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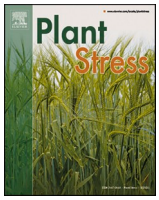

Tetralone-ABA enhances winter cold acclimation, reduces deacclimation, and delays budbreak in *V. vinifera* and *V. hybrid* grapevines

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ABSTRACT

Climate change-related acute winter freezes and unseasonal false springs have become significant and predictable risks for grape growers across North America and Eurasia. Novel strategies to enhance resilience during the dormant season, particularly by improving bud cold hardiness and delaying budbreak, are urgently needed for the sustainability of grape and wine production. In this study, we evaluated a synthetic abscisic acid (ABA) analog, tetralone-ABA (ABA-1102), as a sprayable product for inducing these traits in three grapevine cultivars: 'Riesling', 'Cayuga White', and their progeny, 'Aravelle'. Post-harvest foliar application of tetralone-ABA accelerated leaf senescence, enhanced bud cold hardiness during cold acclimation, and slowed deacclimation under both controlled and field conditions. It also delayed the budbreak in spring without altering growing season phenology, physiology, or harvest yield. Transcriptomic analysis during deacclimation assays suggests that tetralone-ABA's effect on delaying deacclimation may result from its suppression of the activation of growth-related pathways under growth-permissive conditions. Detailed investigations of these pathways indicate that tetralone-ABA may have modulated critical biological processes such as cell wall remodeling, sugar metabolism, and ABA signaling. Overall, this study provides novel insights into the genetic control of grapevine deacclimation, highlights ABA's role in grapevine dormant season physiology, and demonstrates tetralone-ABA's potential as a promising tool for improving dormant season viticulture resilience, offering a new strategy to protect grapevines against the increasing threats posed by climate change.

1. Introduction

The growing frequency and intensity of extreme weather events due to climate change are emerging as the primary challenge to sustainable agriculture and global food security (AghaKouchak et al., 2020; De Rosa et al., 2021). Perennial crops, such as grapevine, could be more vulnerable to these events due to their long lifespan and the high re-establishment cost for the adoption of new cultivars (Gunathilaka et al., 2017). Among all abiotic stresses, cold stress during the dormant season is a leading factor constraining the expansion of the grape and wine industries in cool climate viticulture regions in North America and Eurasia (Poling, 2008; Londo et al., 2023; Kovaleski, 2024). Low temperatures in winter can exceed the cold hardiness of grapevines, resulting in tissue damage to the compound bud, phloem and xylem or the whole plant (Zabadal et al., 2007). Cold damage decreases productivity in the following season, and the cost of replanting and additional management in the following years intensively impact the profitability of the damaged vineyards (Martinson and White, 2004). Globally, the increasing frequency of late spring frost damage due to false spring (advanced budbreak in overall warming winter followed by late frost) is becoming a leading challenge in the major viticultural regions in the world (Poni et al., 2022). Late spring frosts directly threaten the growth of grapevines, especially the most fruitful primary buds, leading to significant yield loss (Poling, 2008; Wang and Dami, 2020). Thus, the enhancement of dormant season viticultural resilience is crucial for the sustainability of the global grape and wine industry.

Active methods that directly increase vineyard ambient temperature or canopy temperature through wind machines, heaters, helicopters, overhead sprinklers or hydrophobic particle film and acrylic polymer have been applied in commercial vineyards to manage cold-related damage (Poling, 2008; Poni et al., 2022). However, to date, these methods are not economically feasible for smaller acreage growers due to their high capital cost. To this end, researchers have been developing novel tools to mitigate cold-related damage with a focus on delaying

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budbreak to avoid frost damage in spring and enhancing cold hardiness to mitigate cold damage in winter. Pre-budbreak application of plant oil-based products such as Amigo Oil® and FrostShield® has been seen to be effective in delaying budbreak from days to weeks (Dami and Beam, 2004; Centinari et al., 2018; Wang and Dami, 2020). However, some studies report carry-on effects from such applications, like phytotoxic effects on certain cultivars contributing to lower bud survival rate and cluster number during harvest (Loseke et al., 2015; Centinari et al., 2018; Persico et al., 2021). Delayed pruning and double pruning are other examples of methods to delay budbreak on basal buds through the prolonged apical dominance of apical buds. Such approaches have been shown effective in delaying budbreak for 15 to 20 days, but the delaying effect can last until harvest, which impacts fruit maturity and wine quality (Poni et al., 2022). Additionally, the increased complexity of vineyard management may also slow the adoption of these methods among growers. Methods that achieve budbreak delay while avoiding carry-on effects on grapevine growth and exhibit ease of application are needed to manage late spring frost damage in a changing world.

