud injury, Grapevine, Potassium, Sugars, Cryoprotection

Introduction

Cold injury is a major problem in grapevine production, causing an average of 5-15% annual crop loss worldwide (Zabadal et al., 2007). Cold sensitive *Vitis vinifera* cultivars are more prone to sub-zero temperatures, especially in regions of Europe and the eastern and midwestern United States (Lamichhane, 2021; Zabadal et al., 2007). Within the eastern United States, major freezing events since 2007 have caused around $250 million of economic damage to the grapevine industry annually (Poni et al., 2022). For example, grape growers in Ohio reported an economic loss of $4 million in 335 hectares by the 2014 polar vortex that damaged 29%, 57%, and 97% of native, hybrid, and *vinifera* grape cultivars, respectively (Dami & Lewis, 2014). Along with affecting crop growth and development, winter injury also affects the wine quality, therefore, grape growers suffer from direct crop losses as well as from indirect losses due to the reduction in wine sales (Zabadal et al., 2007).

Although a grapevine's genetic makeup is the main determinant of its potential cold hardiness, management practices provide short-term or mid-term crop protection against freeze damage (Dami, 2022). For example, several cost-effective and easily applied sprayable products are available that can be useful in mitigating freezing injury in plants. These include cryoprotectants (glycine betadine, Agro-K's Potassium Dextrose-Lac®- KDL), dormant oils, growth regulators (ProTone®, FrostShield®), and ice nucleation inhibitors (ice nucleation active bacteria) (Centinari et al., 2016; Dami, 2022). Potassium (K^+), an essential plant nutrient, has also been considered a potential cryoprotectant to mitigate freezing injury in plants. The highly mobile K^+ macronutrient

plays an important role in cell division, enzyme activation, neutralization of organic acids, increasing proline, protein, and sugar synthesis and translocation, etc. The uptake and transportation of K^+ within the plant depend on the K^+ availability in the soil and K^+ in the leaves and storage reserves within the perennial vine structures, which can be redistributed (Rogiers et al., 2017).

K^+ is the most abundant cation during all stages of berry development (Rogiers et al., 2017), which influences sugar accumulation in berries, wine pH, and color (Mpelasoka et al., 2003; Rogiers et al., 2017). Evidence of potassium playing a role in cold hardiness has been reported, where potassium application reduced shoot mortality and resulted in the accumulation of carbohydrates, abscisic acid (ABA), and phenolic compounds, increasing the overall survivability of grapevines under cold stress (Karimi, 2017; Sarikhani et al., 2014). Although the mechanism is not clear, K^+ accumulation may lead to an increase in cell solute concentration (by increasing the accumulation of soluble sugars) (Karimi, 2017; Sarikhani et al., 2014) and decrease the osmotic potential (Centinari et al., 2016), thus reducing the cell freezing point. Along with inducing cold hardiness, potassium was also found to increase endogenous abscisic acid concentrations (Karimi, 2017), which may delay grapevine budburst (Wang et al., 2020), thus preventing sensitive young shoots from exposure to late-spring frost conditions. Previous studies have been conducted to evaluate the effect of foliar applications of potassium-based fertilizers on cold hardiness of grapevine. Studies on table grape cultivars have shown that foliar application increased cold hardiness in different tissues (Karimi, 2017;

Sarikhani et al., 2014), however, the effectiveness of these products on wine grape cultivars, especially in field conditions is still questionable (Centinari et al., 2016, 2018).

The main objective of this study was to investigate the effects of foliar application of liquid potassium-based fertilizer ReaX^{TM} on the cold hardiness of field-grown grapevines. We hypothesized that vines fertilized with potassium would have higher cold hardiness compared to non-treated (control) vines. Since K^+ accumulation influences fruit quality (pH, acidity) and plant growth, this study also evaluated if the potassium applications impacted grapevine yield or fruit quality.

Materials and Methods

Plant material and Experimental design

This study was conducted on field-grown mature vines of *Vitis spp.* 'Chambourcin' trained on a high cordon trellis with a spacing of 3 m × 1.22 m. *Vitis spp.* 'Chambourcin' (Seyve-Villard 12417 × Seibel 7053) is a French-American hybrid cultivar commonly grown in Ohio with moderate cold hardiness and can survive temperatures as low as -23°C (Zabadal et al., 2007). The vines were planted in 1996 at the Horticultural unit located at The Ohio State University, Wooster, Ohio, USA. There was a total of seven rows of 'Chambourcin' vines, each consisting of 60 vines. We used a completely randomized design to select 20 vines (10 controls and 10 K-treated) within two rows. In addition to pruning, shoot thinning was performed on these vines to control yield. This cultivar has the propensity to overcrop, thus cluster thinning is required (Dami et al. 2005). We used a liquid potassium-based fertilizer, ReaX^{TM} (25% K_2O) (Loveland

Products, Inc.) and applied it to the shoots (leaves and clusters) of the treated vines until they were fully wet. The fertilizer was applied four and five times in 2021 and 2022, respectively. As per the recommendations of the product label, K-treated vines were sprayed with 1.5% (v/v) of ReaX^{TM} (0.38% K_2O) between the fruit set (modified Eichhorn-Lorenz or EL 27) and the veraison stage (modified EL 37) (Dry & Coombe, 2004) (Table 3.1). Control vines were not sprayed. The foliar application was scheduled each year on dry sunny days (Temperature = 20-22°C) from late June to late August to ensure maximum nutrient absorption by plant leaves and avoid wash-off due to rainfall.

Weather data

Air temperature, Growing Degree Days (GDD), and precipitation data were collected from the CFAES Weather System (https://weather.cfaes.osu.edu/). Further, to understand the temperature patterns during the dormant seasons and their effect on grapevine acclimation, we computed mean temperatures for both experimental years and the number of days below freezing before the coldest event each year.

Petiole nutrient analysis

To determine the nutritional status of grapevines, petioles were collected during the growing season of 2022. Two hundred petioles were collected at the EL 36-37 (50% veraison) stage and then randomly subdivided into four replicates per treatment. Petiole

samples were shipped to Brookside Laboratories (New Bremen, OH) for nutrient analysis. The analysis provided a complete determination of petiole macro- and micro-nutrients and is based on published method (Miller & Horneck, 2013).

Yield, Fruit Quality, and Berry K

The crop was harvested on October 19, 2021, and October 25, 2022. The number of clusters per vine (n =10) were counted and the clusters were weighed to determine yield (kg) per vine. Yield per vine was further multiplied by vine density (2732 vines per hectare with a spacing of 3 m × 1.22 m) to estimate average yield (tons. ha^{-1}). Mean cluster weight (g) was calculated by dividing the yield per vine by the number of clusters per vine. During harvest, 400 berries from each of the 10 K-treated and control vines were randomly collected and divided into four replicates, each consisting of 100 berries placed in a plastic bag. Each of the four plastic bags was then weighed to calculate the 100-berry weight. Following the weight measurement, the berry samples (4 replicates) were taken on ice to the laboratory where they were crushed and the juice was extracted for fruit quality analysis (i.e., total soluble solids [TSS], pH, titratable acidity [TA], and fruit maturity index [FMI]). Thirty five (35) mL of the extracted juice was transferred to 50 mL falcon tubes that were centrifuged at 800 rpm for 8 minutes. Following centrifugation, 10 mL of supernatant was transferred to the titration workstation (Denver Instrument Model 350, Denver, CO) to measure pH and TA. TSS content was measured using a digital refractometer (MISCO, Cleveland, OH) and expressed in degrees Brix (°Brix). FMI was calculated as TSS/TA×10.

During harvest, an additional 200 berries were randomly collected from the K-treatment and control vines for analysis of potassium levels in the fruit. Those berries were randomly divided into four replicates, each consisting of 50 berries, placed in plastic bags, transported to the laboratory on ice, and stored at -20°C before being shipped to the ETS Laboratories (St. Helena, CA). After pressing and maceration of the berries, potassium content from the juice was measured using the Flame Atomic Emission Spectroscopy method (Hanson & Horneck, 1997) and expressed in mg/L.

Cold hardiness

The cold hardiness level of the vines was determined using differential thermal analysis (DTA) during both years of the study (Wolf & Cook, 1994). Water contains latent heat energy that gets released when water freezes. This latent heat energy can be detected by thermoelectric modules in terms of an increase in voltage (mV), thus, generating Low-Temperature Exotherm (LTE) that denotes the death of dormant bud tissues (Pierquet & Stushnoff, 1980). Dormant canes from five randomly selected K-treated and control vines each (one cane per vine) were collected monthly from November till April during dormancy (2021-2022 and 2022-2023). The buds were excised from the canes and placed on moist filter paper in a petri dish to avoid dehydration. Five excised buds from each cane were placed in each thermoelectric module (TEM) and the module box was placed into a Tenney environmental chamber (Thermal Products Solutions, New Columbia, PA). The Tenney environmental chamber was programmed to gradually decrease the

temperature from room temperature (~18.5℃) to -40℃ at the rate of 4℃/hour. The median LTE value was used to determine the LT50 (Lethal temperature for 50% injury).

The 2022 winter provided the opportunity to evaluate bud freezing injury in the field when the vines experienced a five-day freeze event which started on December 23, 2022 (minimum temperature dropped from -0.7℃ to -21.2℃ in 24 hours). To assess bud injury in the field due to this freeze event, canes were collected on January 11, 2023. Canes (node positions 3 to 10) were randomly selected from the K-treated and control vines. In total, five replicates for the K-treated and controls, each consisting of 20 buds, were assessed for injury of primary and secondary buds as the percentage of dead buds out of total buds collected.

Data Analysis

Data analyses were conducted using R (v4.3.1; R Core Team, 2023a). Significance in the comparisons between control and K-treated vines was determined using *t*-tests, with a significance level set at $p \leq 0.05$. Before conducting the *t*-tests, the normality of the data was evaluated via the Shapiro-Wilk test, and homogeneity variance was examined using Levene's test (rstatix package v0.7.2; Kassambara, 2023). Welch's *t-tests* were applied for normally distributed data (stats package v4.3.1; R Core Team, 2023b), while the non-parametric Wilcoxon rank-sum test was used for non-normally distributed data (rstatix package v0.7.2).

Results

Weather

In the 2021-2022 winter season, the lowest temperature recorded was -21℃ on January 27, 2022 (Figure 3.1a). During the second-year winter season (2022-2023), the vineyard experienced the lowest temperature on December 23, 2022, which was -21.2℃, (Table 3.2, Figure 3.1a and 3.1b). The average air temperatures prior to the freeze event of January 27, 2022, gradually decreased and remained below 0℃ for at least 15 consecutive days, whereas the temperatures inconsistently fluctuated above or below 0℃ before the freeze event on December 23, 2022 (Table 3.2).

Crop yield and fruit quality are influenced by the environmental conditions during the growing season of the crop. For this study, we recorded different weather parameters of the growing season from January 1 to October 31. During the 2021-2022 growing season, the maximum temperature was 34.3℃ on June 29, 2021. During the 2022-2023 growing season, the maximum temp was 34.2℃ on 15 June 2022 (Table 3.2). During the growing season, cumulative growing degree days (GDD) were higher in 2021 (1817) as compared to year 2022 (1689), which were comparable to the average cumulative GDD (1634) of the last 30 years (1991-2020). The fruit was harvested on October 19, 2021, and October 25, 2022; therefore, the cumulative GDD from January 1 until the harvest date was 1795 and 1681, respectively (Table 3.2). On the other hand, the annual rainfall was higher during the second year (104 cm) than that during 2021 (84 cm), which in turn was lower than the 30-year average (89 cm).

Petiole nutrient analysis

Potassium levels were not significantly different between the K-treatment and control petioles (Table 3.3). Among the macronutrients, nitrogen (N), phosphorous (P), and magnesium (Mg) levels were significantly different between the K treatment and controls, with N levels being higher and P and Mg being lower in the petioles of K-treated vines. Sodium (Na) levels in K-treated petioles were 3.5-fold higher than that in controls. All the other nutrient levels were not significantly different between K-treated and control petioles (p-value > 0.05). Also of note, K, P, boron (B), and iron (Fe) were below the normal range, whereas N, Mg, Manganese (Mn), and Calcium (Ca) were above the normal range for both the control and K-treated petioles (Dami et al., 2005). Actually, the observed petiole K levels (0.73-0.84%) were considered deficient. This was unexpected especially since we applied a foliar K fertilizer multiple times, and we did not observe any leaf symptoms of K deficiency.

Quantile-quantile plots illustrate the data distribution for petiole nutrient content (Figure 3.2). Data collected for petiole nutrient analysis was normal with equal variance for most of the nutrients, however, data failing these assumptions was analyzed using non-parametric tests. Data for B, Mn, and Al were not normal ($p < 0.05$), while the petiole samples had unequal variance for Fe levels ($p = 0.01$) (Table 3.4).

Yield, Fruit Quality, and Berry K

Crop weight per vine averaged 4.97 kg for K-treated and to 5.68 kg for controls in 2021 (Table 3.5). The mean crop weights were lower in 2022, averaging 4.32 kg in K-treated and 4.73 kg in control vines. As expected and due to cluster thinning, yield components were not significantly different between the control and K-treated vines in both years, except for cluster weight in 2021, where the K-treated vines had significantly lower cluster weight compared to the control vines. Generally, yield components were higher in 2021 than in 2022 likely due to heavier clusters and berries in the first year. This also holds true as to why control vines had higher (though non-significant) yields compared to K-treated vines during both years of the experiment.

The average sugar content (TSS) for the K-treated vines was 23.3 and 22.4 °Brix for 2021 and 2022, respectively, and was 22.5 and 21.6 °Brix for control vines, so was significantly higher in K-treated vines as compared to the controls in both years (Table 3.6). TSS increased by 4% in K-treated berries in both years. The average pH levels for the control and K-treated samples were not significantly different in 2021 (3.26 and 3.29) or in 2022 (3.21 and 3.20). Fruit TA levels were significantly lower in K-treated vines (8.4 g/L) compared to the controls (9 g/L) in 2021, however, the differences were non-significant in 2022 (11.3 g/L in K-treated and 11.5 g/L in controls). Due to higher TSS and lower TA, the fruit maturity index (FMI) was significantly higher in K-treated vines in 2021.

Potassium levels in the berries in 2021 were 925 mg/L in controls and 1017 mg/L in K-treated vines and were not significantly different. However, in 2022, K-treated vines

had significantly higher K (1450 mg/L), approximately 20% higher, than the control vines (1212 mg/L) (Table 3.6).

Data distribution for different yield attributes, fruit quality and berry K levels is illustrated as quantile-quantile plots (Figures 3.3 and 3.4). Among yield attributes, crop weight and yield data was not normal for K-treated vines in 2022, while cluster number data was not normal during both years ($p < 0.05$; Table 3.4). Berry K data lacked homogeneity of variance during 2021 and failed normality assumption during 2022. Therefore, non-parametric tests were performed to determine the significance between control and K-treated group.

Cold hardiness

Differential thermal analysis during winter 2021-2022 showed that the mean LT50 values for buds collected on December 20, 2021 were similar for K-treated (-23.6℃) and control vines (-24℃) (Figure 3.5a). The LT50 values increased a little for bud samples collected on January 3, 2022, and the differences remained non-significant (-23.1℃ for controls and -22.2℃ for K-treated vines). After the freezing event of January 27, 2022, the buds of K-treated vines collected on February 9 and February 24 during the winter of 2021-2022 had significantly lower LT50 values compared to the controls (p-values = 0.01 and 0.02, respectively). Cold hardiness in K-treated vines increased by 3.3℃ and 1.5℃, respectively, compared to the controls. Later in March, LT50 values were not significantly different in buds from K-treated (-18.5℃) and control vines (-18.8℃). The bud LT50 values in both K-treated and control vines reached their lowest values after the

vineyard experienced the lowest air temperature of the season, i.e., -21℃ during the last week of January 2022. Because the cold hardiness levels (LT50) were maintained below -22℃ until the end of February 2022, the extreme air temperatures (-21℃), did not cause bud injury in the field.

During winter 2022-2023, no significant difference in the LT50 values was observed between the K-treated and control bud samples at any collection date (Figure 3.5b). LT50 values remained between -20℃ and -22℃ from late November 2022 until mid-February 2023. Later, the cold-hardiness levels decreased and LT50 values were higher in April 2023, but still non-significantly different between K-treated and control vines (-16℃ in controls and -15℃ in K-treated canes). After the freeze event (lowest temperature = -21.2℃) on December 23, 2022, buds were collected on January 11, 2023, for injury assessment. Primary bud injury was significantly lower for K-treated vines (77%) than the controls (91%) (p-value = 0.05). Secondary bud injury (55% for K-treated and 73% for controls) was less than that of primary buds, but not significantly different between controls and K-treated vines (p-value = 0.13) (Figure 3.6). Due to a significantly high level of primary bud injury from the December 2022 freezing event, the LT50 values for January, February, and April of 2023 were not reliable, since it is likely that buds were already dead before the DTA tests were performed.

Assumptions for normality and homogeneity of variance were satisfied for most of the data related to cold hardiness. Descriptive quantile-quantile plots illustrate the distribution of cold hardiness data (Figures 3.7 and 3.8). However, data for measuring bud cold hardiness on November 29, 2022, and secondary bud injury were not normally

distributed, while cold hardiness data collected on January 3, 2022 had unequal variance (Table 3.4). Therefore, non-parametric t-tests were used to determine the significance of differences between the two groups.

Discussion

In this study, liquid-based potassium fertilizer, ReaX^{TM} (0-0-25), was applied to the leaves of grapevines four to five times at a concentration of 1.5% (v/v) during the growing seasons of two consecutive years. ReaX^{TM} contains 25% K_2O, therefore, the total K_2O (%) applied to the plants with five foliar applications was approximately 1.88% K_2O per year or 0.38% K_2O per application. The rate of 1.5% (v/v) was selected because it corresponded to the highest rate recommended for grapes by the product label as higher concentrations lead to phytotoxicity (Centinari et al., 2016; Wilson, 2001).

Previous studies tested the effects of various concentrations of potassium on grapevine cold hardiness (Centinari et al., 2016, 2018; Sarikhani et al., 2014)**.** Sarikhani et al. (2014) applied 0%, 0.25%, 0.5%, and 1% K_2O (w/v) in each of five foliar applications on 'Bidaneh Sefid' grapevine (*Vitis vinifera* L.). They found a continuous increase in bud and cane cold hardiness, proline, soluble sugars, and protein concentrations with higher K concentrations. Although vines showed some response to the lower concentrations of the fertilizer, application of K_2O at 1% (w/v) concentration was considered most effective in increasing the cold hardiness of the canes and the buds. Similarly, Karimi (2017) applied four foliar sprays of K_2SO_4 using 0%, 0.5%, 1%, and 1.5% K_2O (w/v). The study found that a higher concentration of K_2SO_4 (1%, and 1.5%

K_2O) profoundly increased cold hardiness, ABA, polyamine, soluble sugars, and phenolic compounds. In another study, foliar macronutrient, Potassium Dextrose-Lac (KDL; 24% K_2O) was applied at a concentration of 0.24% K_2O just before exposing the vines to either natural or artificial freezing conditions (Centinari et al., 2016, 2018). The results of those studies showed a reduction in shoot mortality among single-bud cuttings but no effect on whole vines (Centinari et al., 2016).

In our study, we used a comparatively low concentration (0.38% K_2O per application) and observed a significant increase in TSS, berry K levels, and cold hardiness, but a deficiency in K in the petioles. The fertilizer application showed no significant effect on yield or the other fruit quality traits measured. Additional studies are needed to determine if ReaX™ has an effect on protein, hormone, and secondary metabolite quantity, as was reported in other studies (Sarikhani et al., 2014; Karimi 2017), or if increasing the concentration >1% K_2O per application could affect fruit quality and cold hardiness.

Potassium is the most abundant cation in plant tissue and plays a critical role in overall plant health (Mpelasoka et al., 2003). Potassium is necessary for cellular osmoregulation, electrochemical processes, enzyme activation, cell division, photosynthesis, and protein and carbohydrate synthesis and transportation (Centinari, 2016; Karimi et al., 2014). Inadequate amounts of potassium can lead to reduced shoot, root, and fruit growth; thus, it is vital for growers to monitor potassium (Centinari, 2016). Demand for potassium is highest from fruit set through ripening (Keller, 2015; Mpelasoka et al., 2003), therefore, foliar application was applied during these stages of

development (Rogiers et al., 2017). In our study, although we observed a 15% increase in potassium levels in K-treated petioles, the difference between K-treated vines and controls was not significant and all the vines actually showed deficient petiole K levels (0.73 and 0.84%), even after multiple foliar applications. For the Eastern US, petiole concentrations of potassium less than 1% are considered inadequate, whereas the normal range for potassium concentration in grapevine petioles is 1.5-2.5% (Dami et al., 2005; Wolf, 2008). When vines are K deficient, soil application, instead of foliar application, of the fertilizer is warranted due to the large amount of K needed (Wolf, 2008). The root system of grapevines has a larger surface area for absorbing nutrients and K that is not immediately taken up can remain in the soil to be absorbed as needed. In addition, higher concentrations of K fertilizer can be used since the risk of phototoxicity is low when soil fertilizers are applied at proper concentrations.

According to Mpelasoka et al. (2003), the relation between petiole K and berry K content is still questionable. Similar results for N levels were found by Davenport et al. (2012) where petiole N levels were below the normal range, even though the crop produced was of high quality for premium wine production. In our study, even though the nutrient analysis showed K deficiency in petioles of K-treated and control vines, the K levels in the berries were around or above the optimum level (> 1000 mg/L), with the berries from K-treated vines having greater K levels than controls. K within the plants was likely transferred from their source locations to the strongest sinks, i.e., developing and ripening berries, leaving other tissues, such as the petioles, deficient. The K applied to the leaves of the treatment plants was also moved from source to sink, resulting in the

higher K detected in the K-treated berries compared to controls. Buds are also nutrient sinks and K could be deposited within the buds, as well, enhancing the cold hardiness, though testing the potassium levels of the bud tissue between K-treated and control vines is needed to validate this hypothesis. Further investigation of K fertilizer application timing (e.g. post-harvest) and the transportation and sequestration of potassium to different plant tissues can better determine its value as a management practice for increasing cold hardiness.

It is also noted that the accumulation of sodium was approximately 3.5 times higher in K-treated vines than in the controls. We hypothesize that this could be due to a gradient effect of potassium-sodium exchange. Potassium was in higher demand in the sink tissues, i.e., the berries, therefore, sodium could have substituted for potassium as an osmoticum in the petioles. The concentration of sodium ions increases in berry pulp and skin along with potassium ions, but at a lower level. Under a limited K supply, sodium, along with magnesium and calcium, can take the role of an inorganic osmoticum replacing potassium ions (Keller, 2020). This could be the reason why we observed higher sodium levels in the petioles. In our study, Mg and Ca accumulation was lower in K-treated vines as compared to the controls, which could have further elevated the sodium levels to raise the water potential of the cell.

Although the cluster weight of K-treated vines was significantly lower in the first year, in general, the application of potassium fertilizer did not affect the yield components of Chambourcin. This finding is consistent with that of Centinari et al. (2018) where they found no significant effect of KDL foliar spray on yield of *Vitis*

vinifera cultivars Lemberger and Riesling and interspecific hybrid cultivars Noiret and Traminette. Potassium silicate (K_2SiO_3) foliar application did not affect berry weight or yield in Touriga National and Touriga Franca grapes, either (Singh et al., 2020). However, Karimi (2017) did find an increase in cluster weight and crop weight per vine in 'Sultana' grapevine (*Vitis vinifera* L.) with increasing concentration of foliar application of K_2SO_4. Additionally, all concentrations of K_2SO_4 increased individual berry weight over the controls (Karimi, 2017). We did observe a significant effect on cluster weight in 2021, but this did not translate to crop wt. or yield, which was not significantly different between the controls and K-treated vines. The discrepancy observed between K treatments and crop weight across studies may be due to variations in the concentration of K applied or could be a cultivar-dependent response.