To mitigate winter cold damage in vineyards, grapevine breeders in cold/cool climate viticulture regions in the world have developed cold hardy cultivars by introgressing the superior cold hardiness of wild *Vitis* species adapted to cold winter conditions such as *V. labrusca* and *V. amurensis* into cold sensitive *V. vinifera* cultivars (Reisch et al., 2012; Gutierrez et al., 2021). In parallel, the development of practical tools to enhance grapevine cold hardiness has centered on the evaluation of post-harvest application of plant hormones such as abscisic acid (ABA) (Li and Dami, 2016; Sun et al., 2016; Vergara et al., 2017; Rubio et al., 2019a; Wang and Dami, 2020). ABA is an essential plant phytohormone that regulates the plant life cycle and functions in response to various abiotic stresses through the ABA signaling pathway and mediated by ABA-responsive genes and ABA-responsive element binding proteins (AREBs) (Abrams and Loewen, 2019). The processes of dormancy establishment, transition, and release in perennials are controlled by the interaction of ABA and other plant hormones (Basu and Rabara, 2017; Liu and Sherif, 2019; Yang et al., 2021). In grapevine, bud ABA concentration increases during cold acclimation (gain of cold hardiness) and decreases during deacclimation (loss of cold hardiness) as a result of altered ABA biosynthesis and catabolism during the dormant season, supporting ABA's involvement in cold hardiness (Zheng et al., 2015, 2018; Rubio and Pérez, 2019). Application of ABA on grapevines upregulated the regulatory or functional pathways for cold hardiness such as C-Repeat Binding Factor/*DRE* Binding Factors (*CBF/DREB1*), starch, raffinose family oligosaccharides, and hydrogen peroxide biosynthesis (Rubio et al., 2019a,b; Wang and Dami, 2020; Pérez et al., 2021). Some studies have reported that post-harvest foliar application of ABA on grapevine promoted cold acclimation, induced deeper dormancy, decreased bud water content and enhanced bud cold hardiness, but others reported limited effect (Zhang and Dami, 2012; Li and Dami, 2016; Bowen et al., 2016; Wang et al., 2020). These inconsistencies may be a result of natural ABA's short half-life or natural rapid enzymatic catabolism as a phytotoxicity that interferes with the equilibrium of transcriptome and metabolome (Diddi et al., 2023). In recent years, synthetic ABA analogs have been tested for their efficacy to enhance cold hardiness in winter (Bowen et al., 2016; Willwerth and Abrams, 2019; Gunn, 2023). The analogs are functionally similar to the natural form of ABA but are structurally different, which contributes to increased resistance to catabolism (Diddi et al., 2023). For example, 8′-acetylene-ABA, synthesized by replacing the 8′-methyl group in ABA with an acetylene group, avoids being targeted by the ABA catabolism pathway (Cutler et al., 2010). Some studies report significant enhancement of grapevine bud cold hardiness from post-harvest application of this analog, yet others reported limited effect (Bowen et al., 2016; Willwerth and Abrams, 2019; Gunn, 2023). Tetralone-ABA is another form of ABA analog that is synthesized by introducing an aromatic ring in place of the vinyl methyl group in the endogenous ABA, allowing for slower catabolism thus potentially maintaining prolonged bioactivity (Nyangulu et al., 2006; Diddi et al., 2023). Previous studies of the effect of a tetralone-ABA product (ABA-1016, purity $\geq$ 95 %) on grapevine indicate that this analog, enhanced bud cold hardiness close to the end of the dormant season, suggesting a potential role in slowing deacclimation (Willwerth and Abrams, 2019; Gunn, 2023). While encouraging, a more in-depth study of the impact of ABA analogs on grapevine physiology is needed to evaluate tetralone-ABA as a cold hardiness and budbreak delay mitigation tool.

In this study, we conducted a two-year evaluation of the impact of the post-harvest foliar application of an ABA analog, tetralone-ABA (ABA-1102, purity = 100 %), on three grapevine cultivars (*V. vinifera* 'Riesling', *V. hybrid* 'Cayuga White' and their progeny, *V. hybrid* 'Aravelle') through assessing changes in physiological, phenological and transcriptomic responses. 'Riesling' is a moderately cold hardy cultivar of great economic importance for cool and cold climate grape and wine industries such as the Finger Lake regions. 'Riesling' exhibits mid-season budbreak compared to other *V. vinifera* cultivars (Londo and Johnson, 2014). 'Cayuga White', with *V. labrusca, V. rupestris* and *V. aestivalis* genetic background, sustains sufficient cold hardiness to survive in the winters in the cool climate viticultural regions in North America. This cultivar tends to deacclimate slower and initiate budbreak later compared to other cold hybrid cultivars that derive from *V. riparia* (Londo and Kovaleski, 2025). Limited information is available for the overwintering behavior of 'Aravelle' as this cultivar was recently released. We hypothesized that post-harvest foliar application of tetralone-ABA would enhance cold hardiness in winter and delay budbreak in spring, and the delaying effect would be a consequence of tetralone-ABA-induced slower activation of growth resumption-related pathways. The first objective was to determine whether tetralone-ABA could enhance grapevine bud cold hardiness and if the enhancements differ among genotypes. The second objective was to understand tetralone-ABA's impact on the grapevine bud transcriptome during deacclimation under forcing conditions. The third objective was to evaluate if tetralone-ABA could delay budbreak, and if there were any fundamental changes in growing season physiology or harvest yield.

2. Materials and methods

2.1. Plant materials and treatment application

The experiment was conducted at the Finger Lakes Teaching and Demonstration Vineyard at Anthony Road Wine Co. in Geneva, NY, US (42.71° N, −76.97 ° W). The experiment was conducted in two years: 2022–2023 and 2023–2024. Treatment applications occurred on 10/05/2022 (2022–2023 experiment) and 10/17/2023 (2023–2024 experiment) on *V. vinifera* 'Riesling', *V. hybrid* 'Cayuga White', and *V. hybrid* 'Aravelle'. Experimental vines were subjected to standard vineyard management during the growing season. The experimental design, chronological data collection process, and data collection methods used in the study are graphically presented in Fig. 1.

Previous studies have tested post-harvest foliar ABA applications to induce favored phenotypes in the dormant season (Dami et al., 2015; Li and Dami, 2016). Applying tetralone-ABA post-harvest but before defoliation ensured efficient uptake while avoiding interference with fruit development and active growth, which makes it an optimal timing for targeting dormant season physiology. Thus, in this study, treatments of either tetralone-ABA or water control were applied as a foliar canopy spray a week after the harvest of 'Riesling' on 10/05/2022 (for the 2022–2023 experiment) and 10/17/2023 (for the 2023–2024 experiment). Tetralone-ABA ((+)-tetralone ABA, ABA-1102, purity = 100 %, ABAzyne Biosciences Inc., Saskatoon, Canada) was obtained as a fine powder and was kept under dry, cool, and dark conditions to maintain chemical activity (Diddi et al., 2023). Stock solutions were prepared by dissolving 6 g of tetralone-ABA in 1 L of distilled water with 120.0 mL of DMSO (Fisher Scientific, NH, US) and 3840.0 μL of 5.00 M NaOH solution (Sigma-Aldrich, MO, US). Stock solutions were diluted to the final

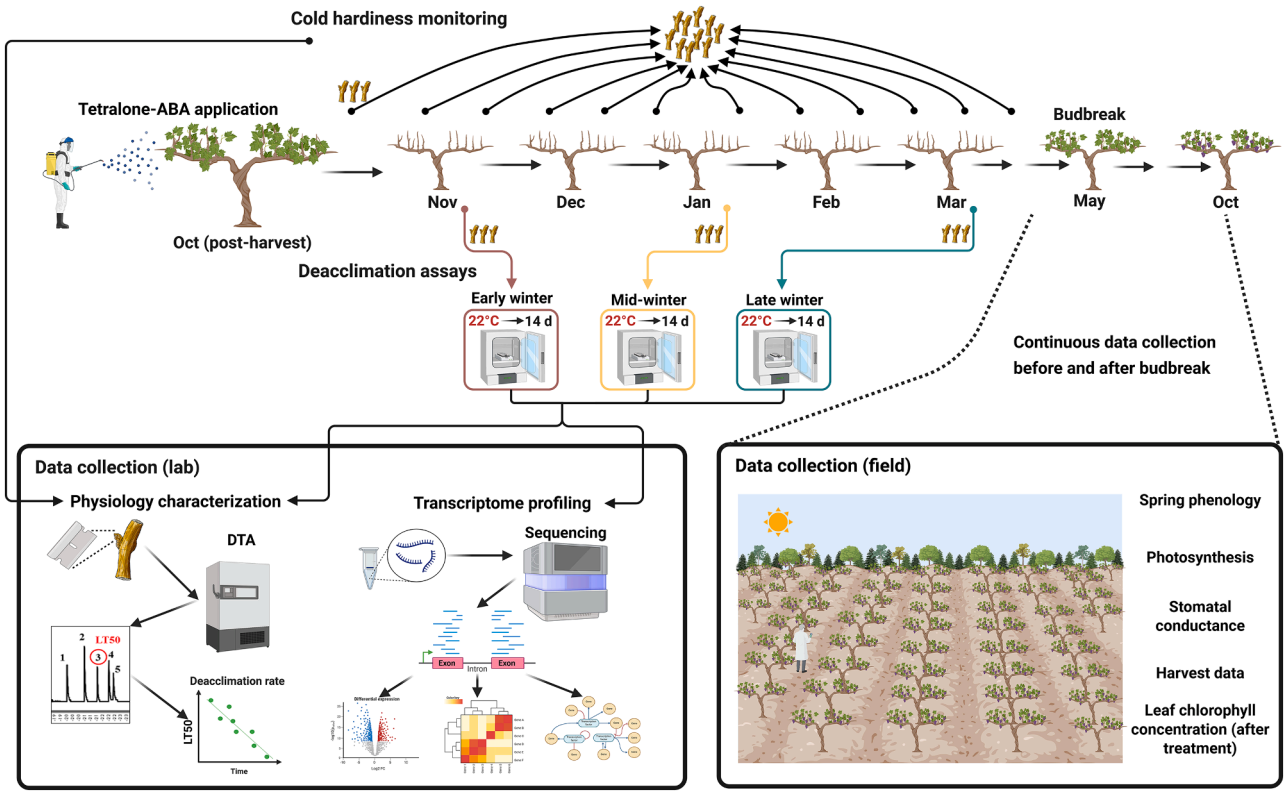

Fig. 1. Schematic representation of the experimental design and data collection process. Arrows indicate the flow of sample collection and data acquisition.

concentration of 0.5 $g{\cdot}L^{-1}$ using distilled water. During treatment application, 1 L of 0.5 $g{\cdot}L^{-1}$ tetralone-ABA solution was applied to the vegetative canopy of each experimental vine, corresponding to a final application rate of 0.5 g tetralone-ABA per vine. Control treatment was distilled water mixed with the same concentrations of DMSO and NaOH. Silwet L-77 (Helena Agri-Enterprises, TN, US) was added at a rate of 0.05 % (v/v) to both treatments as a surfactant. For each cultivar, 12 individual vines were treated in both tetralone-ABA and control treatments. To avoid any carry-over effect from the treatment in the previous year, different vines were used for experimentation in each of the two dormant seasons.

2.2. Relative chlorophyll concentration measurement

In the 2022–2023 experiment, relative chlorophyll concentration of the leaves in experimental vines was quantified after treatment application using an MC-100 chlorophyll concentration meter (Apogee Electronics, CA, USA). For each vine, the measurement was conducted three times on three randomly selected leaves in the lower, middle, and upper canopy. Relative chlorophyll concentration was expressed as SPAD (Soil Plant Analysis Development), a relative measure of chlorophyll concentration derived from the absorbance of specific wavelengths of light (Richardson et al., 2002; Parry et al., 2014; Yuan et al., 2016). The three readings were averaged as subsamples to represent the relative chlorophyll concentration of the vine. Measurements were conducted four times until defoliation at one-day post-treatment (10/06/2022), one-week post-treatment (10/12/2022), two-week post-treatment (10/19/2022), and three-week post-treatment (10/26/2022).