However, foliar application of K increased sugar accumulation in berries. This finding is consistent with previous studies (Karimi, 2017; Sarikhani et al., 2014). Sarikhani et al. (2014) suggested that K^+ ions could be involved in inducing the enzymes for carbohydrate metabolism. This has been shown in other crop studies such as potato, where potassium increased the activity of sucrose synthase (Liu et al., 2013). An increase of K in grapevine could induce carbohydrate metabolism in the berries, which could lead to the higher sugar content observed. Additionally, high sugar levels in buds have been linked to enhanced cold hardiness, due to their role in osmoregulation (De Rosa et al., 2022). The positive correlation between K and sugar accumulation suggests that the K accumulated in buds (a sink tissue) protects against freezing injury by increasing the sugar levels, and therefore decreasing the freezing point of the cells (Cakmak, 2005).

Titratable Acidity is the ratio of free organic acids to organic acids neutralized by K^+ (Villette et al., 2020). Among organic acids, tartaric acid is a stronger acid while malic acid is a weak acid and gets degenerated by malic enzyme (malic acid-degrading enzyme) (Lakso & Kliewer, 1975). K^+ is known to decrease tartaric acid in juice and wine by combining with it to form potassium bitartrate precipitates, thus, lowering the tartrate-malate ratio (Mpelasoka et al., 2003). This could be the reason why we observed a decrease in fruit TA for K-treated vines, though it was only significantly lower in 2021. It is also noted that TA values were generally lower in 2021 than in 2022. This could be attributed to the warmer growing season of the former, since warmer temperatures can affect enzyme activity. The activity of the malic acid-degrading enzyme continuously increases with an increase in temperature from 10°C to 46°C (Lakso & Kliewer, 1975), therefore, due to higher temperatures, malic acid gets used as a respiratory substrate resulting in lower TA (Famiani et al., 2016; Villette et al., 2020).

In our study, K content in berries was 1017 mg/L in K-treated vines and 925 mg/L in controls in 2021. In 2022, the values were significantly higher in fruits of K-treated vines (1450 mg/L) than in the controls (1212 mg/L). Previous studies state that K levels within the range 860-1279 mg/L are considered “normal” (Somers, 1977) and values higher than 1056-2776 mg/L are considered high levels of fruit potassium (Mpelasoka et al., 2003; Somers, 1975). Although the berry levels were within the optimum range, the values were still low as compared to the K levels found in other red grape cultivars (1700-1800 mg L^-) (ETS Laboratories, 2022). High concentrations of potassium in the fruit can cause high pH (>3.70) of fruit juice and wine and create changes in the physical,

chemical, and microbial stability, (Mpelasoka et al. 2003) color stability, and oxidative potential of the wine (Gardner, 2016). In our study, even though K concentration in the berries of K-treated vines significantly increased in year 2, as compared to the controls, foliar application of K did not affect the juice pH as reported in the literature, which is positive, since a high pH is deleterious for wine quality.

The effect of K on increasing cold hardiness was observed during both dormant seasons. In year 1, LT50 values of the K-treated buds were significantly lower than controls during mid-winter, but not during the acclimation (fall) and deacclimation (late winter-early spring) stages. In year 2, since the DTA test did not work due to bud injury, we relied on determining cold hardiness by tissue browning. Like year 1, K was most effective in increasing cold hardiness during mid-winter in year 2.

During this study, another observation regarding cold hardiness status was worth noting. In general, the cold hardiness of Chambourcin was higher during the 2021-2022 season than that during 2022-2023. Even though the lowest temperature recorded in both dormant seasons was similar (-21 °C), injury after the second-year freezing event (December 23, 2022) was much higher than the first one (January 27, 2022). Since the coldest temperature in the second dormant season occurred in late December, 2022, the vines likely had not completely acclimated for maximum cold hardiness yet. Plants undergo cold acclimation under low non-freezing temperatures which leads to physiological changes in plants including membrane fluidity, sugar accumulations and cryoprotein synthesis, thus improving their ability to survive freezing conditions (Londo et al., 2018). Mild fall/winters, like seen in fall/winter 2022, were not conducive for

maximum cold hardiness (Zabadal et al., 2007), whereas temperatures below freezing enhance cold hardiness of grapevines (Hamman et al., 1996; Zabadal et al., 2007). The mean temperature in January, 2022 was below the 30-year average, whereas the mean temperature in December 2022 was above the 30-year average (Figure 3.9). Furthermore, the mean temperature was below 0°C for 7 consecutive days prior to the freeze event of January 27, 2022, while the mean temperature was not below 0°C in the days leading up to the freeze event of December 23, 2022 (Table 3.2). The warmer temperatures proceeding the extreme cold event in December 2022 likely impacted the cold acclimation of the vines prior to the major freeze event that season. Because of this, the majority of primary buds were dead, but our bud injury assessment did find that primary and secondary bud injury in K-treated vines was less than control vines (77% K-treated vs 91% control primary buds injured, and 55% K-treated vs 73% control secondary buds injured) (Figure 3.6). These results suggest the K treatment did have a significant effect on preventing bud injury, especially in mid-winter. More studies are needed to understand K transportation in different plant tissues and how it protects dormant buds and canes from cold stress.

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Declaration of Interest

The authors declare no competing interests that could have appeared to influence the work reported in this paper.

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References

Cakmak, I. (2005). The Role of Potassium in Alleviating Detrimental Effects of Abiotic stresses in plants. *Journal of Plant Nutrition and Soil Science*, 168, 521–530. https://onlinelibrary.wiley.com/doi/abs/10.1002/jpln.200420485

Centinari, M. (2016). Assessing and Managing Potassium Concentration in the Vineyard. Penn State Extension Wine & Grapes U. https://psuwineandgrapes.wordpress.com/2016/09/16/assessing-and-managing-potassium-concentration-in-the-vineyard/

Centinari, M., Gardner, D. M., Smith, D. E., & Smith, M. S. (2018). Impact of Amigo Oil and KDL on Grapevine Postbudburst Freeze Damage, Yield Components, and Fruit and Wine Composition. *American Journal of Enology and Viticulture*, 69(1), 77–88. https://doi.org/10.5344/ajev.2017.17030

Centinari, M., Smith, M. S., & Londo, J. P. (2016). Assessment of Freeze Injury of Grapevine Green Tissues in Response to Cultivars and a Cryoprotectant Product. *HortScience*, 51(7), 856–860. https://doi.org/10.21273/HORTSCI.51.7.856

Dami et al. (2005). Midwest Grape Production Guide. https://extensionpubs.osu.edu/midwest-grape-production-guide/

Dami, I. (2022). Spring Frost Injury of Grapevines and Protection Methods. OSU Extension Publications. https://extensionpubs.osu.edu/spring-frost-injury-of-grapevines-and-protection-methods/

Dami, I., & Lewis, D. (2014). 2014 Grape Winter Damage Survey Report. https://kb.osu.edu/handle/1811/60447

Davenport, Joan & Lunden, Jeffrey & Winkler, Tanya. (2012). Wine Grape Tissue Nutrient Concentrations in the Inland Pacific Northwest. *Communications in Soil Science and Plant Analysis*. 43. 21-27. 10.1080/00103624.2012.631395

De Rosa, V., Falchi, R., Moret, E., & Vizzotto, G. (2022). Insight into Carbohydrate Metabolism and Signaling in Grapevine Buds during Dormancy Progression. *Plants*, 11(8), 1027. https://doi.org/10.3390/plants11081027

Dry, P. and Coombe, B. 2004. Revised version of "Grapevine growth stages—The modified E-L system" *Viticulture 1 – Resources. 2nd edition. Winetitles*.

ETS Laboratories. (2022). Harvest Guide. https://issuu.com/etslabs/docs/harvestguide2022digital

Famiani, F., Farinelli, D., Frioni, T., Palliotti, A., Battistelli, A., Moscatello, S., & Walker, R. P. (2016). Malate as Substrate for Catabolism and Gluconeogenesis during Ripening in the Pericarp of Different Grape Cultivars. *Biologia Plantarum*, 60(1), 155–162. https://doi.org/10.1007/s10535-015-0574-2

Gardner, D. (2016, September 23). Making [red] Wine from Fruit High in Potassium. Penn State Extension Wine & Grapes U.

https://psuwineandgrapes.wordpress.com/2016/09/23/making-red-wine-from-fruit-high-in-potassium/

Hamman, R. A., Dami, I., Walsh, T. M., & Stushnoff, C. (1996). Seasonal Carbohydrate Changes and Cold Hardiness of Chardonnay and Riesling Grapevines. *American Journal of Enology and Viticulture*, 47, 31–36. https://doi.org/10.5344/ajev.1996.47.1.31

Hanson, D., & Horneck, D. A. (1997). Determination of Potassium and Sodium by Flame Emission Spectrophotometry. https://api.semanticscholar.org/CorpusID:99517948

Karimi, R. (2017). Potassium-induced Freezing Tolerance is Associated with Endogenous Abscisic acid, Polyamines and Soluble Sugars Changes in Grapevine. *Scientia Horticulturae*, 215, 184–194. https://doi.org/10.1016/j.scienta.2016.12.018

Karimi, R., Ershadi, A., & Esna-Ashari, M. (2014). Effects of Late- season Nitrogen and Potassium Spray on Dormant Buds Cold Tolerance of 'Bidaneh Sefid' Grapevine. *Iran. J. Hortic. Sci. Technol*., 15, 419–434. Scopus.

Kassambara A (2023). *rstatix: Pipe-Friendly Framework for Basic Statistical Tests*. R package version 0.7.2, https://rpkgs.datanovia.com/rstatix/

Keller. (2015). The Science of Grapevines—2nd Edition. https://shop.elsevier.com/books/the-science-of-grapevines/keller/978-0-12-419987-3

Lakso, A. N., & Kliewer, W. M. (1975). The Influence of Temperature on Malic Acid Metabolism in Grape Berries: I. Enzyme Responses. *Plant Physiology*, 56(3), 370–372.

Lamichhane, J. R. (2021). Rising Risks of Late-spring Frosts in a Changing Climate. *Nature Climate Change*, 11(7), Article 7. https://doi.org/10.1038/s41558-021-01090-x

Liu et al. (2013). Effects of Potassium on Yield, Photosynthate Distribution, Enzymes Activity and ABA Content in Storage Roots of Sweet Potato (*Ipomoea batatas* Lam.). *Aust. J. Crop Sci*. 7, 735-743.

Londo, J. P., Kovaleski, A. P., Lillis, J. A. (2018). Divergence in the Transcriptional Landscape between Low Temperature and Freeze Shock in Cultivated Grapevine (*Vitis vinifera*). *Horticulture Research* 5(1), 1–14. https://doi.org/10.1038/s41438-018-0020-7

Miller, D. R. O., & Horneck, D. D. (2013). *Soil, Plant and Water Reference Methods for the Western Region*.

Mpelasoka, B. S., Schachtman, D. P., Treeby, M. T., & Thomas, M. R. (2003). A Review of Potassium Nutrition in Grapevines with Special Emphasis on Berry Accumulation. *Australian Journal of Grape and Wine Research,* 9(3), 154–168. https://doi.org/10.1111/j.1755-0238.2003.tb00265.x

Pierquet, Patrick. (1980). Relationship of Low Temperature Exotherms to Cold Injury in *Vitis riparia* Michx.. *American Journal of Enology and Viticulture*. 31. 1-6.

Poni, S., Sabbatini, P., & Palliotti, A. (2022). Facing Spring Frost Damage in Grapevine: Recent Developments and the Role of Delayed Winter Pruning – A Review. *American Journal of Enology and Viticulture*. https://doi.org/10.5344/ajev.2022.22011

R Core Team. (2023a). *R: A language and environment for statistical computing* (Version 4.3.1) [Software]. R Foundation for Statistical Computing. https://www.R-project.org/

R Core Team (2023b). *stats: The R Stats Package*. R package version 4.3.1. https://cran.r-project.org/package=stats

Rogiers, S. Y., Coetzee, Z. A., Walker, R. R., Deloire, A., & Tyerman, S. D. (2017). Potassium in the Grape (*Vitis vinifera* L.) Berry: Transport and Function. *Frontiers in Plant Science*, 8. https://www.frontiersin.org/articles/10.3389/fpls.2017.01629

Sarikhani, H., Haghi, H., Ershadi, A., Esna-Ashari, M., & Pouya, M. (2014). Foliar Application of Potassium Sulphate Enhances the Cold-hardiness of Grapevine (*Vitis vinifera* L.). *The Journal of Horticultural Science and Biotechnology*, 89(2), 141–146. https://doi.org/10.1080/14620316.2014.11513060

Singh, R. K., Afonso, J., Nogueira, M., Oliveira, A. A., Cosme, F., & Falco, V. (2020). Silicates of Potassium and Aluminium (Kaolin); Comparative Foliar Mitigation Treatments and Biochemical Insight on Grape Berry Quality in *Vitis vinifera* L. (cv. Touriga National and Touriga Franca). *Biology*, 9(3), 58. https://doi.org/10.3390/biology9030058

Somers, T. C. (1977). A Connection between Potassium Levels in the Harvest and Relative Quality in Australian red wines. *Aust. Wine Brew. Spirit Rev*. 24, 32–34.

Somers, T.C. (1975). In search of quality for red wines. *Food Technology in Australia* 27, 49–56.

Villette, J., Cuéllar, T., Verdeil, J.-L., Delrot, S., & Gaillard, I. (2020). Grapevine Potassium Nutrition and Fruit Quality in the Context of Climate Change. *Frontiers in Plant Science*, 11. https://www.frontiersin.org/articles/10.3389/fpls.2020.00123

Wang, H., Blakeslee, J., Jones, M., Chapin, L., & Dami, I. (2020). Exogenous Abscisic Acid Enhances Physiological, Metabolic, and Transcriptional Cold Acclimation Responses in Greenhouse-grown Grapevines. *Plant Science*, 293. https://doi.org/10.1016/j.plantsci.2020.110437

Wilson. (2001, August). Frost Management in Cool Climate Vineyards.

Wolf, T. K. (2008). Wine grape production for Eastern North America. Virginia Polytechnic Institute and State University.

Wolf, T. K., & Cook, M. K. (1994). Cold Hardiness of Dormant Buds of Grape Cultivars: Comparison of Thermal Analysis and Field Survival. *HortScience*, 29(12), 1453–1455. https://doi.org/10.21273/HORTSCI.29.12.1453

Zabadal, T., Dami, I., & Goffinet, M. (2007). Winter Injury to Grapevines and Methods of Protection.

Tables

Table 3.1 Treatment dates and phenology of Chambourcin grapevines in 2021 and 2022.

Timing of treatment	EL stage[1]	Year	Treatment date	GDD[2]
Fruit set	27-29	2021	June 17	453
		2022	June 29	637
Marble berry size	31	2021	July 9	731
		2022	July 14	828
Bunch-closing	32-33	2021	July 22	882
		2022	July 29	1027
1% veraison	35	2021	August 6	1053
		2022	August 9	1176
50% veraison[3]	36-37	2021	-	-
		2022	August 26	1345

[1] Phenological stages of grapevine according to the modified Eichhorn-Lorenz or EL system (Dry & Coombe, 2004).
[2] Growing Degree Days (GDD) (°C) accumulation calculated from January 1 until the date of spraying.
[3] Vines were not sprayed at 50% veraison stage in 2021.

Table 3.2 Weather parameters during 2021-2022 and 2022-2023 experimental seasons.

Parameter	**2021-2022**	**2022-2023**	**30-year average[1]**
Harvest date	October 19, 2021	October 25, 2022	-
GDD (growing season)[2]	1817	1689	1634 ± 0.3
GDD (harvest) [3]	1795	1681	-
Annual precipitation (cm)	84	104	89 ± 2.6
Precipitation (cm) during ripening (Aug 1 – Oct 31)	22	23	22 ± 0.9
Mean temperature (growing season)[2]	12.9	12.1	12.0 ± 0.6
Mean temperature (harvest) [3]	12.9	12.2	-
Maximum temperature (growing season)[2]	34.3	34.2	36.5
Date of maximum temperature	June 29, 2021	June 15, 2022	July 20, 1991
Lowest temperature (winter season)[4]	-21.0	-21.2	-32.1
Date of lowest temperature	January 27, 2022	December 23, 2022	January 19, 1994
Number of days the mean temperature <0°C before the lowest temperature	7	0	-

[1] 30-year data were taken from the years 1991-2020. The values denote means ± standard errors.

[2] Accumulated growing degree days or GDD (°C) and temperatures (°C) during the growing season were recorded from January 1 to October 31 of that year.

[3] Accumulated GDD (°C) and temperatures (°C) were recorded from January 1 until harvest date.

[4] Winter season temperatures were recorded from September 1 to April 30 of the experimental year.

Table 3.3 Petiole nutrients in Chambourcin during the 2022 growing season.

	Control	K-treated	p-value[1]	Suggested normal ranges in petioles at veraison[2]
Primary macro-nutrients				
Nitrogen (%)	1.37 ± 0.03	1.53 ± 0.04	0.02 (S)	0.90-1.30
Phosphorus (%)	0.15 ± 0.00	0.14 ± 0.00	0.03 (S)	0.16-0.29
Potassium (%)	0.73 ± 0.05	0.84 ± 0.06	0.23 (NS)	1.50-2.50
Secondary macro-nutrients	1.97 ± 0.04	1.73 ± 0.06	0.02 (S)	0.26-0.45
Magnesium (%)	2.28 ± 0.04	2.14 ± 0.05	0.06 (NS)	1.20-1.80
Calcium (%)	0.22 ± 0.01	0.22 ± 0.00	0.79 (NS)	-
Sulphur (%)				
Micro-nutrients				
Boron (ppm)	21 ± 0.6	19 ± 0.7	0.15 (NS)	25-50
Iron (ppm)	23 ± 1.1	21 ± 0.1	0.08 (NS)	31-50
Manganese (ppm)	827 ± 33	737 ± 46	0.20 (NS)	31-150
Copper (ppm)	9 ± 0.2	9 ± 0.2	0.14 (NS)	5-15
Zinc (ppm)	51 ± 1.5	46 ± 1.82	0.11 (NS)	30-50
Non-essential nutrients				
Aluminum (ppm)	17 ± 2	22 ± 6	0.69 (NS)	-
Sodium (ppm)	223 ± 14	778 ± 40	0.00 (S)	-

Values within Control and K-treated columns represent means ± standard errors. [1]Significance at $p \leq 0.05$; S = Significant, NS = Non-significant. [2] Normal ranges were available for most of the nutrients in the Midwest Grape Production Guide (Dami et al. (2005)).

Table 3.4 Data analysis for normality and variance.

Petiole Nutrient Analysis			
Nutrient	**Normality (Control)**	**Normality (K-treated)**	**Variance**
Nitrogen	0.30[1]	0.81	0.53
Phosphorus	0.10	0.50	0.37
Potassium	0.66	0.18	0.69
Magnesium	0.57	0.19	0.43
Calcium	0.42	0.39	0.64
Sulphur	0.60	0.30	0.73
Boron	0.02	0.23	0.72
Iron	0.11	0.69	0.01
Manganese	0.99	0.03	0.59
Copper	0.97	0.80	0.95
Zinc	0.77	0.22	0.78
Aluminum	0.61	0.01	0.11
Sodium	0.06	0.72	0.12
Yield Attributes			
	Year 2021		
Crop wt./vine (kg)	0.23	0.29	0.57
Yield (ton. ha^{-1})	0.23	0.29	0.57
Cluster number	0.02	<0.01	0.39
Cluster wt. (g)	0.18	0.52	0.42
100-berry wt. (g)	0.27	0.14	0.82
	Year 2022		
Crop wt./vine (kg)	0.36	0.01	0.17
Yield (ton. ha^{-1})	0.36	0.01	0.17
Cluster number	<0.01	<0.01	0.23
Cluster wt. (g)	0.21	0.43	0.08
100-berry wt. (g)	0.14	0.96	0.78

Continued…

[1] Values denote p-values from the results of the Shapiro-Wilk test and Levene's test for homogeneity of variance for different analyses: Petiole nutrient analysis, yield, fruit quality, bud cold hardiness, and bud injury. Significant differences were reported for p-values ≤ 0.05.

[2] Berry potassium levels in K-treated vines were similar in all four replicates, therefore, no variance was observed.

(Table 3.4 continued)

	Normality (Control)	Normality (K-treated)	Variance
Fruit Quality			
	Year 2021		
Total Soluble Solids	0.57	0.90	0.46
pH	0.80	0.19	0.08
Titratable Acidity	0.85	0.77	0.72
Fruit Maturity Index	0.46	0.73	0.99
Berry-K	0.26	0.13	0.02
	Year 2022		
Total Soluble Solids	0.64	0.41	0.34
pH	0.85	0.10	0.81
Titratable Acidity	0.86	0.98	0.47
Fruit Maturity Index	0.85	0.72	0.59
Berry-K[2]	<0.01	-	0.36
Bud Cold Hardiness			
Date			
12/20/2021	0.58	0.85	0.69
1/3/2022	0.20	0.92	0.03
2/9/2022	0.80	0.23	0.27
2/24/2022	0.36	0.98	0.97
3/24/2022	0.29	0.27	0.26
11/29/2022	0.02	0.27	0.48
1/11/2023	0.84	0.90	0.54
2/15/2023	0.93	0.98	0.15
4/5/2023	0.87	0.97	0.54
Bud Injury Assessment			
Bud Type			
Primary Bud	0.15	0.88	0.20
Secondary Bud	0.02	0.48	0.62

Table 3.5 Yield components of Chambourcin for the years 2021 and 2022.

	Year 2021			Year 2022		
	Control	K-treated	p-value[1]	Control	K-treated	p-value[1]
Crop wt./vine (kg)	5.68 ± 0.30	4.97 ± 0.35	0.14 (NS)	4.73 ± 0.27	4.32 ± 0.16	0.27 (NS)
Yield (ton. ha^{-1})	15.53 ± 0.83	13.59 ± 0.95	0.14 (NS)	12.93 ± 0.73	11.81 ± 0.43	0.27 (NS)
Cluster number/vine	24 ± 0.4	24 ± 0.3	0.37 (NS)	24 ± 0.1	23 ± 0.6	0.30 (NS)
Cluster wt. (g)	242 ± 12	204 ± 13	0.04 (S)	193 ± 8	185 ± 8	0.56 (NS)
100-berry weight (g)	262 ± 5	260 ± 3	0.78 (NS)	232 ± 2	231 ± 3	0.78 (NS)

Values within Control and K-treated columns represent means ± standard errors.

[1] Significance at $p \leq 0.05$; S = Significant, NS = Non-significant.

Table 3.6 Fruit parameters in Chambourcin for the years 2021 and 2022.

	Year 2021			Year 2022		
	Control	K-treated	p-value[3]	Control	K-treated	p-value[3]
TSS (°Brix)[1]	22.5 ± 0.1	23.3 ± 0.2	0.01 (S)	21.6 ± 0.4	22.4 ± 0.2	0.04 (S)
pH	3.26 ± 0.02	3.29 ± 0.01	0.13 (NS)	3.21 ± 0.02	3.20 ± 0.02	0.46 (NS)
TA (g L^{-1})[1]	9.0 ± 0.2	8.4 ± 0.2	0.05 (S)	11.5 ± 0.4	11.3 ± 0.3	0.37 (NS)
FMI[1,2]	25 ± 0.6	28 ± 0.8	0.01 (S)	19 ± 0.6	20 ± 0.4	0.12 (NS)
Berry K level (mg L^{-1})	925 ± 68	1017 ± 37	0.14 (NS)	1212 ± 37	1450 ± 0	0.01 (S)

Values within Control and K-treated columns represent means ± standard errors.
[1] TSS: total soluble solids, TA: titratable acidity, FMI: fruit maturity index.
[2] FMI = TSS/TA × 10.
[3] Significance at $p \leq 0.05$; S = Significant, NS = Non-significant.