2.3. Cold hardiness monitoring

After the treatment application, the monitoring of grapevine bud cold hardiness was conducted over the entire dormant season to evaluate the treatment effect. Grapevine bud cold hardiness was determined bi-weekly from 10/26/2022 to 03/22/2023 in the 2022–2023 dormant season and from 10/17/2023 to 03/18/2024 in the 2023–2024 dormant season using differential thermal analysis based on a standardized protocol (Londo et al., 2023). Briefly, grapevine buds were loaded in sample plates and treated with a decreasing temperature ramp of 4 °C·h^{-1} from 0 °C to −50 °C in a Tenney T2C environmental chamber (Tenney Environmental, PA, US). Thermocouples on samples plates detected low temperature exotherms (LTEs), which represent the intracellular ice nucleation of grapevine buds, corresponding to the cold hardiness of the buds (Londo et al., 2023). For each cultivar and each treatment, at least 15 buds were used to determine the concurrent cold hardiness.

2.4. Deacclimation assays

In addition to bud cold hardiness monitoring, deacclimation rate, the speed of cold hardiness loss that occurs under growth permissive condition, was measured three times in early winter, mid-winter and late winter to monitor the progression of dormancy transition in response to tetralone-ABA treatment. Deacclimation rate was only determined for 'Riesling' due to limited plant material for the other two cultivars. In the 2022–2023 experiment, buds were collected three times from the field at 518.5 (11/07/2022, early winter), 978.5 (01/02/2023, mid-winter) and 1,339.9 (03/13/2023, late winter) chilling units (Utah model) (Richardson et al., 1974). In the 2023–2024 experiment, buds were collected three times from the field at 629.1 (11/08/2023, early winter), 1,282.6 (01/24/2023, mid-winter) and 1,737.9 (03/20/2024, late winter) chilling units (Utah model). These three timepoints roughly correspond to the major physiological stages in the dormant season: endodormancy (unable to deacclimate and initiate budbreak under growth permissive condition), transition from endodormancy to ecodormancy (partially able to deacclimate and initiate budbreak under growth permissive condition) and ecodormancy (fully able to

deacclimate and initiate budbreak under growth permissive condition) (North and Kovaleski, 2023). During each deacclimation assay, canes collected from field were chopped into single-bud cuttings, and the cuttings were incubated in growth chamber at 22 °C without supplemental light with cutting ends dipped in water. On day 0, day 3 (day 2 in the 2023–2024 experiment), day 5, day 7 and day 14 in the growth chamber, bud cold hardiness was determined by DTA if the buds remained unbroken. Deacclimation rate, the coefficient of the linear regression between LTE and days in the chamber, was computed for each deacclimation assay and for each treatment (Kovaleski et al., 2018).

2.5. Spring phenology monitoring

Phenology recording started before budbreak to evaluate the treatment effect on growth restoration and vegetative growth during the growing season. To assess the impact of tetralone-ABA on the timing of budbreak, we combined two previously established grapevine phenology systems, EL system (Eichhorn and Lorenz, 1977) for the stages after budbreak and Modified Shaulis Field Score (https://lergp.com/modified-shaulis-field-score) for the stage before budbreak to increase the resolution of phenological stages in the early growing season. Visual assessment of phenology was conducted on each experimental vine three times every week from 04/19/2023 to 06/26/2023 in the 2023 growing season and 04/23/2024 to 06/03/2024 in the 2024 growing season. For each cultivar and each treatment, the date on which at least 50 % of experimental vines reached budbreak stage was recorded as the date of budbreak (Kovaleski and Londo, 2019; Wang et al., 2020; Wang and Dami, 2020). We first quantified the delay of budbreak for the two treatments each year using 'days'. To estimate the delaying effect from tetralone-ABA treatment, we also quantified the length of the treatment effect in each year using thermal units based on GDH4 (growing degree hour using 4 °C as the based temperature). GDH4 utilizes two cosine functions between a base temperature (4 °C) and an upper limit temperature (36 °C), with an optimum at 26 °C as suggested in previous modeling approaches of grapevine and tree fruit budbreak (Luedeling et al., 2013; Santos et al., 2019).

2.6. Growing season physiology and harvest data

During the growing season, grapevine photosynthesis and stomatal conductance were measured to determine if the tetralone-ABA affected growing season physiology. A Ll-600 Porometer/Fluorometer (LI-COR Biosciences, NE, US) was used to quantify the photosynthetic efficiency, expressed as *ETR* ($\mu mol \cdot m^{-2} \cdot s^{-1}$), calculated from operating efficiency of PSII (Φ_{PSII}) and ambient light condition (Q_{amb}), and stomatal conductance, expressed as g_{sw} ($mol \cdot m^{-2} \cdot s^{-1}$). Harvest yield data, including fruit yield per vine and the number of clusters per vine, were obtained during the harvest in both years.