Figures

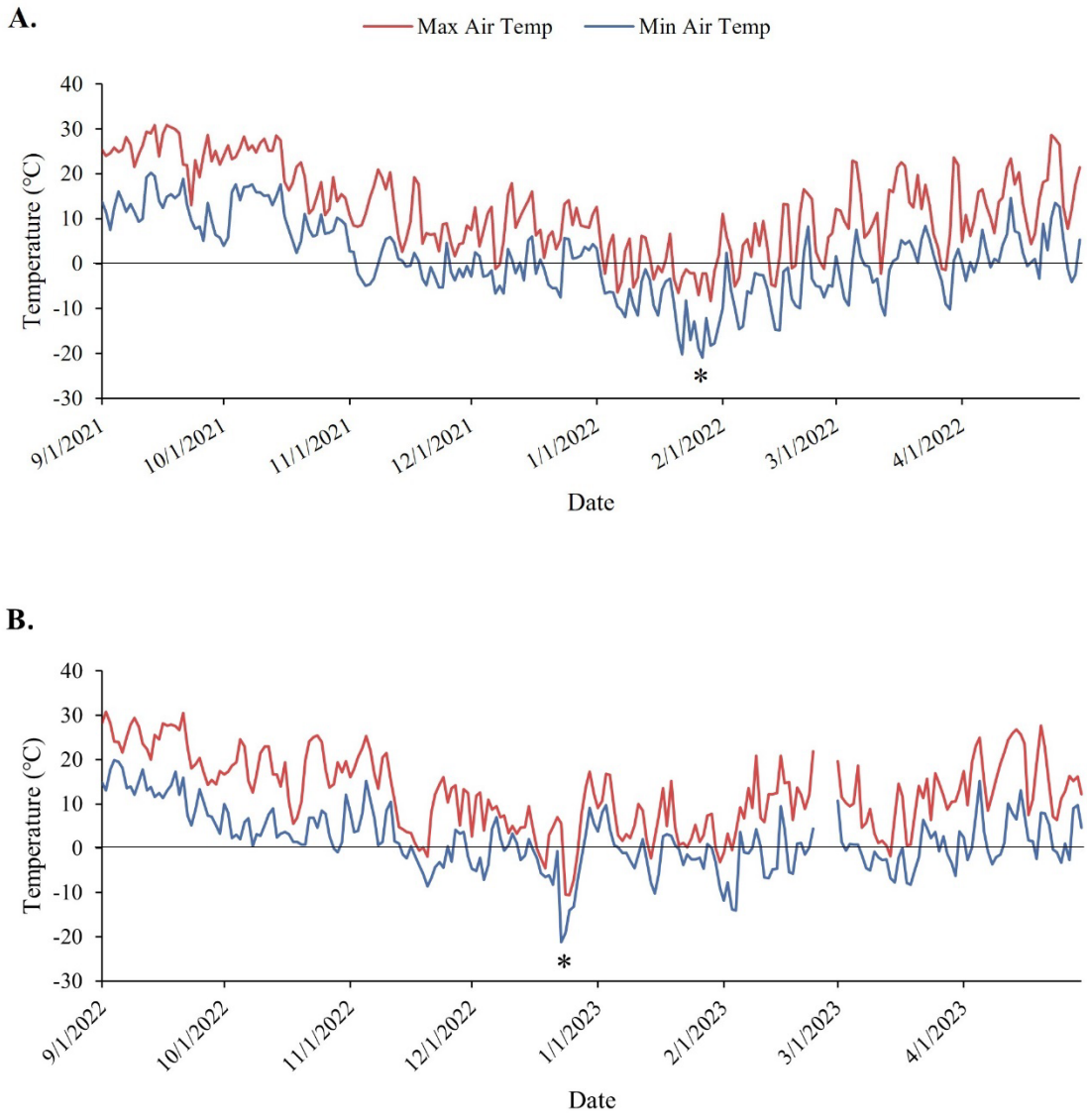

Figure 3.1 Daily maximum (red) and minimum (blue) air temperatures from September 1 to April 30 in: A) 2021-22 and B) 2022-23.

'*' denotes the lowest temperatures recorded during the winter seasons of both experimental years (-21°C on January 27, 2022; -21.2°C on December 23, 2022). Air temperature data was not available for Feb 24-28, 2023.

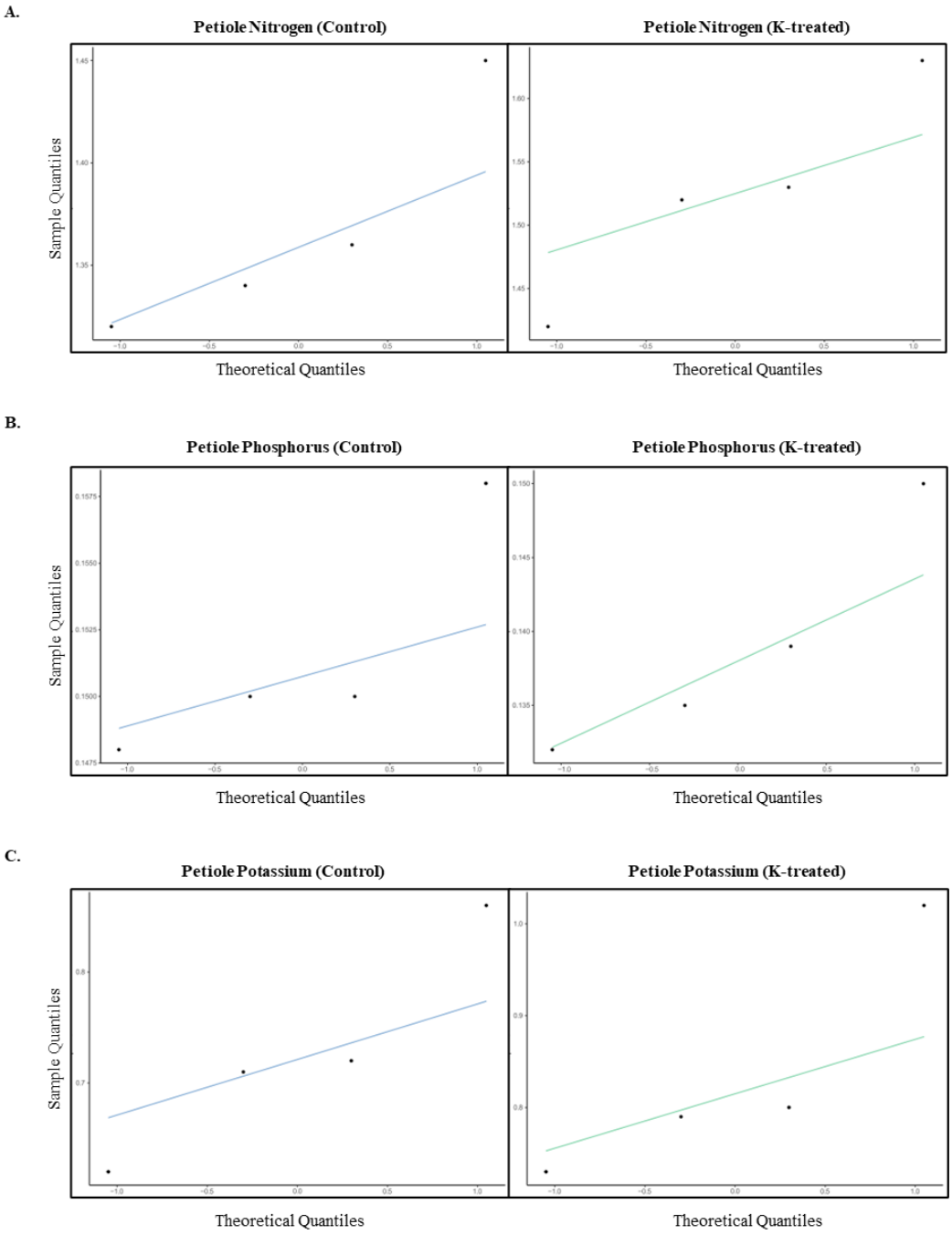

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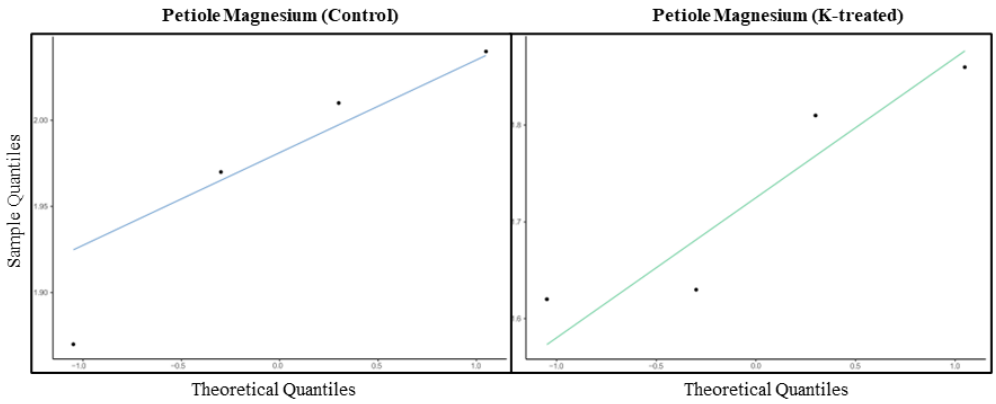
Figure 3.2 Quantile-quantile (QQ) plots showing distribution of data collected for petiole nutrient analysis for both control (blue) and K-treated (green) samples.

(Figure 3.2 continued)

D.



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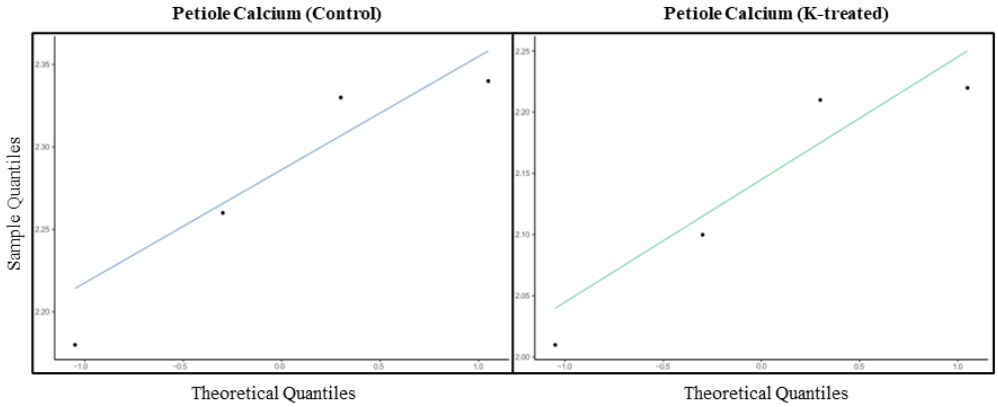

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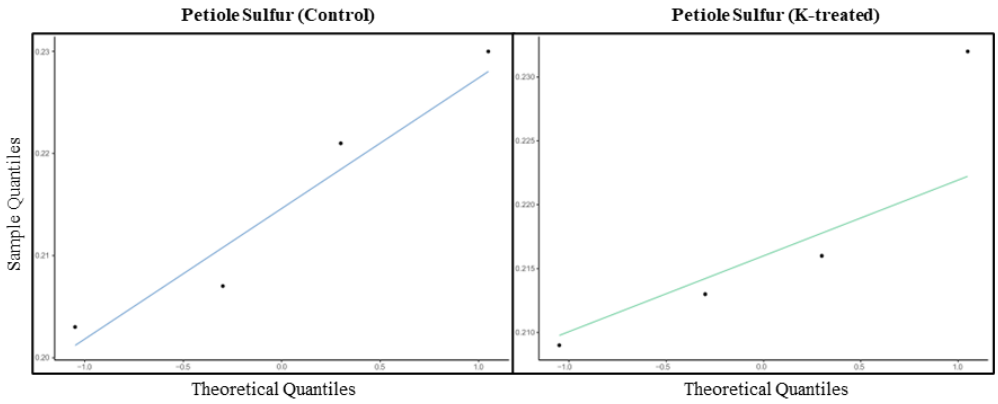

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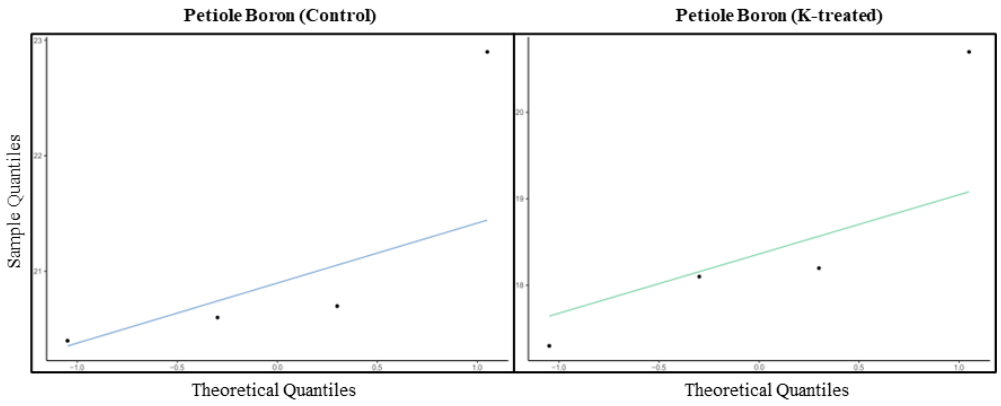
(Figure 3.2 continued)

G.



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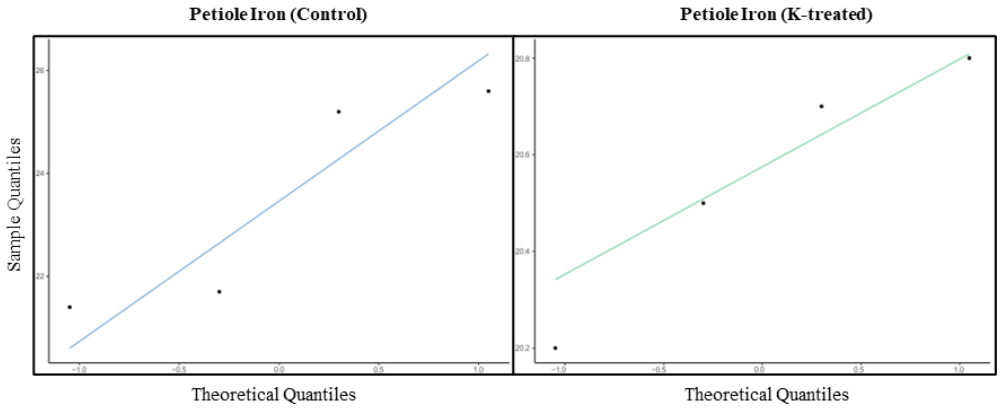
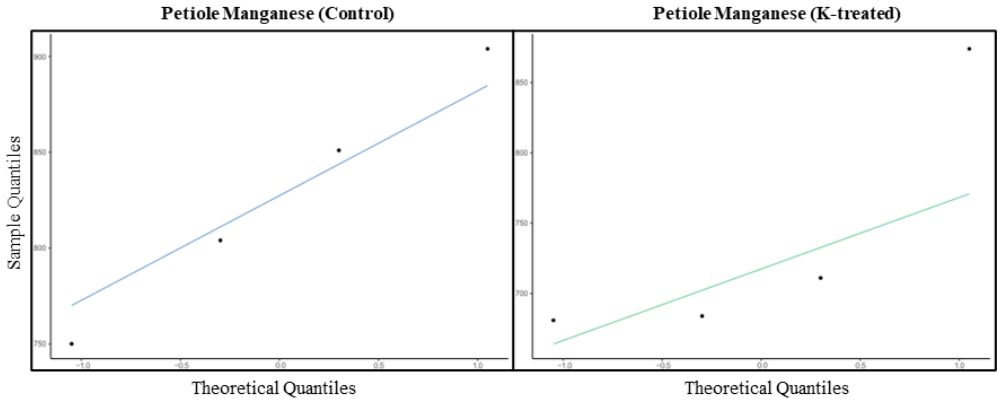


I.



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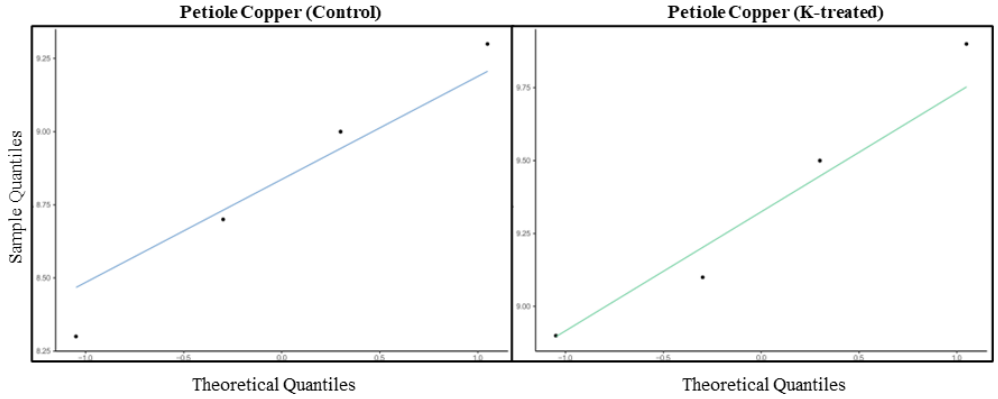
(Figure 3.2 continued)

J.



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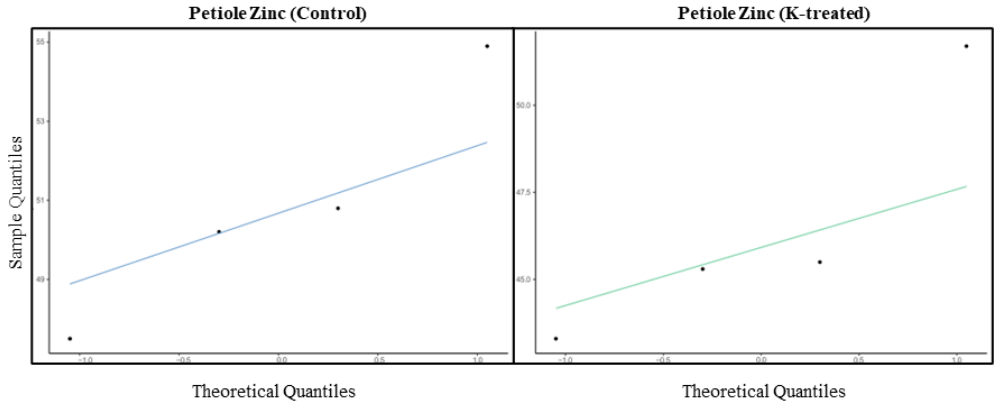

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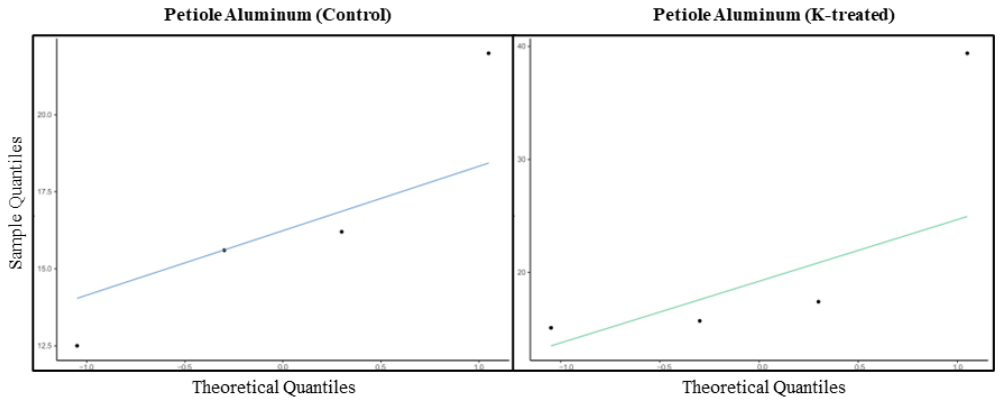

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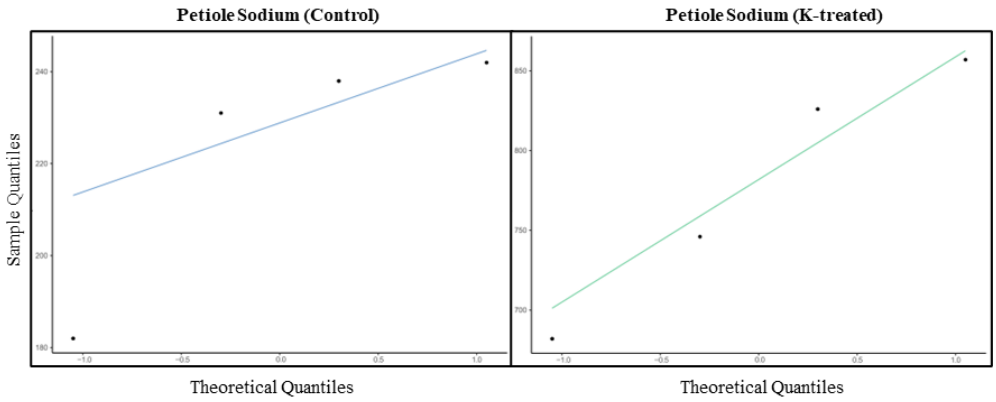
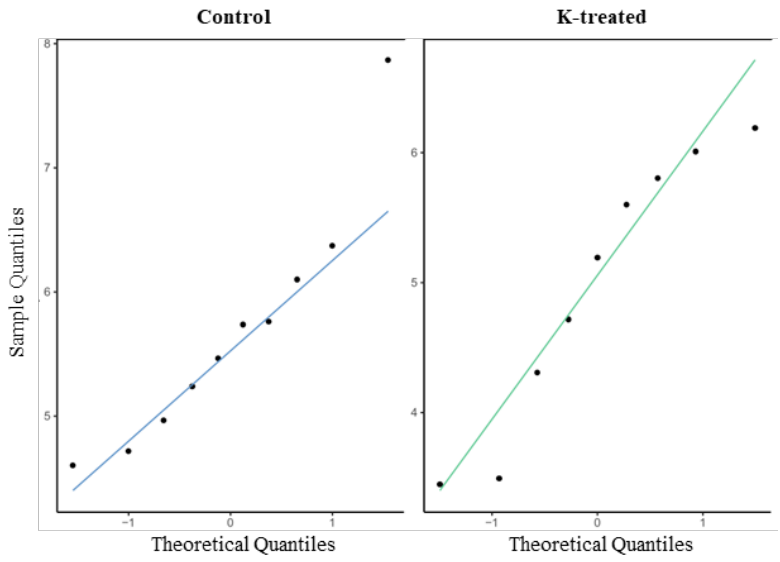
(Figure 3.2 continued)

M.



A. Crop weight per vine (2021)



B. Crop weight per vine (2022)

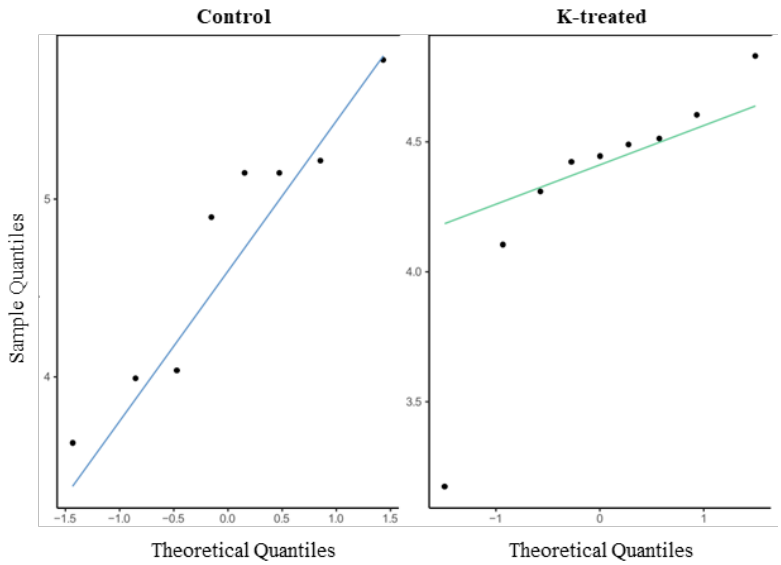


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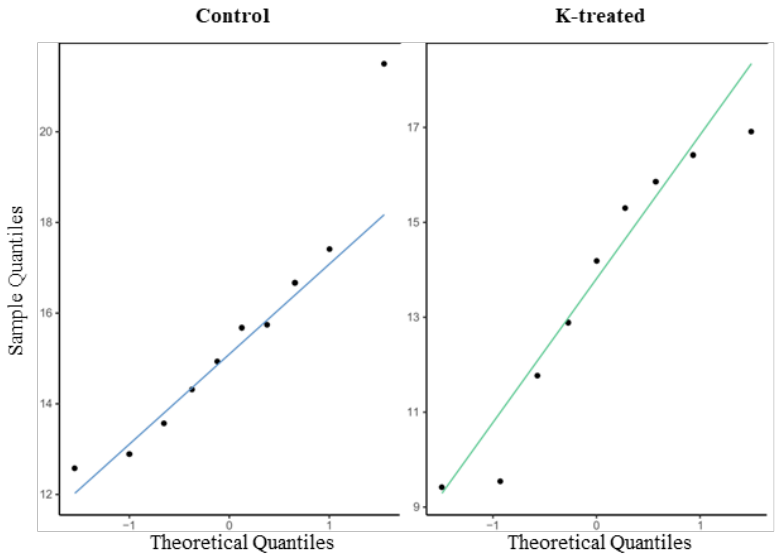
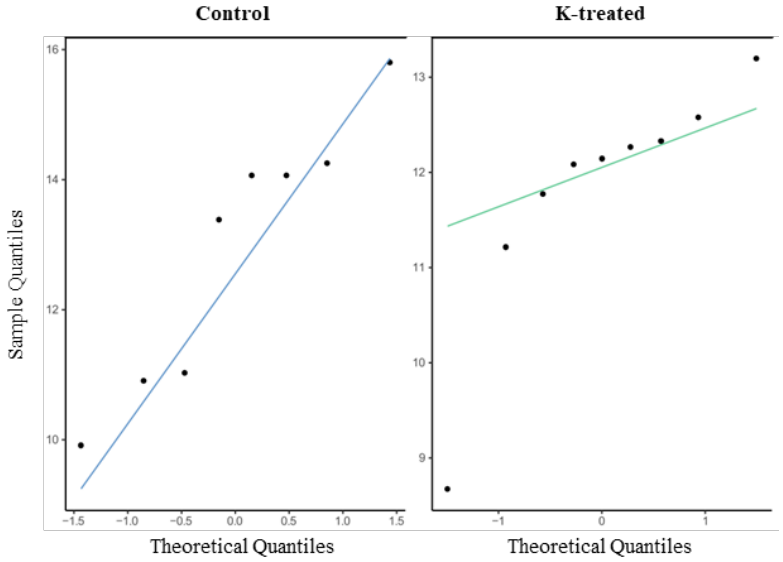
Figure 3.3 Quantile-quantile (QQ) plots showing distribution of data collected for yield attributes for both control (blue) and K-treated (green) samples.

(Figure 3.3 continued)

C. Yield (ton. ha-1) (2021)



D. Yield (ton. ha-1) (2022)



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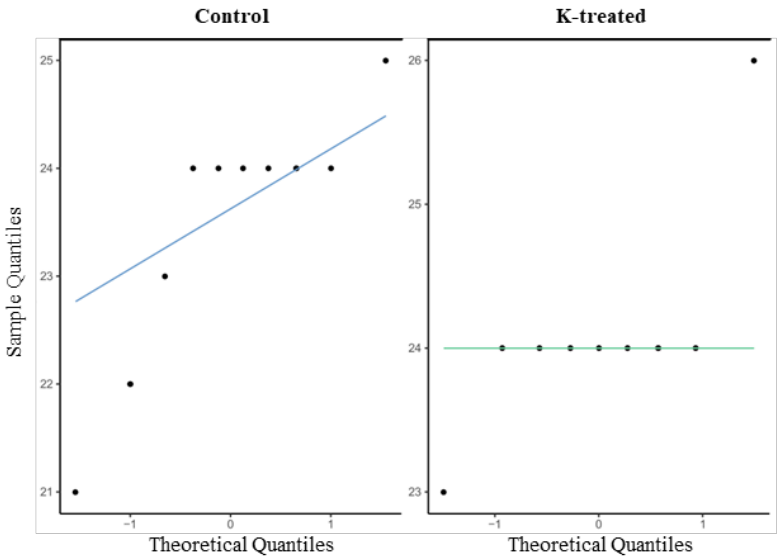
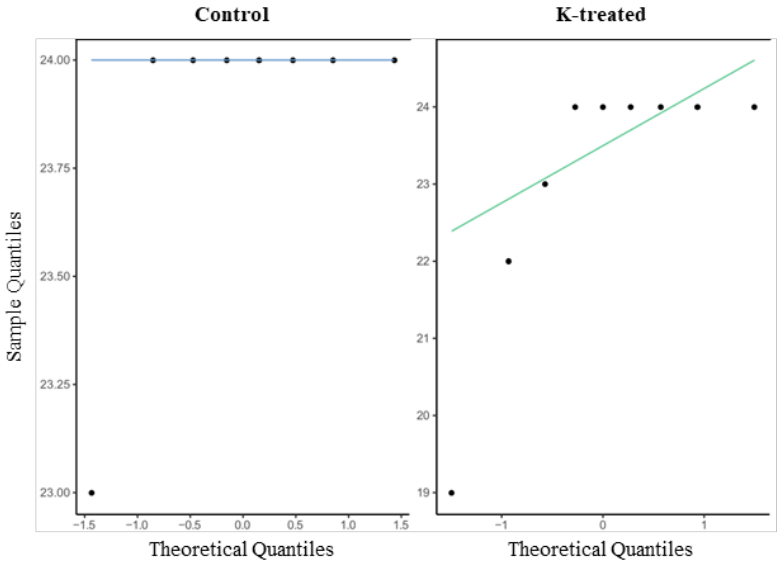
(Figure 3.3 continued)

E. Cluster number/vine (2021)



F. Cluster number/vine (2022)



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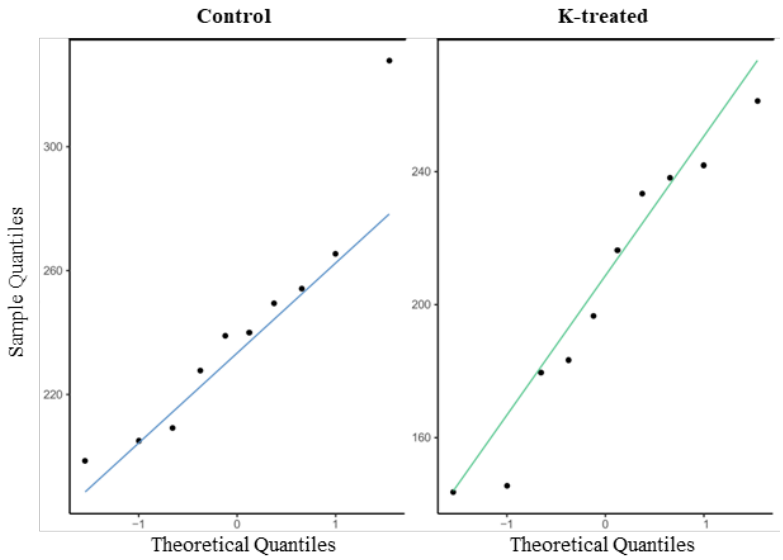
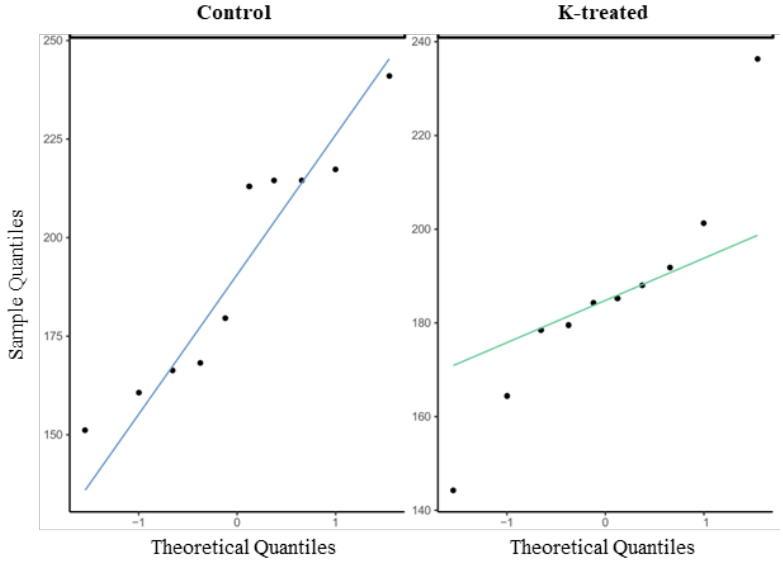
(Figure 3.3 continued)

G. **Cluster wt. (g) (2021)**



H. **Cluster wt. (g) (2022)**



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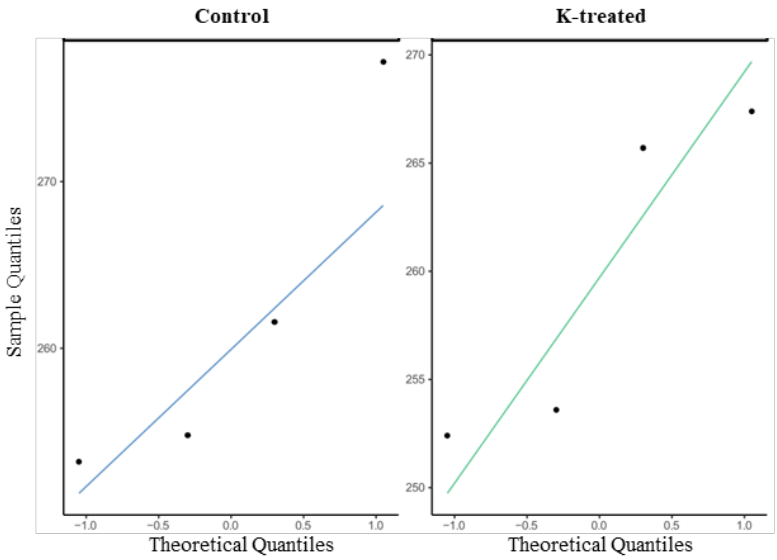
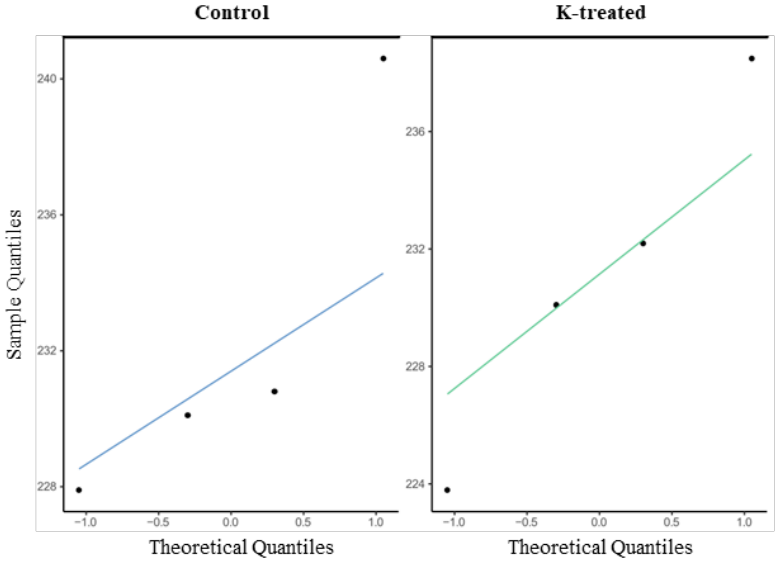
(Figure 3.3 continued)

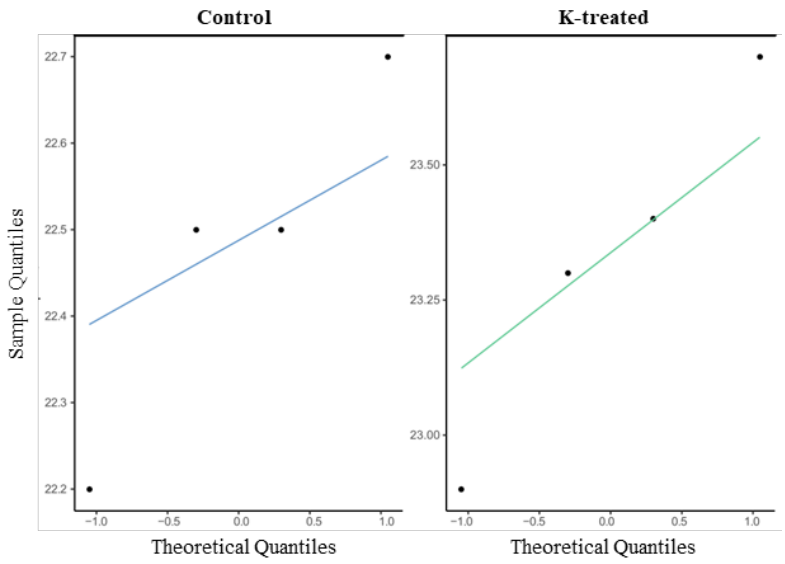
I. 100-berry wt. (g) (2021)



J. 100-bery wt. (g) (2022)



A. **Total Soluble Solids (2021)**



B. **Total Soluble Solids (2022)**

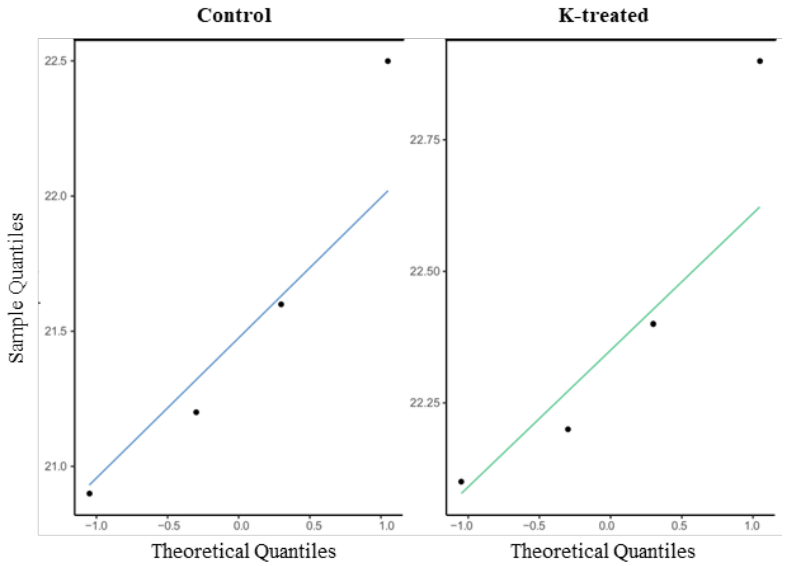


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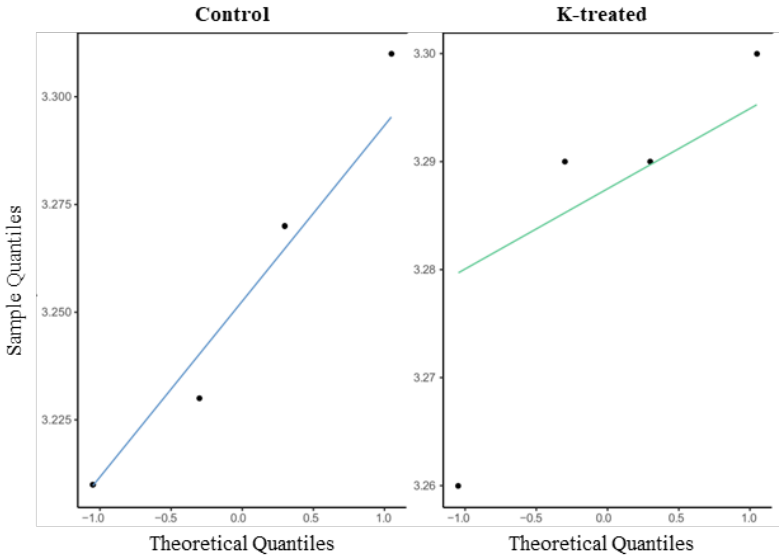
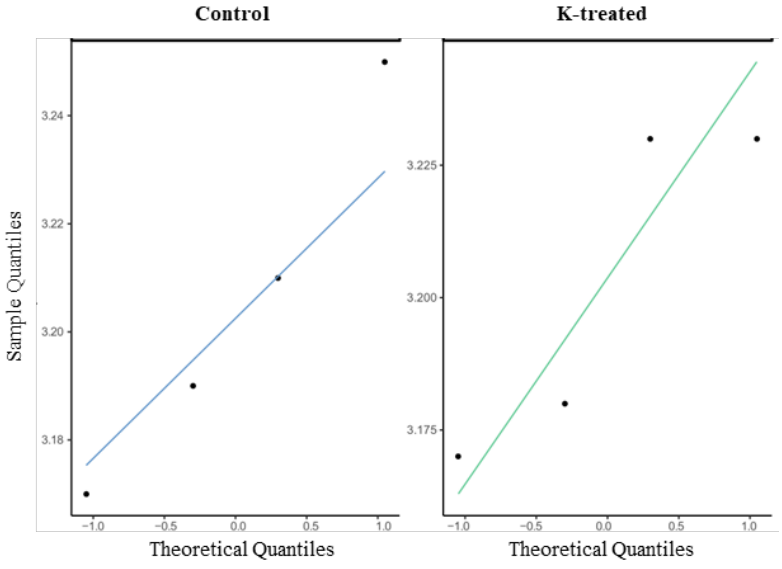
Figure 3.4 Quantile-quantile (QQ) plots showing distribution of data collected for fruit quality traits for both control (blue) and K-treated (green) samples

(Figure 3.4 continued)

C. **pH (2021)**



D. **pH (2022)**



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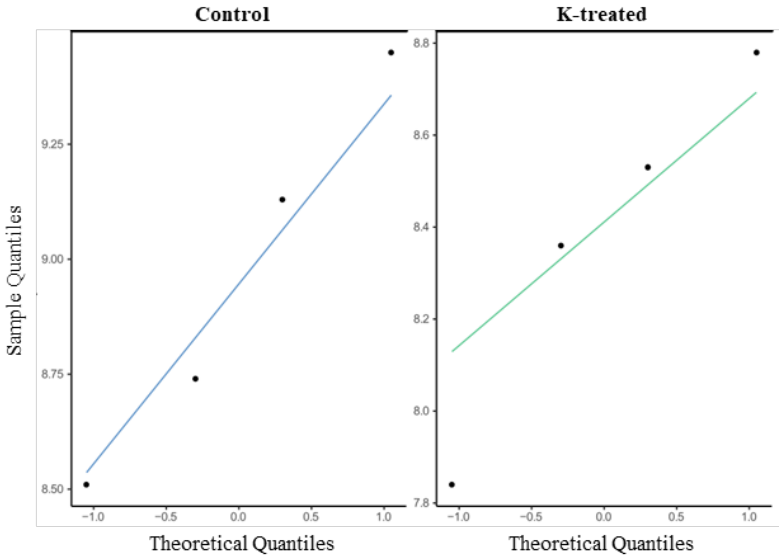
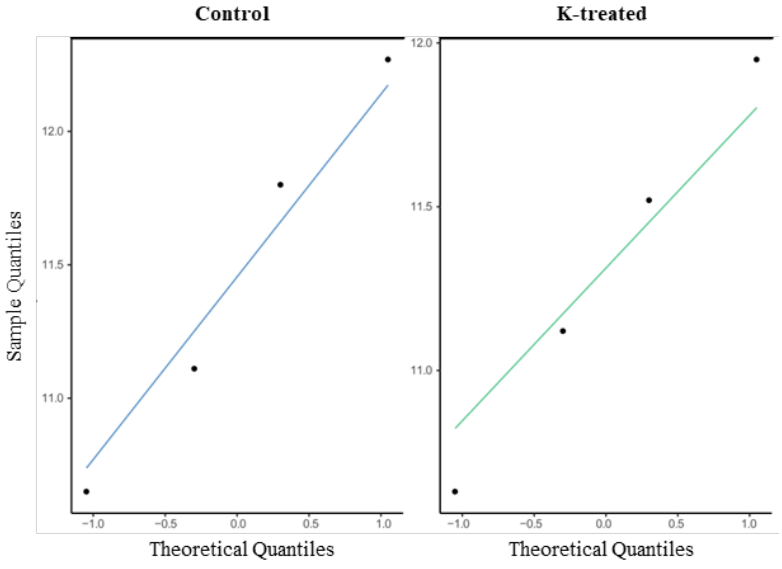
(Figure 3.4 continued)

E. Titratable Acidity (2021)



F. Titratable Acidity (2022)



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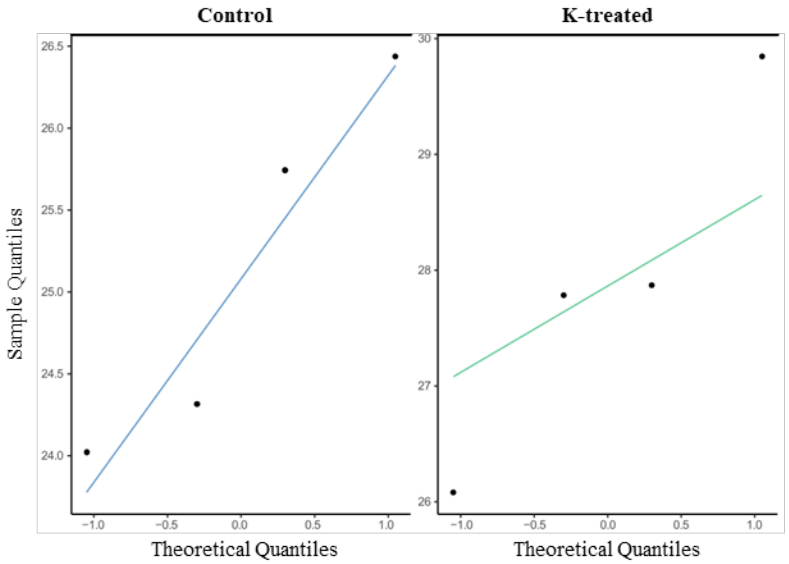
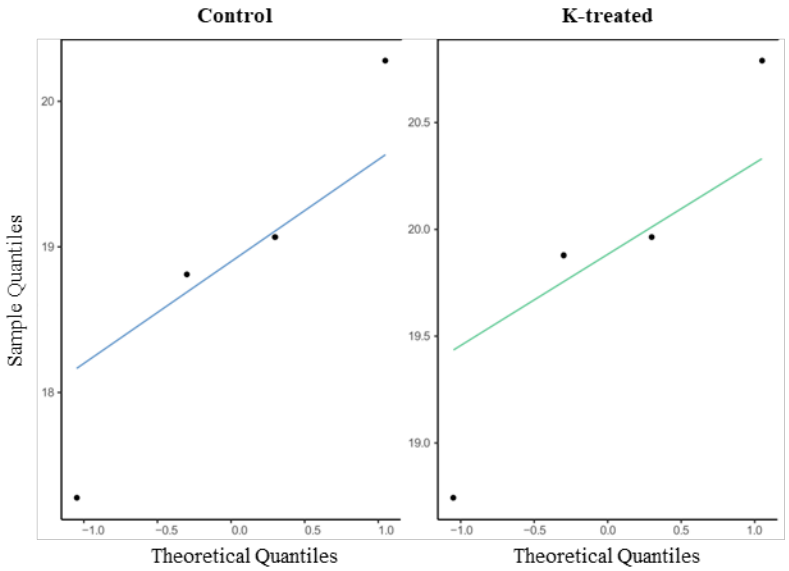
(Figure 3.4 continued)