2.7. RNA-seq during deacclimation assays, library preparation and data processing

During each deacclimation assay conducted in the 2022–2023 experiment and in parallel with bud cold hardiness measurements, bud samples were collected for RNA-seq to investigate the transcriptome effect of tetralone-ABA during deacclimation. Three biological replicates, each composed of five pooled buds, were used for extraction and library preparation. The Cornell University Institute of Genomic Diversity (Ithaca, NY, USA) provided technical support for library construction using Lexogen QuantSeq 3′mRNA-Seq Prep Kit (Lexogen, Greenland, NH). Sequencing of libraries was conducted at Cornell University Institute of Biotechnology (Ithaca, NY) using NextSeq500 (Illumina, Inc., San Diego, CA, USA) with 95 samples per lane. The read length was 85 bp, and sequencing was replicated three times in each library for technical validity.

RNA-seq data were analyzed following a previously generated pipeline specifically optimized for grapevine (Wang et al., 2022a). Standardized library QC (FastQC) and trimming (BBDuk) were conducted for quality control of the libraries, and transcript alignment was conducted in STAR using *V. vinifera* 12X.v2 genome and VCost.v3 annotation (Dobin et al., 2013; Canaguier et al., 2017). The resulting gene count matrix, composed of libraries (as columns) and genes (as rows) was analyzed using DESeq2 to identify differentially expressed genes (Love et al., 2014). Principal Component Analysis (PCA), Uniform Manifold Approximation and Projection (UMAP), and Weighted Gene Co-expression Network Analysis (WGCNA) were used for the characterization of transcriptome and the identification of gene co-expression modules (Langfelder and Horvath, 2008). After the identification of target gene co-expression modules that showed significant correlation with deacclimation and responded to tetralone-ABA treatment, we applied a two-step filtering to identify target genes. As the first step, the genes exhibiting a module membership (Pearson correlation coefficient of gene expression with the target module eigengene (ME), the PC1 of all the genes in a module) of greater than 0.8 were reserved. This step ensured that the selected genes follow the pattern of target gene co-expression modules. As the second step, we retained genes with a false discovery rate (FDR) $\leq$ 1e-30 in the differential expression analysis using DESeq2, with the ME values of target modules as the only factors in the gene expression model. This step ensured a highly significant correlation between gene expression and the ME values of target modules. The resulting genes closely followed the behavior of the ME values of the target gene co-expression modules, making them suitable for downstream analysis. Enriched pathways among the target genes were detected through over representation analysis (ORA) using VitisNet function pathways (Subramanian et al., 2005; Grimplet et al., 2009). Significantly enriched pathways were examined for correlations with known biological functions, and hub genes were determined based on the synergy of statistical significance and biological functionality. In addition, we also manually examined individual genes, or the genes involved in pathways that were previously reported to be functional in grapevine cold hardiness.

While the primary analysis incorporated all libraries, additional analyses were conducted on libraries from each deacclimation assay to identify tetralone-ABA-induced differential expression. These analyses used 'days in growth permissive condition' and 'treatment' as factors in the gene expression model to capture specific responses in early, mid-, and late winter buds. Additional analysis was also conducted to identify genes that were significantly upregulated or downregulated during the third deacclimation assay while remaining unresponsive to tetralone-ABA treatment. The identified deacclimation-responsive genes were further filtered using DESeq2 contrasting. Differential expression contrasts were performed between the two treatments at each time point in the third deacclimation assay. Genes with p-values greater than 0.3 across all time point comparisons were retained, which ensures that the selected genes were responsive to deacclimation but unaffected by tetralone-ABA.

3. Results

3.1. Dormant season physiology

The impact of tetralone-ABA treatment on the relative chlorophyll concentration (SPAD), dormant season cold hardiness and deacclimation rate of three cultivars is shown in Fig. 2. For 'Riesling', tetralone-ABA treated vines started showing significantly lower SPAD one-day post-treatment (10/06/2022) (Fig. 2A). The treatment effect was also observed in the following three measurements at one-week post-treatment (10/12/2022), two-week post-treatment (10/19/2022) and three-week post-treatment (10/26/2022). Visually, tetralone-ABA treated 'Riesling' vines showed a yellower pattern on one-week and two-week post-treatment and reached 100 % defoliation at three-week post-