G. Fruit Maturity Index (2021)



H. Fruit Maturity Index (2022)



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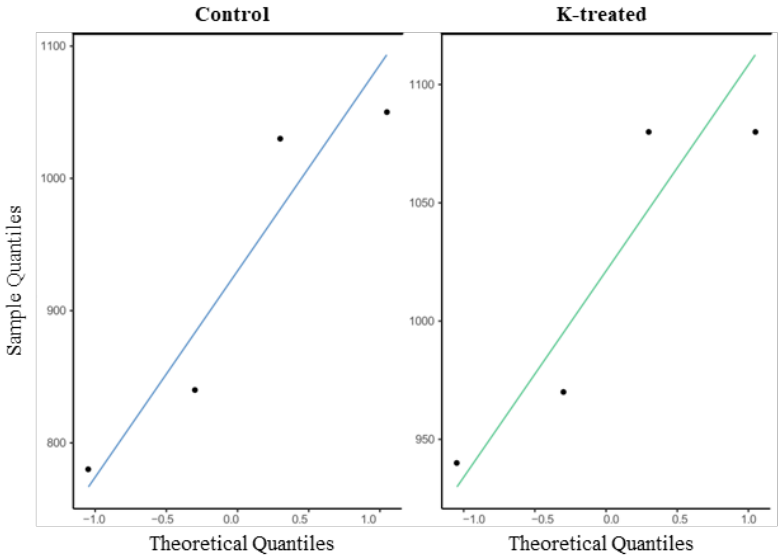
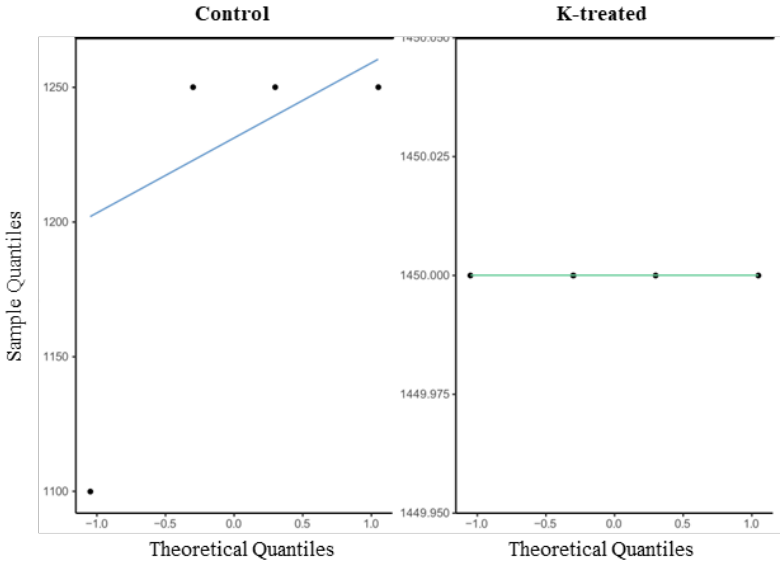
(Figure 3.4 continued)

I. Berry Potassium (2021)



J. Berry Potassium (2022)



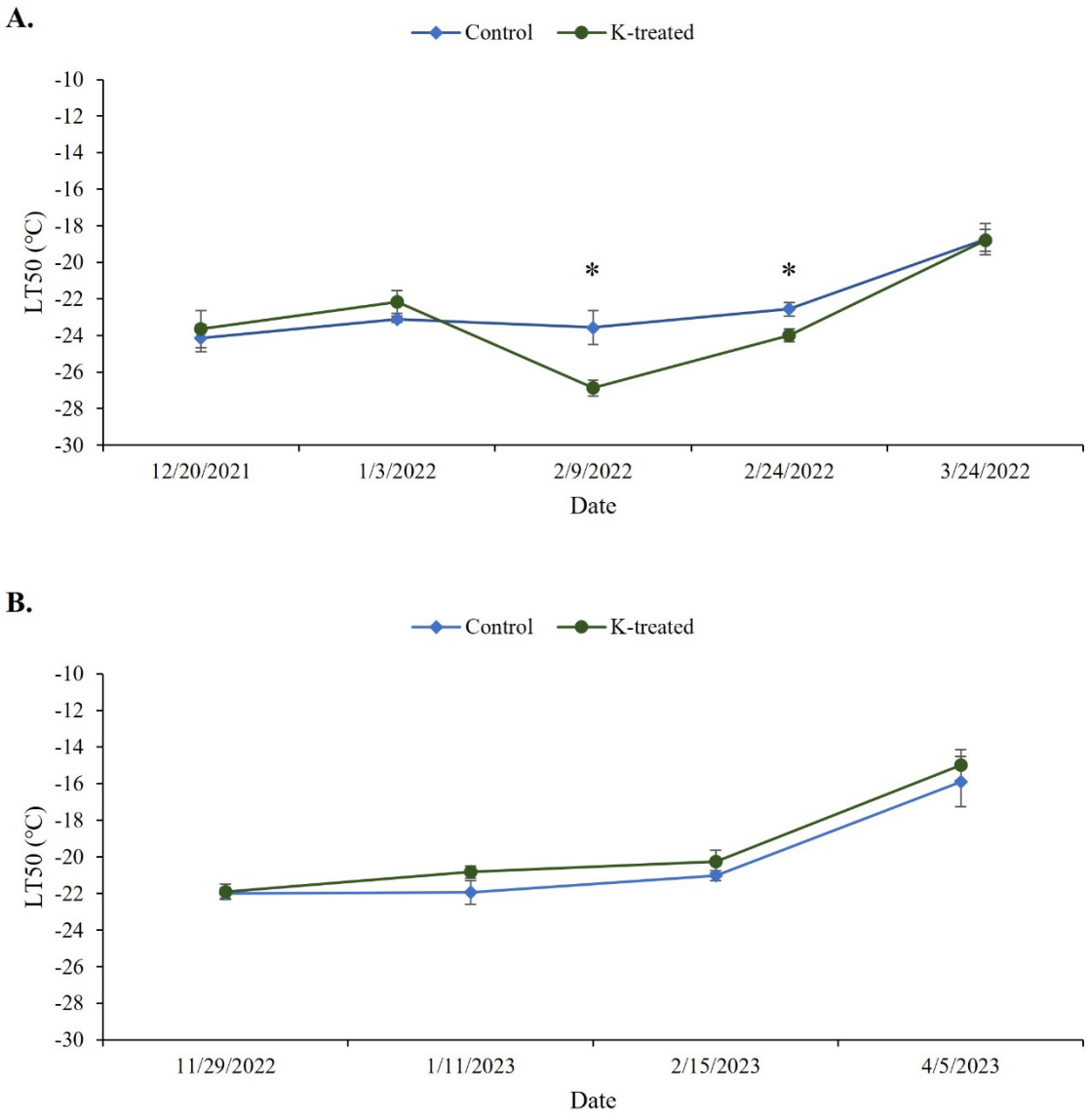


Figure 3.5 Mean LT50 values for K-treated and control buds collected during the winters of A) 2021-22, and B) 2022-23.

Error bars denote standard error. '*' denotes a significant difference among treatments, $p \leq 0.05$.

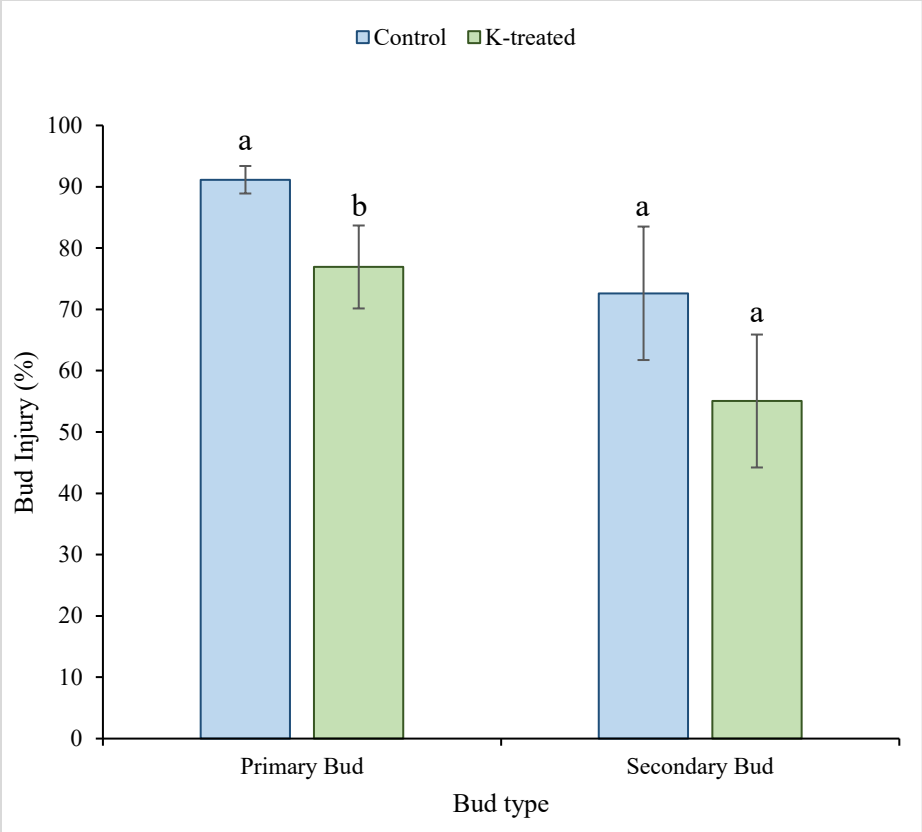


Figure 3.6 Percent bud injury for K-treated (green) and control (blue) buds collected on January 11, 2023.
Mean values marked with different letters denote a significant difference, $p \leq 0.05$. Error bars denote standard errors.

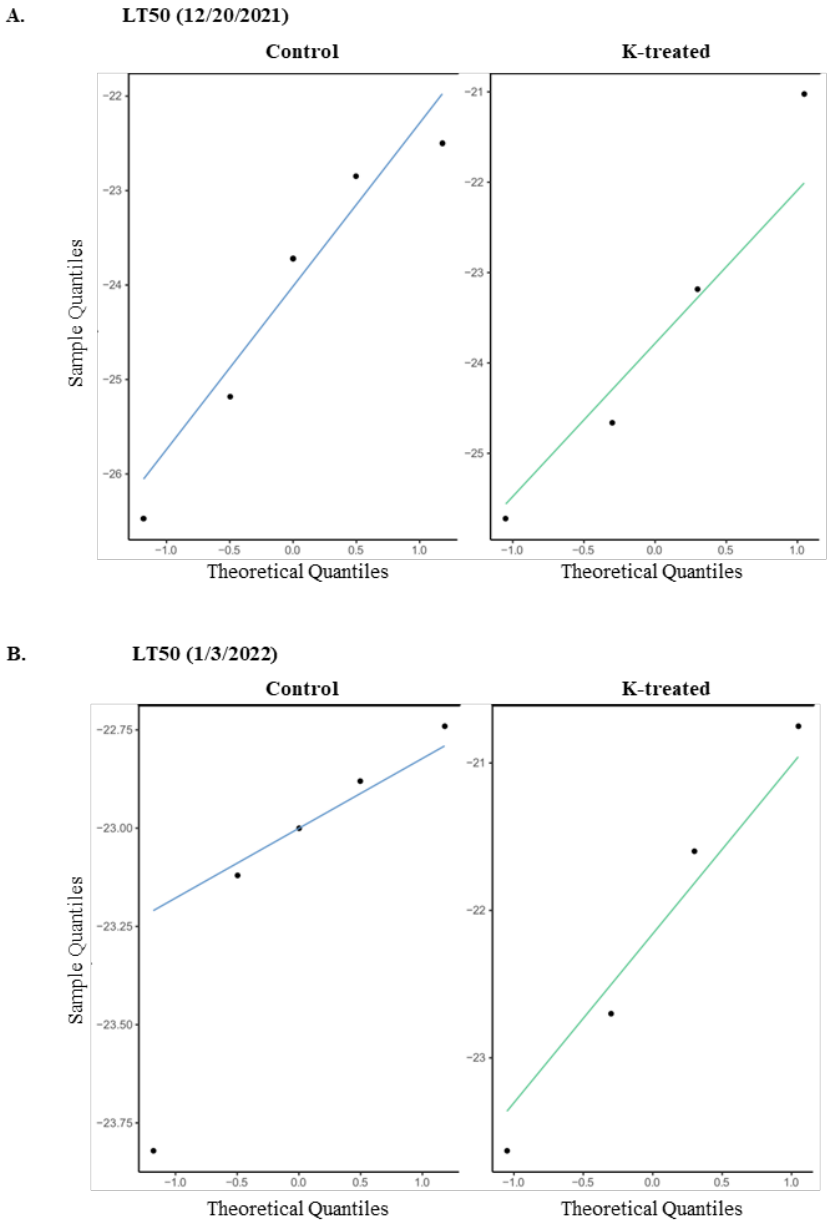


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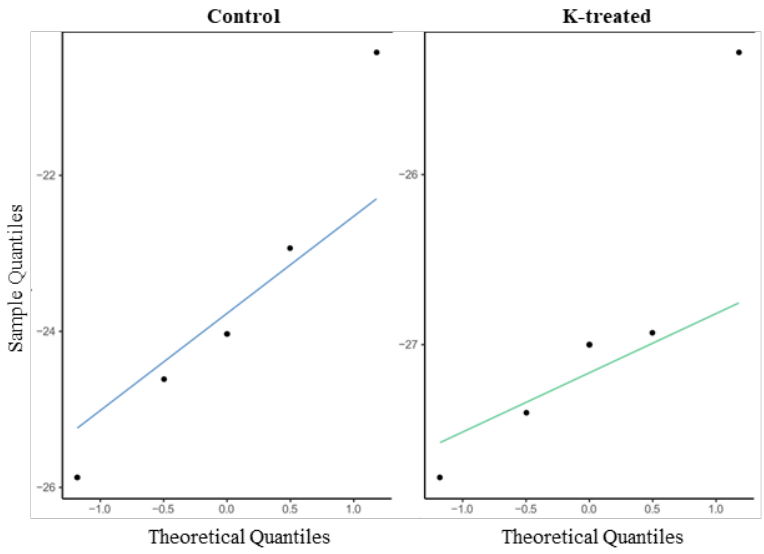
Figure 3.7 Quantile-quantile (QQ) plots showing distribution of data collected for differential thermal analysis for both control (blue) and K-treated (green) samples.

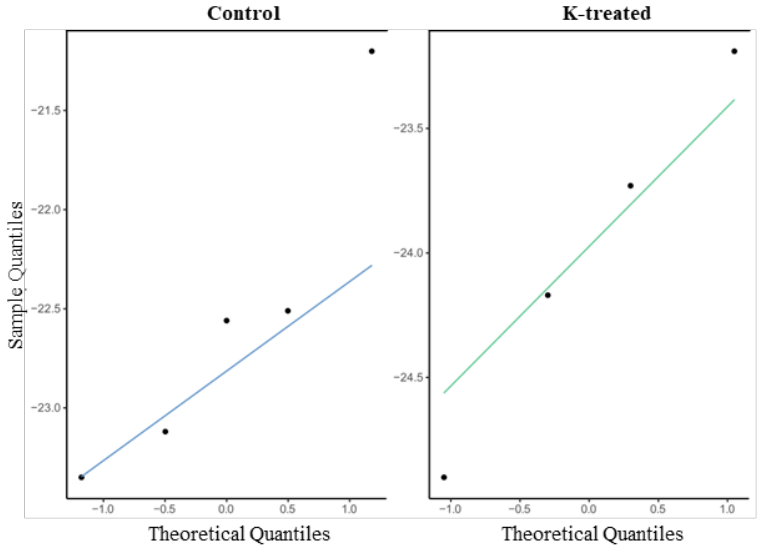
LT50 denotes lethal temperature for 50% bud injury.

(Figure 3.7 continued)

C. **LT50 (2/9/2022)**



D. **LT50 (2/24/2022)**



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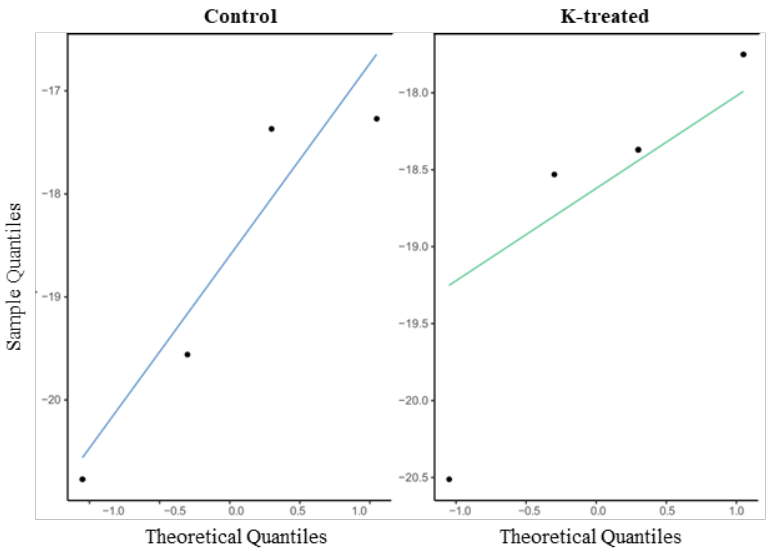
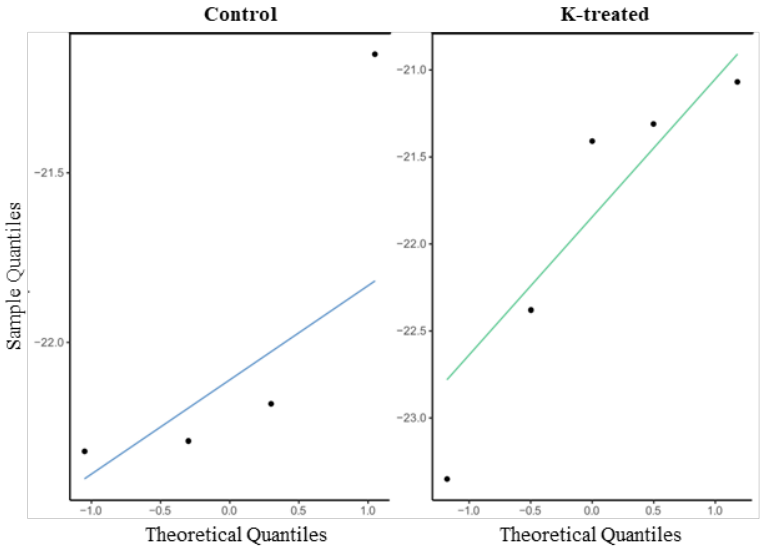
(Figure 3.7 continued)

E. **LT50 (3/24/2022)**



F. **LT50 (11/29/2022)**



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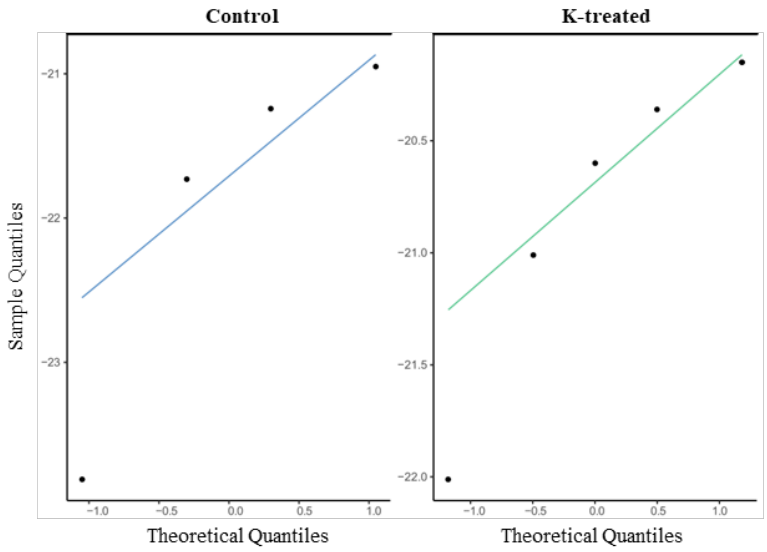
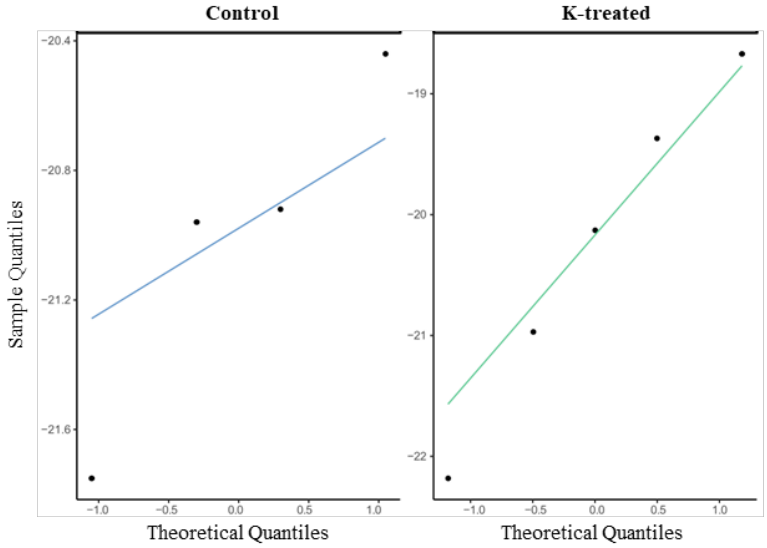
(Figure 3.7 continued)

G. **LT50 (1/11/2023)**



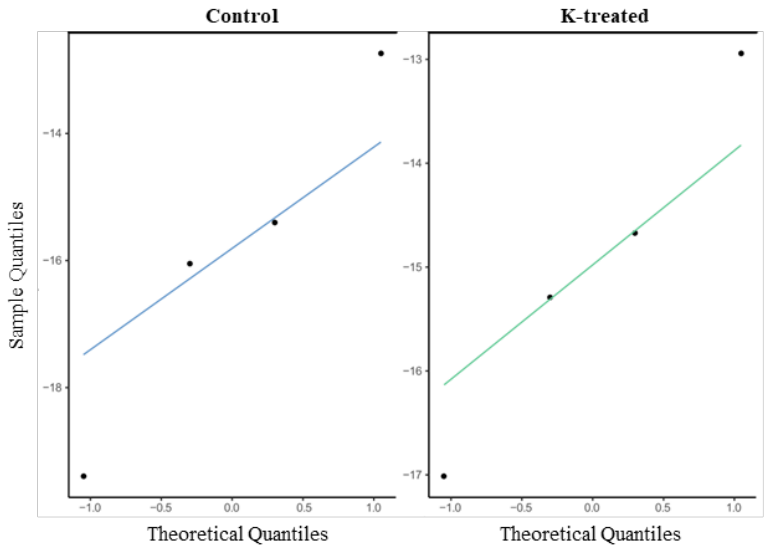
H. **LT50 (2/15/2023)**



Continued…

(Figure 3.7 continued)

I. **LT50 (4/5/2023)**



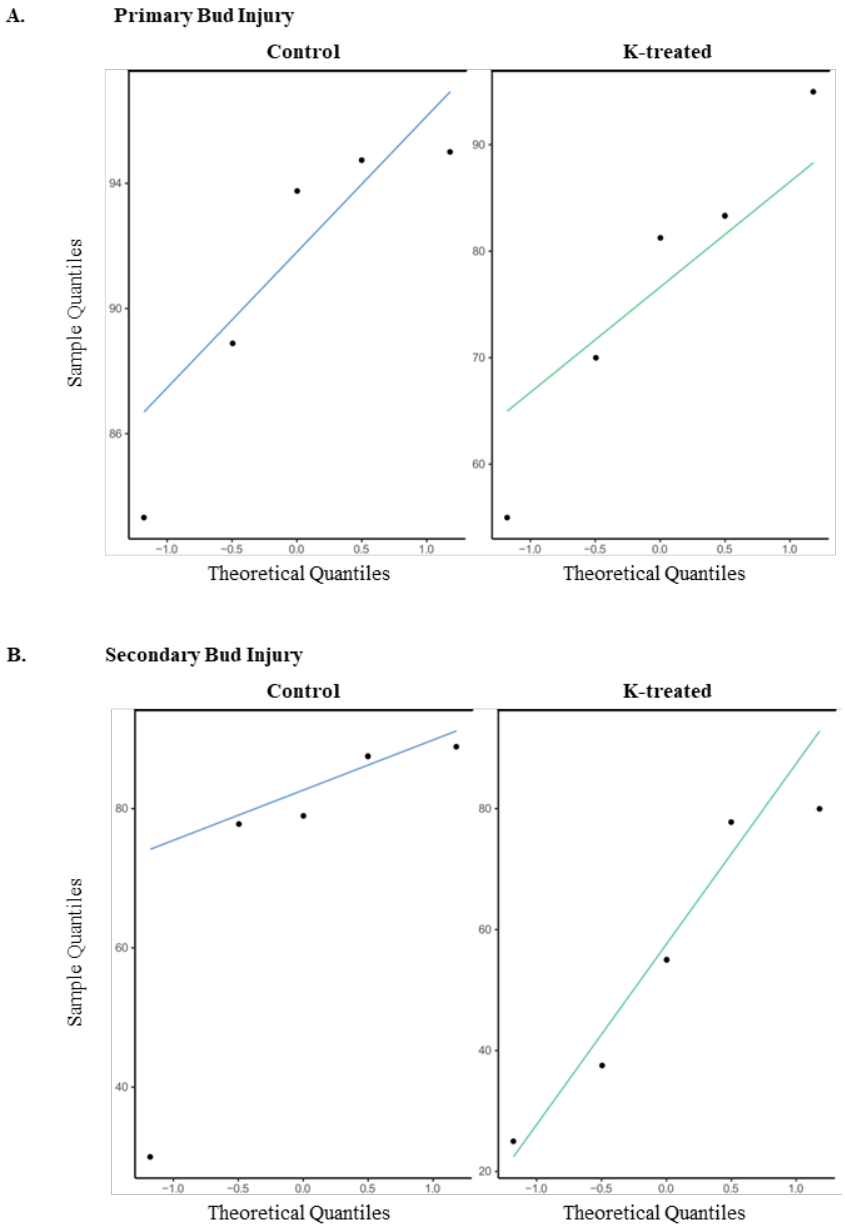


Figure 3.8 Quantile-quantile (QQ) plots showing distribution of data collected for bud injury assessment for both control (blue) and K-treated (green) samples.

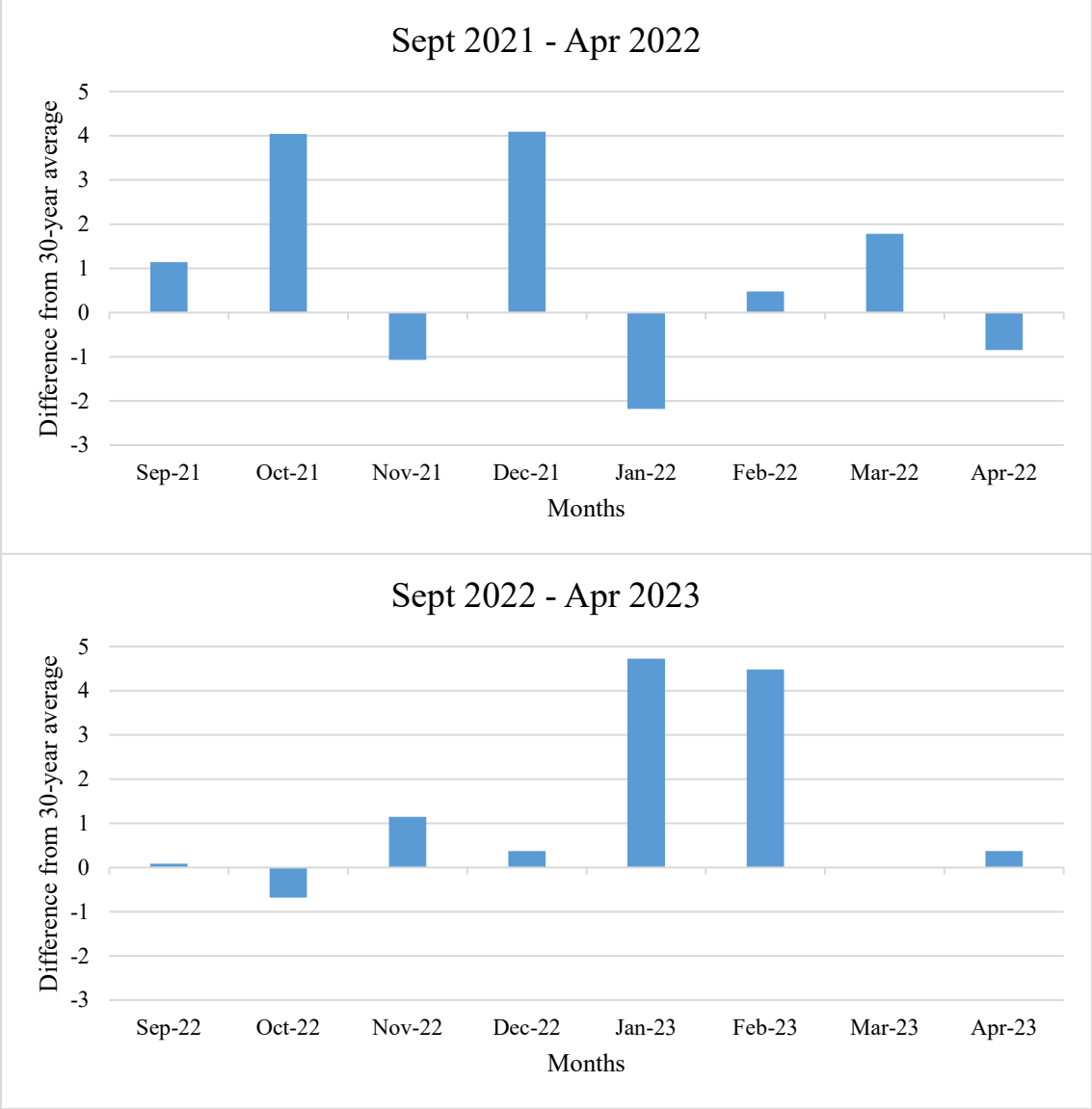


Figure 3.9 Comparison of monthly mean air temperatures of two winter seasons (2021-2022 and 2022-2023) compared to 30-year average (1991-2020).

Chapter 4 - Conclusion and Future Prospects

Grapevine is an economically important fruit crop grown worldwide. In the United States, the annual production of grapevine is around 6 million tons (USDA, 2022). Due to higher consumer demand, the wine industry in the US is expanding, especially in the regions of the Midwest, which are more prone to winter injury (Loseke et al., 2015; Zabadal et al., 2007). During mid-winter, low temperatures can injure the dormant buds or cane tissues either partially or completely. Further, temperature changes during spring may accelerate budburst, resulting in young shoots emerging earlier. However, in regions of eastern United States, these sensitive young shoots are more prone to late-spring frost conditions, causing direct and indirect losses to grapevine growers (De Rosa et al., 2022; Zabadal et al., 2007). Therefore, it is imperative to find ways to improve cold hardiness of the dormant tissues during mid-winter and avoid frost injury during late spring.

Injury due to low temperatures can be avoided by utilizing a holistic approach with different long-term and short-term management practices (Dami, 2022). Long-term strategies include genetic improvement of grapevine cultivars to increase their mid-winter cold hardiness as well as late-spring frost tolerance. On the other hand, cold hardiness of already established vines can be improved via short- or mid-term management practices,

such as pruning, cover crop management, or spraying cryoprotectants. In this thesis, we investigated the genetics contributing to frost tolerance by comparing frost tolerance in two grapevine species and identifying potential genes involved in low temperature stress tolerance through comparative transcriptomics of chill (4℃) and freeze (-2℃) stressed shoots. Further, we investigated how management practices, such as foliar potassium applications, can improve grapevine cold hardiness and its potential impact on yield and fruit quality.

In our frost tolerance study, we found that 'GREM4' young shoots had higher survivability under sub-zero temperatures than those of 'Cabernet Sauvignon'. Transcriptomic analysis revealed that genes encoding cell wall associated *receptor kinases* and *KCS* genes involved in VLCFAs synthesis were differentially expressed in 'GREM4' compared to 'Cabernet Sauvignon' and likely play a major role in improving low temperature stress tolerance of 'GREM4' young shoots. This study provided new insights into the frost tolerance of *V. labrusca* young shoots and their transcriptomic responses. In future studies, these candidate genes can be functionally characterized for their role in improving tolerance to low temperature stresses. Other than transcriptomic responses, the two grapevine species differ in are other morphological traits, such as leaf thickness and trichome density. Therefore, these morphological traits might also be involved in avoiding frost injury. Future investigation of these morphological and physiological changes may help us better understand the mechanism of frost tolerance.

In this study, we only investigated the frost tolerance of *V. labrusca* acc. 'GREM4' and *V. vinifera* cv. 'Cabernet Sauvignon', but we saw significant differences in

their bud cold hardiness levels and frost tolerance. Cold hardiness levels, therefore, can vary between species, however, there is likely intraspecies variation, as well (Londo & Johnson, 2014). Therefore, in order to improve cold hardiness or frost tolerance, it is important to choose appropriate accessions for the development of cold hardy cultivars, as the accessions of the same species may respond differently under different environmental conditions. Future research can be conducted to determine how different accessions and cultivars of different grapevine species respond to temperature fluctuations during mid-winters or late springs.

For already established vineyards, cold hardiness can be improved in the plants by different management practices. We focused on foliar application of potassium fertilizer, ReaX^{TM} (25% K_2O) as a cryoprotectant to improve cold hardiness during winter. Along with that, we investigated if the foliar application of potassium significantly impacted yield and fruit quality. The K applications enhanced cold hardiness of the buds, However, despite four to five foliar applications per year, the petiole samples were deficient in potassium (K) content. Therefore, future studies can be conducted using different fertilizer rates applied at different stages of grapevine development.

Another benefit of foliar potassium application was that it had no negative impact on overall yield or pH, but it significantly increased the total soluble solids [TSS] during both years and decreased titratable acidity during first year. Overall, foliar application of K increased cold hardiness and fruit quality without impacting the yield, therefore, K fertilization can be a potential solution to mitigate winter injury. However, this application is only effective to an extent as we observed that despite foliar applications,

there was significant bud injury in the vineyard during second winter season of our study. Therefore, further research on effectiveness of different K fertilizers in improving cold hardiness is required.

We only investigated the effect of foliar K application on *Vitis spp.* 'Chambourcin' which is a cold sensitive hybrid. K application not only increased the bud cold tolerance, but also increased the TSS. Studies have reported that there is a positive correlation between K and sugar accumulation (Cakmak, 2005). Therefore, it would be interesting to investigate if K application can improve the TSS in grapevine cultivars having inherent low TSS. This would be an effective way to increase the sugar levels in the fruits, thus increasing the profitability of the crop. Since buds also serve as a strong sink, it would be worth to investigate the sugar levels in bud tissues and their role in improving bud cold hardiness.

Taken together, findings from these studies can help address the challenge of grapevine survivability under low temperature stress, thus increasing the overall profitability to grapevine growers.

Bibliography

Aazami, M. A., & Mahna, N. (2017). Salicylic acid affects the expression of VvCBF4 gene in grapes subjected to low temperature. *Journal of Genetic Engineering and Biotechnology*, 15(1), 257–261. https://doi.org/10.1016/j.jgeb.2017.01.005

Agarwal, M., Hao, Y., Kapoor, A., Dong, C.-H., Fujii, H., Zheng, X., & Zhu, J.-K. (2006). A R2R3 Type MYB Transcription Factor Is Involved in the Cold Regulation of CBF Genes and in Acquired Freezing Tolerance. *Journal of Biological Chemistry*, 281(49), 37636–37645. https://doi.org/10.1074/jbc.M605895200

Anderson, J. V., Li, Q. B., Haskell, D. W., & Guy, C. L. (1994). Structural organization of the spinach endoplasmic reticulum-luminal 70-kilodalton heat-shock cognate gene and expression of 70-kilodalton heat-shock genes during cold acclimation. *Plant Physiology*, 104(4), 1359–1370. https://doi.org/10.1104/pp.104.4.1359

Ault, T. R., Henebry, G. M., de Beurs, K. M., Schwartz, M. D., Betancourt, J. L., & Moore, D. (2013). The False Spring of 2012, Earliest in North American Record. Eos, *Transactions American Geophysical Union*, 94(20), 181–182. https://doi.org/10.1002/2013EO200001

Babraham Bioinformatics—FastQC A Quality Control tool for High Throughput Sequence Data. (n.d.). Retrieved February 26, 2024, from https://www.bioinformatics.babraham.ac.uk/projects/fastqc/

Bai, Y., Ali, S., Liu, S., Zhou, J., & Tang, Y. (2023). Characterization of Plant Laccase Genes and Their Functions. *Gene*, *852*, 147060. https://doi.org/10.1016/j.gene.2022.147060

Bañuelos, M. L. G., Moreno, L. V., Winzerling, J., Orozco, J. A., & Gardea, A. A. (2008). Winter Metabolism in Deciduous Trees: Mechanisms, Genes and Associated Proteins. 31, 16.

Benveniste, I., Bronner, R., Wang, Y., Compagnon, V., Michler, P., Schreiber, L., Salaün, J.-P., Durst, F., & Pinot, F. (2005). CYP94A1, a Plant Cytochrome P450-catalyzing Fatty Acid Omega-hydroxylase, is Selectively Induced by Chemical Stress in *Vicia sativa* Seedlings. *Planta*, *221*(6), 881–890. https://doi.org/10.1007/s00425-005-1503-y

Blighe et al. (2023). EnhancedVolcano: Publication-ready volcano plots with enhanced colouring and labeling. Bioconductor version: Release (3.16) doi: 10.18129/B9.bioc.EnhancedVolcano. (1.16.0) [Computer software].

Bogs, J., Jaffé, F. W., Takos, A. M., Walker, A. R., & Robinson, S. P. (2007). The Grapevine Transcription Factor VvMYBPA1 Regulates Proanthocyanidin Synthesis during Fruit Development. *Plant Physiology*, 143(3), 1347–1361. https://doi.org/10.1104/pp.106.093203

Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics (Oxford, England)*, 30(15), 2114–2120. https://doi.org/10.1093/bioinformatics/btu170

Boneh, U., Biton, I., Schwartz, A., & Ben-Ari, G. (2012b). Characterization of the ABA signal transduction pathway in Vitis vinifera. *Plant Science*, 187, 89–96. https://doi.org/10.1016/j.plantsci.2012.01.015

Boneh, U., Biton, I., Zheng, C., Schwartz, A., & Ben-Ari, G. (2012a). Characterization of potential ABA receptors in Vitis vinifera. *Plant Cell Reports*, 31(2), 311–321. https://doi.org/10.1007/s00299-011-1166-z

Bradley et al. (1992). Elicitor- and wound-induced oxidative cross-linking of a proline-rich plant cell wall protein: A novel, rapid defense response—*ScienceDirect*. https://www.sciencedirect.com/science/article/abs/pii/009286749290530P

Brady, J. D., & Fry, S. C. (1997). Formation of Di-Isodityrosine and Loss of Isodityrosine in the Cell Walls of Tomato Cell-Suspension Cultures Treated with Fungal Elicitors or H2O2. *Plant Physiology*, 115(1), 87–92. https://doi.org/10.1104/pp.115.1.87

Browse, J., & Xin, Z. (2001). Temperature sensing and cold acclimation. *Current Opinion in Plant Biology*, 4(3), 241–246. https://doi.org/10.1016/s1369-5266(00)00167-9

Bu et al. (2021). KOBAS-i: Intelligent prioritization and exploratory visualization of biological functions for gene enrichment analysis | *Nucleic Acids Research* | *Oxford Academic*. https://academic.oup.com/nar/article/49/W1/W317/6292104

Buchfink, B., Reuter, K., & Drost, H.-G. (2021). Sensitive protein alignments at tree-of-life scale using DIAMOND. *Nature Methods*, 18(4), 366–368. https://doi.org/10.1038/s41592-021-01101-x

Byun, Y.-J., Koo, M.-Y., Joo, H.-J., Ha-Lee, Y.-M., & Lee, D.-H. (2014). Comparative analysis of gene expression under cold acclimation, deacclimation and reacclimation in Arabidopsis. *Physiologia Plantarum*, 152(2), 256–274. https://doi.org/10.1111/ppl.12163

Cakmak, I. (2005). The role of potassium in alleviating detrimental effects of a biotic stresses in plants. *Journal of Plant Nutrition and Soil Science*, 168, 521–530. https://onlinelibrary.wiley.com/doi/abs/10.1002/jpln.200420485

Carlow, C. E., Faultless, J. T., Lee, C., Siddiqua, M., Edge, A., & Nassuth, A. (2017). Nuclear localization and transactivation by Vitis CBF transcription factors are regulated by combinations of conserved amino acid domains. *Plant Physiology and Biochemistry*: PPB, 118, 306–319. https://doi.org/10.1016/j.plaphy.2017.06.027

Carmona, M., Chaib, J., Martinez-Zapater, J., & Thomas, M. (2008). A molecular genetic perspective of reproductive development in grapevine. *Journal of Experimental Botany*, 59(10), 2579–2596. https://doi.org/10.1093/jxb/ern160

Castilleux, R., Plancot, B., Ropitaux, M., Carreras, A., Leprince, J., Boulogne, I., Follet-Gueye, M.-L., Popper, Z. A., Driouich, A., & Vicré, M. (2018). Cell wall extensins in root–microbe interactions and root secretions. *Journal of Experimental Botany*, 69(18), 4235–4247. https://doi.org/10.1093/jxb/ery238

Centinari. (2016). Assessing and Managing Potassium Concentration in the Vineyard. Penn State Extension Wine & Grapes U. [Https://psuwineandgrapes.wordpress.com/2016/09/16/assessing-and-managing-potassium-concentration-in-the-vineyard/].

Centinari, M., Gardner, D. M., Smith, D. E., & Smith, M. S. (2018). Impact of Amigo Oil and KDL on Grapevine Postbudburst Freeze Damage, Yield Components, and Fruit and Wine Composition. *American Journal of Enology and Viticulture*, 69(1), 77–88. https://doi.org/10.5344/ajev.2017.17030

Centinari, M., Smith, M. S., & Londo, J. P. (2016). Assessment of Freeze Injury of Grapevine Green Tissues in Response to Cultivars and a Cryoprotectant Product. *HortScience*, 51(7), 856–860. https://doi.org/10.21273/HORTSCI.51.7.856

Chai, F., Liu, W., Xiang, Y., Meng, X., Sun, X., Cheng, C., Liu, G., Duan, L., Xin, H., & Li, S. (2019). Comparative metabolic profiling of *Vitis amurensis* and *Vitis vinifera* during cold acclimation. *Horticulture Research*, 6(1), Article 1. https://doi.org/10.1038/s41438-018-0083-5

Champely, S. (2020). *pwr: Basic Functions for Power Analysis* (Version 1.3-0) [R package]. https://github.com/heliosdrm/pwr

Chin, C.-S., Peluso, P., Sedlazeck, F. J., Nattestad, M., Concepcion, G. T., Clum, A., Dunn, C., O'Malley, R., Figueroa-Balderas, R., Morales-Cruz, A., Cramer, G. R., Delledonne, M., Luo, C., Ecker, J. R., Cantu, D., Rank, D. R., & Schatz, M. C. (2016). Phased diploid genome assembly with single-molecule real-time sequencing. *Nature Methods*, *13*(12), 1050–1054. https://doi.org/10.1038/nmeth.4035

Cooke, J. E. K., Eriksson, M. E., & Junttila, O. (2012). The dynamic nature of bud dormancy in trees: Environmental control and molecular mechanisms. *Plant, Cell & Environment*, 35(10), 1707–1728. https://doi.org/10.1111/j.1365-3040.2012.02552.x

Coombe, B. g. (1995). Growth Stages of the Grapevine: Adoption of a system for identifying grapevine growth stages. *Australian Journal of Grape and Wine Research*, 1(2), 104–110. https://doi.org/10.1111/j.1755-0238.1995.tb00086.x

Cutler, A. J., & Krochko, J. E. (1999). Formation and breakdown of ABA. Trends in *Plant Science*, 4(12), 472–478. https://doi.org/10.1016/s1360-1385(99)01497-1

Czemmel, S., Stracke, R., Weisshaar, B., Cordon, N., Harris, N. N., Walker, A. R., Robinson, S. P., & Bogs, J. (2009). The Grapevine R2R3-MYB Transcription Factor VvMYBF1 Regulates Flavonol Synthesis in Developing Grape Berries. *Plant Physiology*, 151(3), 1513–1530. https://doi.org/10.1104/pp.109.142059

Dal Santo, S., Vannozzi, A., Tornielli, G. B., Fasoli, M., Venturini, L., Pezzotti, M., & Zenoni, S. (2013). Genome-wide analysis of the expansin gene superfamily reveals grapevine-specific structural and functional characteristics. *PloS One*, 8(4), e62206. https://doi.org/10.1371/journal.pone.0062206

Dami et al. (2005). Midwest Grape Production Guide. https://extensionpubs.osu.edu/midwest-grape-production-guide/

Dami, I. (2014). 2014 Grape Winter Damage Survey. *Crop Science*, 11.