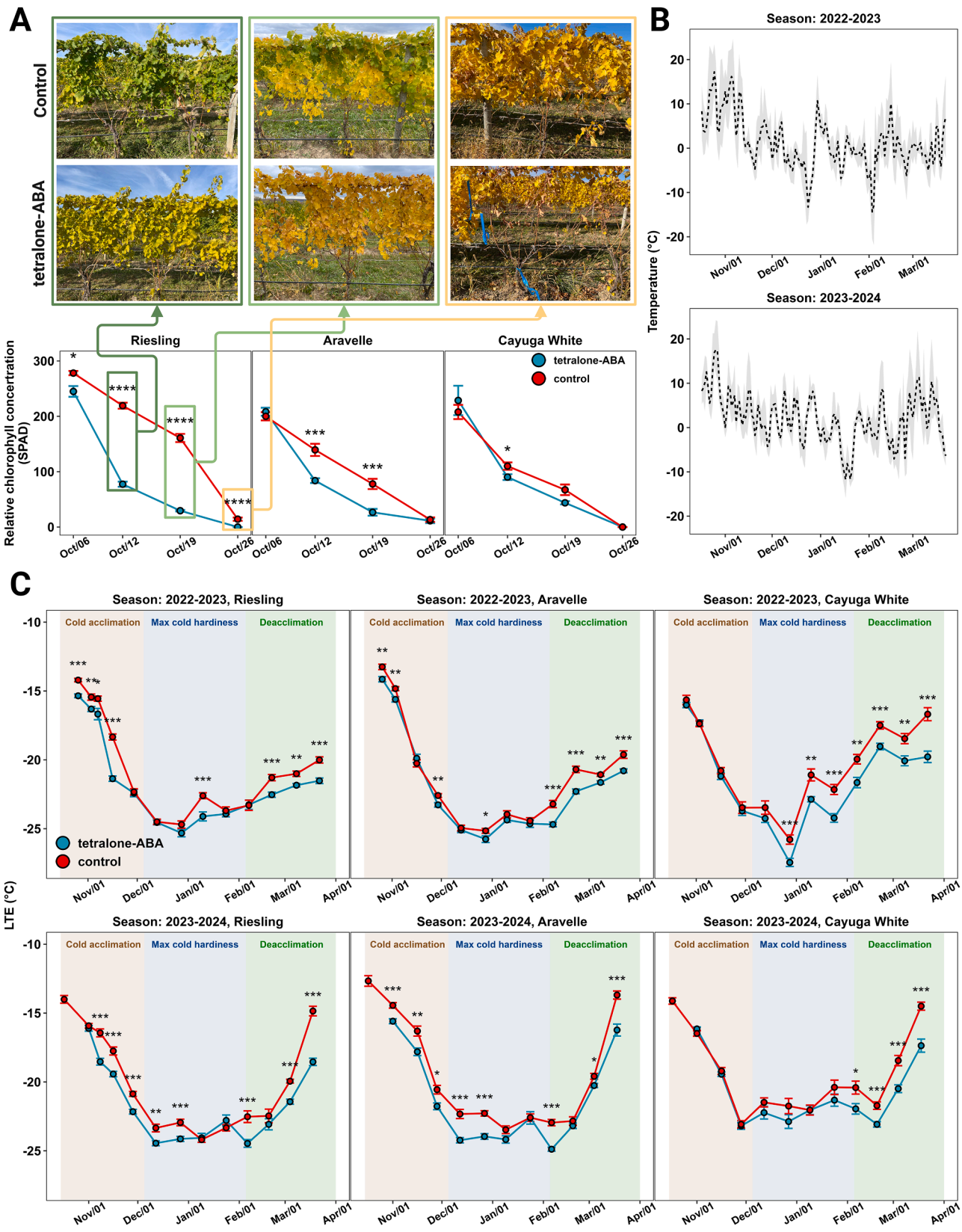

Fig. 2. The impact of tetralone-ABA on grapevine relative chlorophyll concentration and dormant season physiology. A) Relative chlorophyll concentration of 'Riesling' after treatment application on 10/05/2022. Images represent changes in 'Riesling' vines during each relative chlorophyll concentration measurement. B) Daily temperature in the 2022–2023 and 2023–2024 dormant seasons, with dashed lines representing mean temperatures and shaded areas indicating maximum and minimum temperature ranges. C) Bud cold hardiness of three cultivars in the 2022–2023 and 2023–2024 dormant seasons. The mean of bud LTE is shown with the error bars representing standard error ($n \geq 15$).

treatment, as the control vines just started defoliation (Fig. 2A). In 'Cayuga White', tetralone-ABA treatment effect was limited to a marginally lower SPAD at only one-week post-treatment (Fig. 2A). Defoliation occurred simultaneously in tetralone-ABA treated and control 'Cayuga White' vines. In 'Aravelle', the tetralone-ABA treatment effect was intermediate to its parents: significant reductions in SPAD were noted at one-week and two-week post-treatment, yet defoliation timings were equivalent in tetralone-ABA treated and control vines (Fig. 2A).

Winter temperatures during the 2023–2024 dormant season were on average warmer and exhibited less temperature fluctuation compared to the 2022–2023 dormant season (Fig. 2B). Consequently, chilling accumulation occurred faster under field conditions during the 2023–2024 dormant season compared with 2022–2023 (Figure S1). When comparing field cold hardiness between the two seasons, it was seen that the deacclimation process occurred faster (steeper loss of cold hardiness) in the 2023–2024 dormant season regardless of cultivar or treatment (Fig. 2C). For 'Riesling' and in both dormant seasons, significant enhancements in bud cold hardiness were noted in tetralone-ABA treated vines during cold acclimation following decreasing minimum temperature from mid-October to mid-November (Fig. 2C). Field deacclimation was observed in control vines following a warming event in early January 2023, and tetralone-ABA treated vines exhibited superior bud cold hardiness in this period (Fig. 2C). In both dormant seasons, significant enhancement of bud cold hardiness was observed for tetralone-ABA treated vines after mid-February (Fig. 2C). Comparatively, in 'Cayuga White', no treatment effect was identified during cold acclimation in either seasons, but significant enhancements of bud cold hardiness were observed throughout the deacclimation processes until the end of data collection (Fig. 2C). The tetralone-ABA treatment effect on 'Aravelle' was intermediate to its parents: enhancement of bud cold hardiness was observed in cold acclimation and deacclimation, but the degree of enhancement was comparatively modest (Fig. 2C).

In deacclimation assays conducted under growth permissive conditions, tetralone-ABA treated buds deacclimated at slower rates than control and the magnitude of the delay increased with higher field chilling (Fig. 3). The control buds collected from early winter exhibited minimal deacclimation rates, measured as 0.2–0.3 °C·day^{-1} (Fig. 3). Tetralone-ABA treatment did not alter the overall deacclimation rate of these early winter collections (Fig. 3). The control buds collected from mid-winter and late winter showed increased deacclimation rate, while tetralone-ABA treatment consistently delayed the loss of cold hardiness at later timepoints in these assays, resulting in a decreased deacclimation rate (Fig. 3). On average, the deacclimation rate of mid and late winter deacclimation assays was decreased by tetralone-ABA treatment by 25 % relative to control.

3.2. *Transcriptomic analysis of deacclimation and tetralone-ABA treatment impact*

In parallel with each deacclimation assay conducted in the 2022–2023 dormant season, replicate buds were examined for their transcriptomic response to tetralone-ABA during deacclimation. A total of 90 libraries were sequenced from the three deacclimation assays.

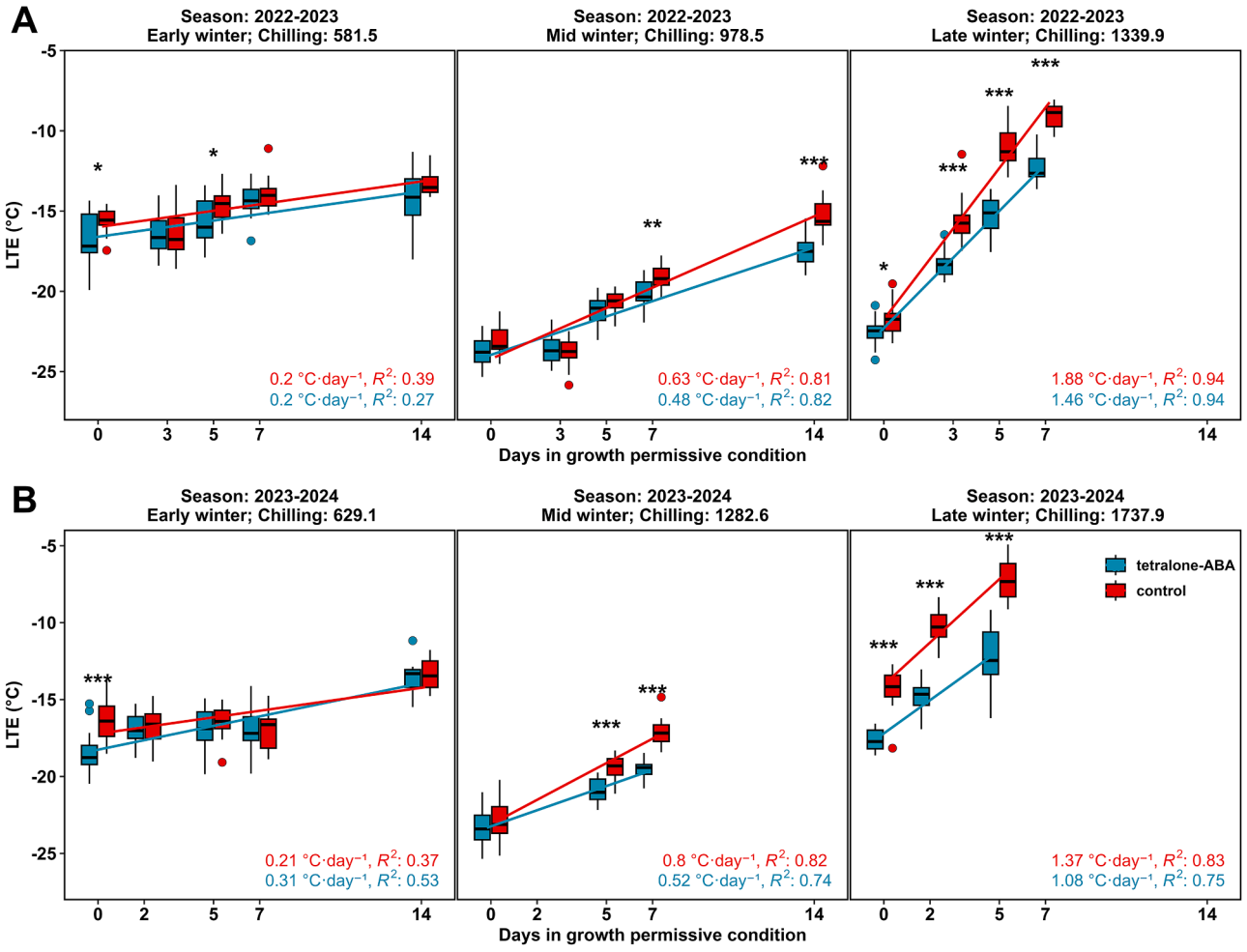

Fig. 3. The impact of tetralone-ABA on the deacclimation of 'Riesling' buds in controlled environment. A) Deacclimation assays conducted in the 2022–2023 dormant season. B) Deacclimation assays conducted in the 2023–2024 dormant season.

Each library met FastQC per base quality standards. On average, each library contained 4.6 million reads, with a unique mapping rate averaging 78.9 %. Six libraries were identified as outliers in PCA, and these libraries were removed for downstream analysis (Figure S2). After filtering out low count genes (genes with fewer than five transcripts per library), the final gene count matrix for downstream analysis was composed of 84 libraries and 15,002 genes (35 % of total transcriptome).