Dami, I. (2022). Spring Frost Injury of Grapevines and Protection Methods. *OSU Extension Publications*. https://extensionpubs.osu.edu/spring-frost-injury-of-grapevines-and-protection-methods/

Davenport, J., Lunden, J., & Winkler, T. (2012). Wine Grape Tissue Nutrient Concentrations in the Inland Pacific Northwest. Communications in Soil Science and Plant Analysis - *Commun Soil Sci Plant Anal*, 43, 21–27. https://doi.org/10.1080/00103624.2012.631395

de Mendiburu, F. (2023). agricolae: Statistical Procedures for Agricultural Research. R package version 1.3-7. https://CRAN.R-project.org/package=agricolae

De Rosa, V., Falchi, R., Moret, E., & Vizzotto, G. (2022). Insight into Carbohydrate Metabolism and Signaling in Grapevine Buds during Dormancy Progression. *Plants*, 11(8), 1027. https://doi.org/10.3390/plants11081027

De Rosa, V., Falchi, R., Peressotti, A., & Vizzotto, G. (2022). Expression patterns of DEMETER-like DNA demethylases homologs hint at potential involvement in grapevine dormancy release. *BIO Web of Conferences*, 44, 04001. https://doi.org/10.1051/bioconf/20224404001

De Rosa, V., Vizzotto, G., & Falchi, R. (2021). Cold Hardiness Dynamics and Spring Phenology: Climate-Driven Changes and New Molecular Insights into Grapevine Adaptive Potential. *Frontiers in Plant Science*, 12, 644528. https://doi.org/10.3389/fpls.2021.644528

Decreux, A., & Messiaen, J. (2005). Wall-associated kinase WAK1 interacts with cell wall pectins in a calcium-induced conformation. *Plant & Cell Physiology*, *46*(2), 268–278. https://doi.org/10.1093/pcp/pci026

Deluc, L., Bogs, J., Walker, A. R., Ferrier, T., Decendit, A., Merillon, J.-M., Robinson, S. P., & Barrieu, F. (2008). The Transcription Factor VvMYB5b Contributes to the Regulation of Anthocyanin and Proanthocyanidin Biosynthesis in Developing Grape Berries. *Plant Physiology*, 147(4), 2041–2053. https://doi.org/10.1104/pp.108.118919

Deschamps-Francoeur, G., Boivin, V., Abou Elela, S., & Scott, M. S. (2019). CoCo: RNA-seq read assignment correction for nested genes and multimapped reads. *Bioinformatics* (*Oxford, England*), 35(23), 5039–5047. https://doi.org/10.1093/bioinformatics/btz433

Diao, P., Chen, C., Zhang, Y., Meng, Q., Lv, W., & Ma, N. (2020). The role of NAC transcription factor in plant cold response. *Plant Signaling & Behavior*, 15(9), 1785668. https://doi.org/10.1080/15592324.2020.1785668

Dixon & Gschwend. (2023). (Under Review). Trichomes and Unique Gene Expression Confer Insect Herbivory Resistance in *Vitis labrusca* Grapevines. *BMC Plant Biology.*

Dobin, A., Davis, C. A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., Batut, P., Chaisson, M., & Gingeras, T. R. (2013). STAR: Ultrafast universal RNA-seq aligner. *Bioinformatics* (*Oxford, England*), 29(1), 15–21. https://doi.org/10.1093/bioinformatics/bts635

Dokoozlian, N. K. (1999). Chilling Temperature and Duration Interact on the Budbreak of `Perlette' Grapevine Cuttings. *HortScience*, *34*(6), 1–3. https://doi.org/10.21273/HORTSCI.34.6.1

Dokoozlian, N. K., Williams, L. E., & Neja, R. A. (1995). Chilling Exposure and Hydrogen Cyanamide Interact in Breaking Dormancy of Grape Buds. *HortScience*, *30*(6), 1244–1247. https://doi.org/10.21273/HORTSCI.30.6.1244

Druart, N., Johansson, A., Baba, K., Schrader, J., Sjödin, A., Bhalerao, R. R., Resman, L., Trygg, J., Moritz, T., & Bhalerao, R. P. (2007). Environmental and hormonal regulation of the activity–dormancy cycle in the cambial meristem involves stage-specific modulation of transcriptional and metabolic networks. *The Plant Journal*, 50(4), 557–573. https://doi.org/10.1111/j.1365-313X.2007.03077.x

Dry and Coombe. (2004). Revised version of "Grapevine growth stages—The modified E-L system" Viticulture 1 – Resources. 2nd edition. *Winetitles*.

Duan, D., Fischer, S., Merz, P., Bogs, J., Riemann, M., & Nick, P. (2016). An ancestral allele of grapevine transcription factor MYB14 promotes plant defence. *Journal of Experimental Botany*, 67(6), 1795–1804. https://doi.org/10.1093/jxb/erv569

Dubrovina, A. S., Kiselev, K. V., Khristenko, V. S., & Aleynova, O. A. (2015). VaCPK20, a calcium-dependent protein kinase gene of wild grapevine *Vitis amurensis* Rupr., mediates cold and drought stress tolerance. *Journal of Plant Physiology*, 185, 1–12. https://doi.org/10.1016/j.jplph.2015.05.020

Duchêne, E., Butterlin, G., Dumas, V., & Merdinoglu, D. (2012). Towards the adaptation of grapevine varieties to climate change: QTLs and candidate genes for developmental stages. *Theoretical and Applied Genetics*, 124(4), 623–635. https://doi.org/10.1007/s00122-011-1734-1

Emms, D. M., & Kelly, S. (2019). OrthoFinder: Phylogenetic orthology inference for comparative genomics. *Genome Biology*, *20*(1), 1–14. https://doi.org/10.1186/S13059-019-1832-Y/FIGURES/5

ETS Laboratories. (2022, August 1). Harvest Guide. https://issuu.com/etslabs/docs/harvestguide2022digital

Ewels, P., Magnusson, M., Lundin, S., & Käller, M. (2016). MultiQC: Summarize analysis results for multiple tools and samples in a single report. *Bioinformatics* (*Oxford, England*), 32(19), 3047–3048. https://doi.org/10.1093/bioinformatics/btw354

Famiani, F., Farinelli, D., Frioni, T., Palliotti, A., Battistelli, A., Moscatello, S., & Walker, R. P. (2016). Malate as substrate for catabolism and gluconeogenesis during ripening in the pericarp of different grape cultivars. *Biologia Plantarum*, 60(1), 155–162. https://doi.org/10.1007/s10535-015-0574-2

FAOSTAT. (2023). Food and Agriculture Organization of the United Nations (FAO). *FAOSTAT* [Internet]. Rome; 2023. Available from: Http://www.fao.org/faostat/en/#data/QC.

Fennell, A. (2004). Freezing tolerance and injury in grapevines. *Journal of Crop Improvement*, 10(1/2), 201–235. https://doi.org/10.1300/J411v10n01_09

Ferguson, J. C., Moyer, M., Mills, L., Hoogenboom, G., & Keller, M. (2014). Modeling Dormant Bud Cold Hardiness and Budbreak in Twenty-Three *Vitis* Genotypes Reveals Variation by Region of Origin. *American Journal of Enology and Viticulture*, 65, 59–71. https://doi.org/10.5344/ajev.2013.13098

Flores-Pérez, Ú., & Jarvis, P. (2013). Molecular chaperone involvement in chloroplast protein import. Biochimica et Biophysica Acta (BBA) - *Molecular Cell Research*, 1833(2), 332–340. https://doi.org/10.1016/j.bbamcr.2012.03.019

Fraire-Velázquez, S., Rodríguez-Guerra, R., & Sánchez-Calderón, L. (2011). Abiotic and biotic stress response crosstalk in plants. *Abiotic Stress Response in Plants—Physiological, Biochemical and Genetic Perspectives*, 3–26.

Gardner. (2016). Making [red] wine from fruit high in potassium. *Penn State Extension Wine & Grapes U.* https://psuwineandgrapes.wordpress.com/2016/09/23/making-red-wine-from-fruit-high-in-potassium/

Grant, T. N. L., Gargrave, J., & Dami, I. E. (2013). Morphological, Physiological, and Biochemical Changes in *Vitis* Genotypes in Response to Photoperiod Regimes. *American Journal of Enology and Viticulture*, 64(4), 466–475. https://doi.org/10.5344/ajev.2013.13060

Gu, L., Hanson, P. J., Post, W. M., Kaiser, D. P., Yang, B., Nemani, R., Pallardy, S. G., & Meyers, T. (2008). The 2007 Eastern US Spring Freeze: Increased Cold Damage in a Warming World? *BioScience*, 58(3), 253–262. https://doi.org/10.1641/B580311

Gubler, F., Hughes, T., Waterhouse, P., & Jacobsen, J. (2008). Regulation of dormancy in barley by blue light and after-ripening: Effects on abscisic acid and gibberellin metabolism. *Plant Physiology*, 147(2), 886–896. https://doi.org/10.1104/pp.107.115469

Guo, H.-S., Zhang, Y.-M., Sun, X.-Q., Li, M.-M., Hang, Y.-Y., & Xue, J.-Y. (2016). Evolution of the KCS gene family in plants: The history of gene duplication, sub/neofunctionalization and redundancy. *Molecular Genetics and Genomics*, 291(2), 739–752. https://doi.org/10.1007/s00438-015-1142-3

Guy, C. L. (1990). Cold Acclimation and Freezing Stress Tolerance: Role of Protein Metabolism.

Hamman, R. A., Dami, I., Walsh, T. M., & Stushnoff, C. (1996). Seasonal Carbohydrate Changes and Cold Hardiness of Chardonnay and Riesling Grapevines. *American Journal of Enology and Viticulture*, 47, 31–36. https://doi.org/10.5344/ajev.1996.47.1.31

Hanson, D., & Horneck, D. A. (1997). Determination of Potassium and Sodium by Flame Emission Spectrophotometry. https://api.semanticscholar.org/CorpusID:99517948

He, H., Liang, G., Lu, S., Wang, P., Liu, T., Ma, Z., Zuo, C., Sun, X., Chen, B., & Mao, J. (2019). Genome-Wide Identification and Expression Analysis of GA2ox, GA3ox, and GA20ox Are Related to Gibberellin Oxidase Genes in Grape (*Vitis vinifera* L.). *Genes*, *10*(9), 680. https://doi.org/10.3390/genes10090680

Hébert-Haché, A., Inglis, D., Kemp, B., & Willwerth, J. J. (2021). Clone and Rootstock Interactions Influence the Cold Hardiness of *Vitis vinifera* cvs. Riesling and Sauvignon blanc. *American Journal of Enology and Viticulture*, 72(2), 126–136. https://doi.org/10.5344/ajev.2020.20025

Herger, A., Dünser, K., Kleine-Vehn, J., & Ringli, C. (2019). Leucine-Rich Repeat Extensin Proteins and Their Role in Cell Wall Sensing. *Current Biology: CB*, 29(17), R851–R858. https://doi.org/10.1016/j.cub.2019.07.039

Höll, J., Vannozzi, A., Czemmel, S., D'Onofrio, C., Walker, A. R., Rausch, T., Lucchin, M., Boss, P. K., Dry, I. B., & Bogs, J. (2013). The R2R3-MYB Transcription Factors MYB14 and MYB15 Regulate Stilbene Biosynthesis in *Vitis vinifera*. The *Plant Cell*, 25(10), 4135–4149. https://doi.org/10.1105/tpc.113.117127

Huang, T., Yang, J., Yu, D., Han, X., & Wang, X. (2021). Bioinformatics analysis of WRKY transcription factors in grape and their potential roles prediction in sugar and abscisic acid signaling pathway. *Journal of Plant Biochemistry and Biotechnology*, 30(1), 67–80. https://doi.org/10.1007/s13562-020-00571-y

Jiang, H.-Y., Li, W., He, B.-J., Gao, Y.-H., & Lu, J.-X. (2014). Sucrose metabolism in grape (*Vitis vinifera* L.) branches under low temperature during overwintering covered with soil. *Plant Growth Regulation*, 72(3), 229–238. https://doi.org/10.1007/s10725-013-9854-z

Jiang, W., & Yu, D. (2009). Arabidopsis WRKY2 transcription factor mediates seed germination and postgermination arrest of development by abscisic acid. *BMC Plant Biology*, 9. https://doi.org/10.1186/1471-2229-9-96

Jones, K. S., Paroschy, J., Mckersie, B. D., & Bowley, S. R. (1999). Carbohydrate Composition and Freezing Tolerance of Canes and Buds in *Vitis vinifera*. *Journal of Plant Physiology*, 155(1), 101–106. https://doi.org/10.1016/S0176-1617(99)80146-1

Kanehisa, M., & Goto, S. (2000). KEGG: Kyoto encyclopedia of genes and genomes. *Nucleic Acids Research*, 28(1), 27–30. https://doi.org/10.1093/nar/28.1.27

Karimi, R. (2017). Potassium-induced freezing tolerance is associated with endogenous abscisic acid, polyamines and soluble sugars changes in grapevine. *Scientia Horticulturae*, 215, 184–194. https://doi.org/10.1016/j.scienta.2016.12.018

Karimi, R., Ershadi, A., & Ashari, M. (2014). Effects of Late- Season Nitrogen and Potassium Spray on Dormant Buds Cold Tolerance of 'Bidaneh Sefid' Grapevine. *Iranian Journal of Horticultural Science and Technology*, 15, 419–434.

Kartschall, T., Wodinski, M., von Bloh, W., Oesterle, H., Rachimow, C., & Hoppmann, D. (2015). Changes in phenology and frost risks of *Vitis vinifera* (cv Riesling). *Meteorologische Zeitschrift*, 189–200. https://doi.org/10.1127/metz/2015/0534

Kassambara A (2023). *rstatix: Pipe-Friendly Framework for Basic Statistical Tests*. R package version 0.7.2, https://rpkgs.datanovia.com/rstatix/

Keller. (2015). The Science of Grapevines—2nd Edition. https://shop.elsevier.com/books/the-science-of-grapevines/keller/978-0-12-419987-3

Koch, K. (2004). Sucrose metabolism: Regulatory mechanisms and pivotal roles in sugar sensing and plant development. *Current Opinion in Plant Biology*, 7(3), 235–246. https://doi.org/10.1016/j.pbi.2004.03.014

Kolattukudy, P. E. (1980). Cutin, Suberin, and Waxes. In P. K. Stumpf (Ed.), *Lipids: Structure and Function* (pp. 571–645). Academic Press. https://doi.org/10.1016/B978-0-12-675404-9.50024-2

Kovaleski, A. P., & Londo, J. P. (2019). Tempo of gene regulation in wild and cultivated *Vitis* species shows coordination between cold deacclimation and budbreak. *Plant Science*, 287, 110178. https://doi.org/10.1016/j.plantsci.2019.110178

Kovaleski, A. P., North, M. G., Martinson, T. E., & Londo, J. P. (2022). Development of a new cold hardiness prediction model for grapevine using phased integration of acclimation and deacclimation responses (p. 2022.09.09.507298). *bioRxiv*. https://doi.org/10.1101/2022.09.09.507298

Kovaleski, A. P., Reisch, B. I., & Londo, J. P. (2018). Deacclimation kinetics as a quantitative phenotype for delineating the dormancy transition and thermal efficiency for budbreak in *Vitis* species. *AoB Plants*. https://doi.org/10.1093/aobpla/ply066

Kovi, M. R., Ergon, Å., & Rognli, O. A. (2016). Freezing tolerance revisited-Effects of variable temperatures on gene regulation in temperate grasses and legumes. *Current Opinion in Plant Biology*, 33, 140–146. https://doi.org/10.1016/j.pbi.2016.07.006

Krishna, P., Sacco, M., Cherutti, J. F., & Hill, S. (1995). Cold-Induced Accumulation of hsp90 Transcripts in *Brassica napus*. *Plant Physiology*, 107(3), 915–923. https://doi.org/10.1104/pp.107.3.915

Kühn et al. (2009). Photoperiod modifies the diurnal expression profile of VvPHYA and VvPHYB transcripts in field-grown grapevine leaves. https://pubmed.ncbi.nlm.nih.gov/19232775/

Kumar, S. (2018). Epigenetic Memory of Stress Responses in Plants. *Journal of J Phytochemistry & Biochemistry*, 2, 1–2.

La Verde, V., Dominici, P., & Astegno, A. (2018). Towards Understanding Plant Calcium Signaling through Calmodulin-Like Proteins: A Biochemical and Structural Perspective. *International Journal of Molecular Sciences*, *19*(5), 1331. https://doi.org/10.3390/ijms19051331

Lakso, A. N., & Kliewer, W. M. (1975). The Influence of Temperature on Malic Acid Metabolism in Grape Berries: I. Enzyme Responses. *Plant Physiology*, 56(3), 370–372.

Lamichhane, J. R. (2021). Rising risks of late-spring frosts in a changing climate. *Nature Climate Change*, 11(7), Article 7. https://doi.org/10.1038/s41558-021-01090-x

Lang, G. A. (1987). Endo-, Para-and Ecodormancy: Physiological Terminology and Classification for Dormancy Research. *Hortic. Sci.*, 22, 271–277.

Lenz, A., Hoch, G., Korner, C., & Vitasse, Y. (2016). Convergence of leaf-out towards minimum risk of freezing damage in temperate trees. *Functional Ecology*, 30(9), 1480–1490. https://doi.org/10.1111/1365-2435.12623

Lewandowska, M., Keyl, A., & Feussner, I. (2020). Wax biosynthesis in response to danger: Its regulation upon abiotic and biotic stress. *New Phytologist*, *227*(3), 698–713. https://doi.org/10.1111/nph.16571

Li, B., & Gschwend, A. R. (2023). *Vitis labrusca* genome assembly reveals diversification between wild and cultivated grapevine genomes. *Frontiers in Plant Science*, 14. https://www.frontiersin.org/journals/plant-science/articles/10.3389/fpls.2023.1234130

Li, B., & Wang, W. (2021). Salicylic acid induces tolerance of *Vitisriparia×V.labrusca* to chilling stress by altered photosynthetic, antioxidant mechanisms and expression of cold stress responsive genes. *Plant Signaling & Behavior*, 16(11), 1973711. https://doi.org/10.1080/15592324.2021.1973711

Li, H., Xu, Y., Xiao, Y., Zhu, Z., Xie, X., Zhao, H., & Wang, Y. (2010). Expression and functional analysis of two genes encoding transcription factors, VpWRKY1 and VpWRKY2, isolated from Chinese wild *Vitis pseudoreticulata*. PLANTA, 232(6), 1325–1337. https://doi.org/10.1007/s00425-010-1258-y

Licausi, F., Ohme-Takagi, M., & Perata, P. (2013). APETALA2/Ethylene Responsive Factor (AP2/ERF) transcription factors: Mediators of stress responses and developmental programs. *New Phytologist*, 199(3), 639–649. https://doi.org/10.1111/nph.12291

Liu, B., Sun, Y., Li, X., Guo, D., Zhao, L., Ma, C., Wang, L., & Wang, S. (2023). β-ketoacyl-CoA synthase improves the drought tolerance of root restricted grown grapevines by regulating the cuticular wax biosynthesis. *Scientia Horticulturae*, 307, 111494. https://doi.org/10.1016/j.scienta.2022.111494

Liu et al. (2013). Effects of potassium on yield, photosynthate distribution, enzymes activity and ABA content in storage roots of sweet potato (*Ipomoea batatas* Lam.). Aust. J. Crop Sci. 7, 735-743.

Liu et al. (2016). Bioinformatic Identification and Analysis of Extensins in the Plant Kingdom | *PloS One*. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0150177

Londo, J. P., & Johnson, L. M. (2014). Variation in the chilling requirement and budburst rate of wild *Vitis* species. *Environmental and Experimental Botany*, 106, 138–147. https://doi.org/10.1016/j.envexpbot.2013.12.012

Londo, J. P., & Kovaleski, A. P. (2017). Characterization of Wild North American Grapevine Cold Hardiness Using Differential Thermal Analysis. *American Journal of Enology and Viticulture*, 68(2), 203–212. https://doi.org/10.5344/ajev.2016.16090

Londo, J. P., Kovaleski, A. P., & Lillis, J. A. (2018). Divergence in the transcriptional landscape between low temperature and freeze shock in cultivated grapevine (*Vitis vinifera*). *Horticulture Research*, 5.

Loseke, B., Read, P., & Blankenship, E. (2015). Preventing spring freeze injury on grapevines using multiple applications of Amigo Oil and naphthaleneacetic acid. *Scientia Horticulturae*, 193, 294–300. https://doi.org/10.1016/J.SCIENTA.2015.07.025

Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology*, 15(12), 550. https://doi.org/10.1186/s13059-014-0550-8

Mahajan, S., & Tuteja, N. (2005). Cold, salinity and drought stresses: An overview. *Archives of Biochemistry and Biophysics*, 444(2), 139–158. https://doi.org/10.1016/j.abb.2005.10.018

Meitha, K., Agudelo-Romero, P., Signorelli, S., Gibbs, D., Considine, J., Foyer, C., & Considine, M. (2018). Developmental control of hypoxia during bud burst in grapevine. *Plant, Cell & Environment*, *41 5*, 1154–1170. https://doi.org/10.1111/pce.13141

Miller, D. R. O., & Horneck, D. D. (2013). *Soil, Plant and Water Reference Methods for the Western Region*.

Min, Z., Zhao, X., Li, R., Yang, B., Liu, M., & Fang, Y. (2017). Comparative transcriptome analysis provides insight into differentially expressed genes related to bud dormancy in grapevine (*Vitis vinifera*). *Scientia Horticulturae*, 225, 213–220. https://doi.org/10.1016/j.scienta.2017.06.033

Mizoi, J., Shinozaki, K., & Yamaguchi-Shinozaki, K. (2012). AP2/ERF family transcription factors in plant abiotic stress responses. *Biochimica et Biophysica Acta (BBA) - Gene Regulatory Mechanisms*, 1819(2), 86–96. https://doi.org/10.1016/j.bbagrm.2011.08.004

Møller, I. M., Jensen, P. E., & Hansson, A. (2007). Oxidative modifications to cellular components in plants. *Annual Review of Plant Biology*, *58*, 459–481. https://doi.org/10.1146/annurev.arplant.58.032806.103946

Morrison, J. C. (1991). Bud Development in *Vitis vinifera* L. Botanical Gazette, 152(3), 304–315. https://doi.org/10.1086/337894

Morsy, M. R., Jouve, L., Hausman, J.-F., Hoffmann, L., & Stewart, J. M. (2007). Alteration of oxidative and carbohydrate metabolism under abiotic stress in two rice (*Oryza sativa* L.) genotypes contrasting in chilling tolerance. *Journal of Plant Physiology*, 164(2), 157–167. https://doi.org/10.1016/j.jplph.2005.12.004

Mosedale, J. R., Wilson, R. J., & Maclean, I. M. D. (2015). Climate Change and Crop Exposure to Adverse Weather: Changes to Frost Risk and Grapevine Flowering Conditions. *PloS One*, 10(10), e0141218. https://doi.org/10.1371/journal.pone.0141218

Mpelasoka, B. S., Schachtman, D. P., Treeby, M. T., & Thomas, M. R. (2003). A review of potassium nutrition in grapevines with special emphasis on berry accumulation.

Australian Journal of Grape and Wine Research, *9*(3), 154–168. https://doi.org/10.1111/j.1755-0238.2003.tb00265.x

Nambara, E., Okamoto, M., Tatematsu, K., Yano, R., Seo, M., & Kamiya, Y. (2010). Abscisic acid and the control of seed dormancy and germination. SEED *Science Research*, 20(2), 55–67.

NCBI Resource Coordinators. (2016). Database resources of the National Center for Biotechnology Information. *Nucleic Acids Research*, 44(D1), D7-19. https://doi.org/10.1093/nar/gkv1290

Nick. (2013). Microtubules, signalling and abiotic stress. *Plant J*. 2013:75(2). https://onlinelibrary.wiley.com/doi/10.1111/tpj.12102

North, M., Workmaster, B., & Atucha, A. (2021). Cold Hardiness of Cold Climate Interspecific Hybrid Grapevines Grown in a Cold Climate Region. *American Journal of Enology and Viticulture*, 72, 318–327. https://doi.org/10.5344/ajev.2021.21001

Okamoto, M., Kuwahara, A., Seo, M., Kushiro, T., Asami, T., Hirai, N., Kamiya, Y., Koshiba, T., & Nambara, E. (2006). CYP707A1 and CYP707A2, which encode abscisic acid 8'-hydroxylases, are indispensable for proper control of seed dormancy and germination in *Arabidopsis*. *Plant Physiology*, 141(1), 97–107. https://doi.org/10.1104/pp.106.079475

Orrantia-Araujo, M. A., Martínez-Téllez, M. Á., Rivera-Domínguez, M., Hernández-Oñate, M. Á., & Vargas-Arispuro, I. (2021). Changes in the Endogenous Content and Gene Expression of Salicylic Acid Correlate with Grapevine Bud Dormancy Release. *Journal of Plant Growth Regulation*, 40(1), 254–262. https://doi.org/10.1007/s00344-020-10100-9

Pagter, M., & Arora, R. (2013). Winter survival and deacclimation of perennials under warming climate: Physiological perspectives. *Physiologia Plantarum*, 147(1), 75–87. https://doi.org/10.1111/j.1399-3054.2012.01650.x

Pearce, R. S. (2001). Plant Freezing and Damage. *Annals of Botany*, 87(4), 417–424. https://doi.org/10.1006/anbo.2000.1352

Pérez, F., & Rubio, S. (2015). Prolonged Exposure of Grapevine Buds to Low Temperatures Increases Dormancy, Cold Hardiness, and the Expression of Vvα-AMYs Genes. https://doi.org/10.1007/978-3-319-14451-1_10

Pierquet, P., & Stushnoff, C. (1980). Relationship of Low Temperature Exotherms to Cold Injury in *Vitis riparia* Michx. *American Journal of Enology and Viticulture*, 31(1), 1–6. https://doi.org/10.5344/ajev.1980.31.1.1

Polisensky, D. H., & Braam, J. (1996). Cold-shock regulation of the Arabidopsis TCH genes and the effects of modulating intracellular calcium levels. *Plant Physiology*, *111*(4), 1271–1279. https://doi.org/10.1104/pp.111.4.1271

Poni, S., Sabbatini, P., & Palliotti, A. (2022). Facing Spring Frost Damage in Grapevine: Recent Developments and the Role of Delayed Winter Pruning – A Review. *American Journal of Enology and Viticulture*. https://doi.org/10.5344/ajev.2022.22011

Poschet, G., Hannich, B., Raab, S., Jungkunz, I., Klemens, P. A. W., Krueger, S., Wic, S., Neuhaus, H. E., & Büttner, M. (2011). A Novel *Arabidopsis* Vacuolar Glucose Exporter Is Involved in Cellular Sugar Homeostasis and Affects the Composition of Seed Storage Compounds. *Plant Physiology*, 157(4), 1664–1676. https://doi.org/10.1104/pp.111.186825

Puranik, S., Sahu, P. P., Srivastava, P. S., & Prasad, M. (2012). NAC proteins: Regulation and role in stress tolerance. *Trends in Plant Science*, 17(6), 369–381. https://doi.org/10.1016/j.tplants.2012.02.004

Quamme, H. A. (1991). Application of Thermal Analysis to Breeding Fruit Crops for Increased Cold Hardiness. *HortScience*, 26(5), 513–517. https://doi.org/10.21273/HORTSCI.26.5.513

R Core Team. (2023a). *R: A language and environment for statistical computing* (Version 4.3.1) [Software]. R Foundation for Statistical Computing. https://www.R-project.org/

R Core Team (2023b). *stats: The R Stats Package*. R package version 4.3.1. https://cran.r-project.org/package=stats

Rahman, M. A., Moody, M. A., & Nassuth, A. (2014). Grape contains 4 ICE genes whose expression includes alternative polyadenylation, leading to transcripts encoding at least 7 different ICE proteins. *Environmental and Experimental Botany*, 106, 70–78. https://doi.org/10.1016/j.envexpbot.2014.01.003

Raza, A., Charagh, S., Najafi-Kakavand, S., Abbas, S., Shoaib, Y., Anwar, S., Sharifi, S., Lu, G., & Siddique, K. H. M. (2023). Role of phytohormones in regulating cold stress tolerance: Physiological and molecular approaches for developing cold-smart crop plants. *Plant Stress*, 8, 100152. https://doi.org/10.1016/j.stress.2023.100152

Ren, C., Fan, P., Li, S., & Liang, Z. (2023). Advances in understanding cold tolerance in grapevine. *Plant Physiology*, 192(3), 1733–1746. https://doi.org/10.1093/plphys/kiad092

Ren, C., Kuang, Y., Lin, Y., Guo, Y., Li, H., Fan, P., Li, S., & Liang, Z. (2022). Overexpression of grape ABA receptor gene VaPYL4 enhances tolerance to multiple abiotic stresses in *Arabidopsis*. BMC *Plant Biology*, 22(1). https://doi.org/10.1186/s12870-022-03663-0

Rogiers, S. Y., Coetzee, Z. A., Walker, R. R., Deloire, A., & Tyerman, S. D. (2017). Potassium in the Grape (*Vitis vinifera* L.) Berry: Transport and Function.

Frontiers in Plant Science, 8. https://www.frontiersin.org/articles/10.3389/fpls.2017.01629

Rubio, S., Dantas, D., Bressan-Smith, R., & Pérez, F. (2016). Relationship Between Endodormancy and Cold Hardiness in Grapevine Buds. *Journal of Plant Growth Regulation*, 35, 266–275. https://doi.org/10.1007/s00344-015-9531-8

Rubio, S., Noriega, X., & Perez, F. (2019). Abscisic acid (ABA) and low temperatures synergistically increase the expression of CBF/DREB1 transcription factors and cold-hardiness in grapevine dormant buds. *Annals of Botany*, 123(4), 681–689. https://doi.org/10.1093/aob/mcy201

Rubio, S., & Pérez, F. J. (2019). ABA and its signaling pathway are involved in the cold acclimation and deacclimation of grapevine buds. *Scientia Horticulturae*, 256, 108565. https://doi.org/10.1016/j.scienta.2019.108565

Saibo et al. (2009). Transcription factors and regulation of photosynthetic and related metabolism under environmental stresses | *Annals of Botany* | *Oxford Academic*. https://academic.oup.com/aob/article/103/4/609/164513

Salzman et al. (1996). Programmed accumulation of LEA-like proteins during desiccation and cold acclimation of overwintering grape buds—*Plant, Cell & Environment—Wiley Online Library*. https://onlinelibrary.wiley.com/doi/10.1111/j.1365-3040.1996.tb00406.x

Santos, J., Fraga, H., Malheiro, A., Moutinho-Pereira, J., Dinis, L., Correia, C., Moriondo, M., Leolini, L., Dibari, C., Costafreda-Aumedes, S., Kartschall, T., Menz, C., Molitor, D., Junk, J., Beyer, M., & Schultz, H. (2020). A Review of the Potential Climate Change Impacts and Adaptation Options for European Viticulture. *Applied Sciences-Basel*, 10(9). https://doi.org/10.3390/app10093092

Sarikhani, H., Haghi, H., Ershadi, A., Esna-Ashari, M., & Pouya, M. (2014). Foliar application of potassium sulphate enhances the cold-hardiness of grapevine (*Vitis vinifera* L.). *The Journal of Horticultural Science and Biotechnology*, 89(2), 141–146. https://doi.org/10.1080/14620316.2014.11513060

Scurlock, D. (2014). Ohio Grape-Wine Electronic Newsletter. 16.

Sengupta, S., Mukherjee, S., Basak, P., & Majumder, A. L. (2015). Significance of galactinol and raffinose family oligosaccharide synthesis in plants. *Frontiers in Plant Science*, 6. https://www.frontiersin.org/articles/10.3389/fpls.2015.00656

Singh, R. K., Afonso, J., Nogueira, M., Oliveira, A. A., Cosme, F., & Falco, V. (2020). Silicates of Potassium and Aluminium (Kaolin); Comparative Foliar Mitigation Treatments and Biochemical Insight on Grape Berry Quality in *Vitis vinifera* L. (cv. Touriga National and Touriga Franca). *Biology*, 9(3), Article 3. https://doi.org/10.3390/biology9030058

Somers, T. C. (1977). A connection between potassium levels in the harvest and relative quality in Australian red wines. *Aust. Wine Brew. Spirit Rev*. 24, 32–34.

Somers, T.C. (1975). In search of quality for red wines. *Food Technology in Australia* 27, 49–56.

Steponkus, P. L. (1984). Role of the Plasma Membrane in Freezing Injury and Cold Acclimation. *Annual Review of Plant Physiology*, 35(1), 543–584. https://doi.org/10.1146/annurev.pp.35.060184.002551

Sturm, A. (1999). Invertases. Primary Structures, Functions, and Roles in Plant Development and Sucrose Partitioning. *Plant Physiology*, 121(1), 1–8. https://doi.org/10.1104/pp.121.1.1

Sun, X., Tomas Matus, J., Wong, D. C. J., Wang, Z., Chai, F., Zhang, L., Fang, T., Zhao, L., Wang, Y., Han, Y., Wang, Q., Li, S., Liang, Z., & Xin, H. (2018). The GARP/MYB-related grape transcription factor AQUILO improves cold tolerance and promotes the accumulation of raffinose family oligosaccharides. *Journal of Experimental Botany*, 69(7), 1749–1764. https://doi.org/10.1093/jxb/ery020

Sun, X., Zhang, L., Wong, D. C. J., Wang, Y., Zhu, Z., Xu, G., Wang, Q., Li, S., Liang, Z., & Xin, H. (2019). The ethylene response factor VaERF092 from Amur grape regulates the transcription factor VaWRKY33, improving cold tolerance. *Plant Journal*, 99(5), 988–1002. https://doi.org/10.1111/tpj.14378

Sun, X., Zhao, T., Gan, S., Ren, X., Fang, L., Karungo, S. K., Wang, Y., Chen, L., Li, S., & Xin, H. (2016). Ethylene positively regulates cold tolerance in grapevine by modulating the expression of ETHYLENE RESPONSE FACTOR 057. *Scientific Reports*, 6(1), 24066. https://doi.org/10.1038/srep24066

Sun, Z., Li, S., Chen, W., Zhang, J., Zhang, L., Sun, W., & Wang, Z. (2021). Plant Dehydrins: Expression, Regulatory Networks, and Protective Roles in Plants Challenged by Abiotic Stress. *International Journal of Molecular Sciences*, 22(23), 12619. https://doi.org/10.3390/ijms222312619

Therneau, T., Hart, S., & Kocher, J.P. (2023). *Calculating sample size estimates for RNA Seq studies*. R package version 1.42.0.

Thomashow, M. F. (1999). PLANT COLD ACCLIMATION: Freezing Tolerance Genes and Regulatory Mechanisms. *Annual Review of Plant Physiology and Plant Molecular Biology*, 50, 571–599. https://doi.org/10.1146/annurev.arplant.50.1.571

Thomashow, M. F. (2001). So what's new in the field of plant cold acclimation? Lots! *Plant Physiology*, 125(1), 89–93. https://doi.org/10.1104/pp.125.1.89

Tronchet, M., Balagué, C., Kroj, T., Jouanin, L., & Roby, D. (2009). Cinnamyl alcohol dehydrogenases-C and D, key enzymes in lignin biosynthesis, play an essential role in disease resistance in *Arabidopsis*. *Molecular Plant Pathology*, *11*(1), 83–92. https://doi.org/10.1111/j.1364-3703.2009.00578.x

USDA. (2022). NonCitrus Fruits and Nuts 2021 Summary (p. 88). https://downloads.usda.library.cornell.edu/usda-esmis/files/zs25x846c/4q77gv96p/8336j672v/ncit0522.txt

van Leeuwen, C., & Darriet, P. (2016). The Impact of Climate Change on Viticulture and Wine Quality. *Journal of Wine Economics*, 11, 150–167. https://doi.org/10.1017/jwe.2015.21

Vergara, R., Noriega, X., Aravena, K., Prieto, H., & Pérez, F. (2017). ABA Represses the Expression of Cell Cycle Genes and May Modulate the Development of Endodormancy in Grapevine Buds. *Frontiers in Plant Science*, 8, null. https://doi.org/10.3389/fpls.2017.00812

Villette, J., Cuéllar, T., Verdeil, J.-L., Delrot, S., & Gaillard, I. (2020). Grapevine Potassium Nutrition and Fruit Quality in the Context of Climate Change. *Frontiers in Plant Science*, 11. https://www.frontiersin.org/articles/10.3389/fpls.2020.00123

Vitasse, Y., Lenz, A., & Körner, C. (2014). The interaction between freezing tolerance and phenology in temperate deciduous trees. *Frontiers in Plant Science*, 5. https://www.frontiersin.org/articles/10.3389/fpls.2014.00541

Wan, H., Wu, L., Yang, Y., Zhou, G., & Ruan, Y.-L. (2018). Evolution of Sucrose Metabolism: The Dichotomy of Invertases and Beyond. *Trends in Plant Science*, 23(2), 163–177. https://doi.org/10.1016/j.tplants.2017.11.001

Wang, H., Blakeslee, J., Jones, M., Chapin, L., & Dami, I. (2020). Exogenous abscisic acid enhances physiological, metabolic, and transcriptional cold acclimation responses in greenhouse-grown grapevines. *Plant Science*, 293. https://doi.org/10.1016/j.plantsci.2020.110437

Wang, J., Ye, H., Zhou, H., Chen, P., Liu, H., Xi, R., Wang, G., Hou, N., & Zhao, P. (2022). Genome-wide association analysis of 101 accessions dissects the genetic basis of shell thickness for genetic improvement in Persian walnut (*Juglans regia* L.). *BMC Plant Biology*, *22*, 436. https://doi.org/10.1186/s12870-022-03824-1

Wang, L., Zhang, X., Qu, Z., Guo, W., van Nocker, S., & Zhang, C. (2022). Grapevine VviERF105 promotes tolerance to abiotic stress and is degraded by the E3 ubiquitin ligase VviPUB19. *Environmental and Experimental Botany*, 201, 105001. https://doi.org/10.1016/j.envexpbot.2022.105001

Wang, L., Zhu, W., Fang, L., Sun, X., Su, L., Liang, Z., Wang, N., Londo, J. P., Li, S., & Xin, H. (2014). Genome-wide identification of WRKY family genes and their response to cold stress in *Vitis vinifera*. *BMC Plant Biology*, 14. https://doi.org/10.1186/1471-2229-14-103

Wang, N., Zheng, Y., Xin, H., Fang, L., & Li, S. (2013). Comprehensive analysis of NAC domain transcription factor gene family in *Vitis vinifera*. *Plant Cell Reports*, 32(1), 61–75. https://doi.org/10.1007/s00299-012-1340-y

Wang, Q., Du, X., Zhou, Y., Xie, L., Bie, S., Tu, L., Zhang, N., Yang, X., Xiao, S., & Zhang, X. (2020). The β-ketoacyl-CoA synthase KCS13 regulates the cold

response in cotton by modulating lipid and oxylipin biosynthesis. *Journal of Experimental Botany*, *71*(18), 5615–5630. https://doi.org/10.1093/jxb/eraa254

Wang, Y., Xin, H., Fan, P., Zhang, J., Liu, Y., Dong, Y., Wang, Z., Yang, Y., Zhang, Q., Ming, R., Zhong, G.-Y., Li, S., & Liang, Z. (2021). The genome of Shanputao (*Vitis amurensis*) provides a new insight into cold tolerance of grapevine. *The Plant Journal: For Cell and Molecular Biology*, 105(6), 1495–1506. https://doi.org/10.1111/tpj.15127

Wang, Z., Wong, D. C. J., Wang, Y., Xu, G., Ren, C., Liu, Y., Kuang, Y., Fan, P., Li, S., Xin, H., & Liang, Z. (2021). GRAS-domain transcription factor PAT1 regulates jasmonic acid biosynthesis in grape cold stress response. *Plant Physiology*, 186(3), 1660–1678. https://doi.org/10.1093/plphys/kiab142

Wickham H (2016). *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York. ISBN 978-3-319-24277-4

Wilson. (2001, August). Frost Management in Cool Climate Vineyards.

Wisniewski, M., Nassuth, A., & Arora, R. (2018). Cold Hardiness in Trees: A Mini-Review. *Frontiers in Plant Science*, 9. https://www.frontiersin.org/article/10.3389/fpls.2018.01394

Wolf, T. (2008). Wine Grape Production Guide for Eastern North America (NRAES 145). *Natural Resource, Agriculture, and Engineering Service* (*NRAES*). https://ecommons.cornell.edu/handle/1813/67189

Wolf, T. K., & Cook, M. K. (1994). Cold Hardiness of Dormant Buds of Grape Cultivars: Comparison of Thermal Analysis and Field Survival. *HortScience*, 29(12), 1453–1455. https://doi.org/10.21273/HORTSCI.29.12.1453

Wu, J., Zhang, Y., Yin, L., Qu, J., & Lu, J. (2014). Linkage of cold acclimation and disease resistance through plant–pathogen interaction pathway in *Vitis amurensis* grapevine. *Functional & Integrative Genomics*, 14(4), 741–755. https://doi.org/10.1007/s10142-014-0392-1

Wu, N., Li, J., Zheng, Q., Wu, J., & Xu, W. (2023). Characterization of the core region of grape VvHOS1 promoter activity and its upstream regulatory proteins. *Environmental and Experimental Botany*, 207. https://doi.org/10.1016/j.envexpbot.2022.105199

Wu, T., Hu, E., Xu, S., Chen, M., Guo, P., Dai, Z., Feng, T., Zhou, L., Tang, W., Zhan, L., Fu, X., Liu, S., Bo, X., & Yu, G. (2021). clusterProfiler 4.0: A universal enrichment tool for interpreting omics data. *Innovation* (*Cambridge (Mass.)*), 2(3), 100141. https://doi.org/10.1016/j.xinn.2021.100141

Wu, Y., Li, X., Zhang, J., Zhao, H., Tan, S., Xu, W., Pan, J., Yang, F., & Pi, E. (2022). ERF subfamily transcription factors and their function in plant responses to abiotic stresses. *Frontiers in Plant Science*, *13*, 1042084. https://doi.org/10.3389/fpls.2022.1042084

Xiao, H., Siddiqua, M., Braybrook, S., & Nassuth, A. (2006). Three grape CBF/DREB1 genes respond to low temperature, drought and abscisic acid. *Plant Cell and Environment*, 29(7), 1410–1421. https://doi.org/10.1111/j.1365-3040.2006.01524.x

Xiao, H., Tattersall, E., Siddiqua, M., Cramer, G., & Nassuth, A. (2008). CBF4 is a unique member of the CBF transcription factor family of *Vitis vinifera* and *Vitis riparia*. *Plant Cell and Environment*, 31(1), 1–10. https://doi.org/10.1111/j.1365-3040.2007.01741.x

Xin, H., Zhu, W., Wang, L., Xiang, Y., Fang, L., Li, J., Sun, X., Wang, N., Londo, J. P., & Li, S. (2013). Genome Wide Transcriptional Profile Analysis of *Vitis amurensis* and *Vitis vinifera* in Response to Cold Stress. *PLOS ONE*, *8*(3). https://doi.org/10.1371/journal.pone.0058740

Xin, Z., & Browse, J. (2000). Cold comfort farm: The acclimation of plants to freezing temperatures. *Plant, Cell & Environment*, 23(9), 893–902. https://doi.org/10.1046/j.1365-3040.2000.00611.x

Xu, W., Li, R., Zhang, N., Ma, F., Jiao, Y., & Wang, Z. (2014). Transcriptome profiling of *Vitis amurensis*, an extremely cold-tolerant Chinese wild *Vitis species*, reveals candidate genes and events that potentially connected to cold stress. *Plant Molecular Biology*, *86*(4–5), 527–541. https://doi.org/10.1007/s11103-014-0245-2

Xu, W., Zhang, N., Jiao, Y., Li, R., Xiao, D., & Wang, Z. (2014). The grapevine basic helix-loop-helix (bHLH) transcription factor positively modulates CBF-pathway and confers tolerance to cold-stress in *Arabidopsis*. *Molecular Biology Reports*, 41(8), 5329–5342. https://doi.org/10.1007/s11033-014-3404-2

Xu, X., Hu, Q., Yang, W., & Jin, Y. (2017). The roles of cell wall invertase inhibitor in regulating chilling tolerance in tomato. *BMC Plant Biology*, 17(1), 195. https://doi.org/10.1186/s12870-017-1145-9

Yang, Y., He, M., Zhu, Z., Li, S., Xu, Y., Zhang, C., Singer, S. D., & Wang, Y. (2012). Identification of the dehydrin gene family from grapevine species and analysis of their responsiveness to various forms of abiotic and biotic stress. *BMC Plant Biology*, 12, 140. https://doi.org/10.1186/1471-2229-12-140

Yang, Z., Yang, X., Dong, S., Ge, Y., Zhang, X., Zhao, X., & Han, N. (2020). Overexpression of β-Ketoacyl-CoA Synthase from *Vitis vinifera* L. Improves Salt Tolerance in *Arabidopsis thaliana*. *Frontiers in Plant Science*, 11, 564385. https://doi.org/10.3389/fpls.2020.564385

Yao, W., Wang, L., Wang, J., Ma, F., Yang, Y., Wang, C., Tong, W., Zhang, J., Xu, Y., Wang, X., Zhang, C., & Wang, Y. (2017). VpPUB24, a novel gene from Chinese grapevine, *Vitis pseudoreticulata*, targets VpICE1 to enhance cold tolerance. *Journal of Experimental Botany*, 68(11), 2933–2949. https://doi.org/10.1093/jxb/erx136

Yu, G., Wang, L.-G., Yan, G.-R., & He, Q.-Y. (2015). DOSE: An R/Bioconductor package for disease ontology semantic and enrichment analysis. *Bioinformatics* (*Oxford, England*), 31(4), 608–609. https://doi.org/10.1093/bioinformatics/btu684

Zabadal, T., Dami, I., & Goffinet, M. (2007). Winter Injury to Grapevines and Methods of Protection.

Zhang et al. (2019). Overexpression of VaWRKY12, a transcription factor from *Vitis amurensis* with increased nuclear localization under low temperature, enhances cold tolerance of plants. *Plant Mol Biol.* 2019:100(1–2):95–110. https://doi.org/10.1007/s11103-019-00846-6

Zhang, H., Hu, Y., Gu, B., Cui, X., & Zhang, J. (2022). VaMYB44 transcription factor from Chinese wild *Vitis amurensis* negatively regulates cold tolerance in transgenic *Arabidopsis thaliana* and *V. vinifera*. *Plant Cell Reports*, 41(8), 1673–1691. https://doi.org/10.1007/s00299-022-02883-w

Zhang, R., Wang, Y., Li, S., Yang, L., & Liang, Z. (2021). ABA signaling pathway genes and function during abiotic stress and berry ripening in *Vitis vinifera*. *Gene*, 769. https://doi.org/10.1016/j.gene.2020.145226

Zhang, Y., & Dami, I. (2012). Improving Freezing Tolerance of "Chambourcin" Grapevines with Exogenous Abscisic Acid. *HortScience: A Publication of the American Society for Horticultural Science*, 47, 1750–1757. https://doi.org/10.21273/HORTSCI.47.12.1750

Zhao, N., Lin, H., Lan, S., Jia, Q., Chen, X., Guo, H., & Chen, F. (2016). VvMJE1 of the grapevine (*Vitis vinifera*) VvMES methylesterase family encodes for methyl jasmonate esterase and has a role in stress response. *Plant Physiology and Biochemistry*, 102, 125–132. https://doi.org/10.1016/j.plaphy.2016.02.027

Zheng, C., Acheampong, A. K., Shi, Z., Halaly, T., Kamiya, Y., Ophir, R., Galbraith, D., & Or, E. (2018). Distinct gibberellin functions during and after grapevine bud dormancy release. *Journal of Experimental Botany*, 69, 1635–1648. https://doi.org/10.1093/jxb/ery022

Zheng, C., Acheampong, A. K., Shi, Z., Mugzech, A., Halaly-Basha, T., Shaya, F., Sun, Y., Colova, V., Mosquna, A., Ophir, R., Galbraith, D. W., & Or, E. (2018). Abscisic acid catabolism enhances dormancy release of grapevine buds. *Plant, Cell & Environment*, 41(10), 2490–2503. https://doi.org/10.1111/pce.13371

Zheng, H., Liang, Y., Hong, B., Xu, Y., Ren, M., Wang, Y., Huang, L., Yang, L., & Tao, J. (2023). Genome-Scale Analysis of the Grapevine KCS Genes Reveals Its Potential Role in Male Sterility. *International Journal of Molecular Sciences*, 24(7), 6510. https://doi.org/10.3390/ijms24076510

Zhu, Z., Quan, R., Chen, G., Yu, G., Li, X., Han, Z., Xu, W., Li, G., Shi, J., & Li, B. (2022). An R2R3-MYB transcription factor VyMYB24, isolated from wild grape *Vitis yanshanesis* J. X. Chen., regulates the plant development and confers the

tolerance to drought. *Frontiers in Plant Science*, 13. https://doi.org/10.3389/fpls.2022.966641

Zhu, Z., Shi, J., Xu, W., Li, H., He, M., Xu, Y., Xu, T., Yang, Y., Cao, J., & Wang, Y. (2013). Three ERF transcription factors from Chinese wild grapevine *Vitis pseudoreticulata* participate in different biotic and abiotic stress-responsive pathways. *Journal of Plant Physiology*, 170(10), 923–933.

Zuther, E., Büchel, K., Hundertmark, M., Stitt, M., Hincha, D. K., & Heyer, A. G. (2004). The role of raffinose in the cold acclimation response of *Arabidopsis thaliana*. *FEBS Letters*, *576*(1–2), 169–173. https://doi.org/10.1016/j.febslet.2004.09.006