We applied PCA, UMAP and WGCNA to the final gene expression matrix for the overall transcriptome characterization of the libraries. The differentiation of the libraries by treatment, timepoints and deacclimation assays using PC1 and PC2 from PCA and UMAP1 and UMAP2 from UMAP is shown in Figure S3. The visualization of PC1 and UMAP2 indicates that the libraries generated from early and mid-winter buds did not show significant changes during the deacclimation assays, and there was limited treatment effect of tetralone-ABA (Fig. 4A and B). The analysis of the first deacclimation assay libraries resulted in no differentially expressed genes between the two treatments. The analysis on the second deacclimation assay libraries resulted in 133 genes that exhibited differential expression at FDR $\leq$ 0.01 between the two treatments. Among the 108 annotated genes in these genes, 73 genes exhibited higher expression in tetralone-ABA treated buds, 35 genes exhibited higher expression in control buds (Figure S4). No pathway was statistically enriched among these genes. We applied WGCNA to these 108 genes and identified three major expression patterns during the second deacclimation assay: 1) increase and converge (50 genes in module 'turquoise'), in which gene expression was initially higher in ABA-treated buds and was later increased during the experiment, but the increase was slower compared to control, leading to the convergence of gene expression of the two treatments by the end of the experiment; 2) decrease and converge (23 genes in module 'brown'), in which the above pattern was reversed; 3) two directions and converge (35 genes in module 'blue'), in which gene expression was initially higher in control buds but was decreased during the experiment when the expression was increased in tetralone-ABA treated buds, which led to the convergence of gene expression of the two treatments by the end of the experiment (Fig. S5).

In contrast, the PC1 and UMAP2 of the libraries generated from late

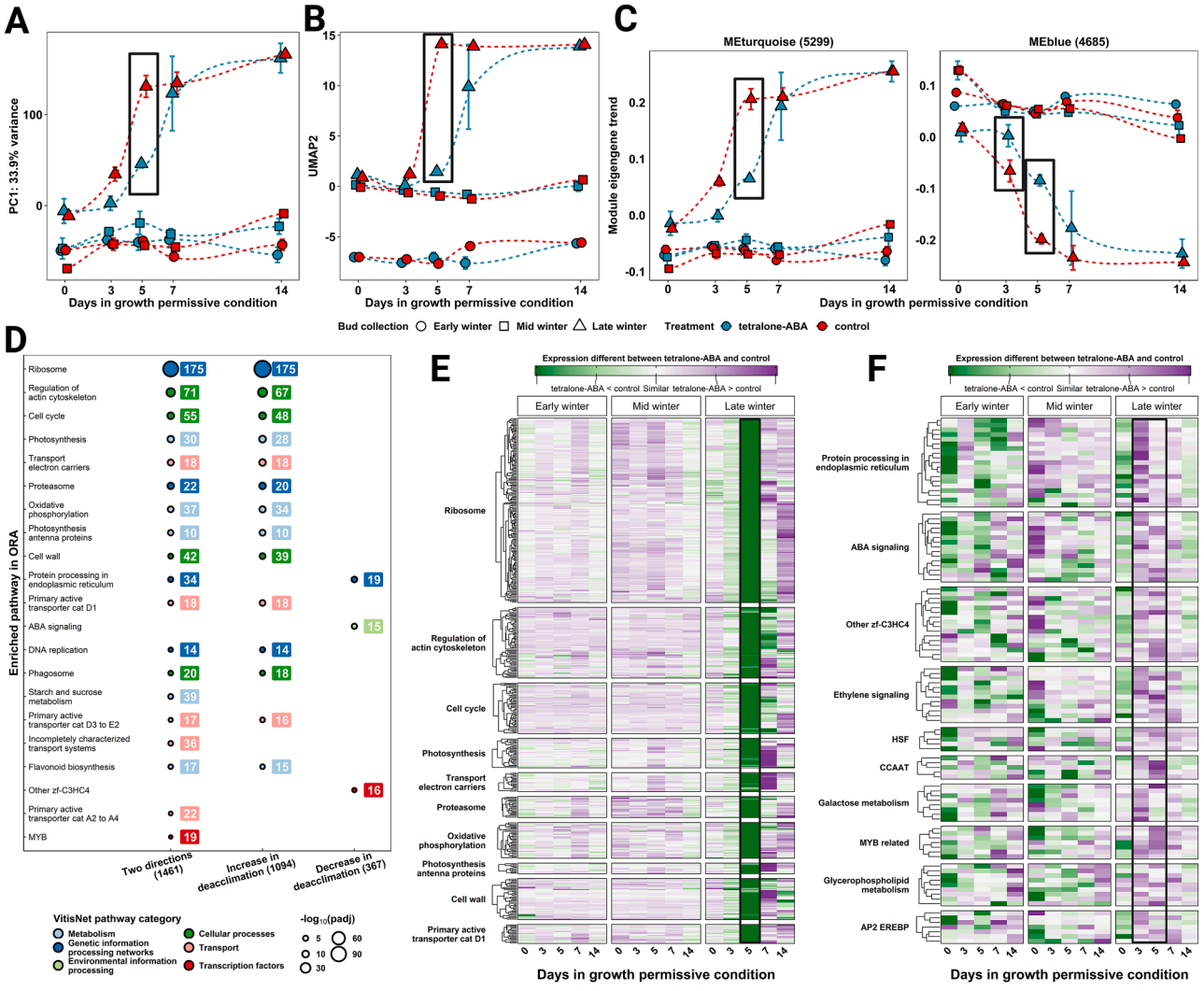

Fig. 4. Identification of two gene clusters that correlated with deacclimation and responded to tetralone-ABA treatment. A) Behavior of PC1 from the PCA of the active transcriptome in the three deacclimation assays. B) Behavior of UMAP2 from the UMAP of the active transcriptome in the three deacclimation assays. C) Expression pattern of the module eigengenes (MEs) in modules 'turquoise' and 'blue' that correlated with deacclimation and responded to tetralone-ABA treatment. D) Pathway enrichment analysis of the genes showed significant correlation with deacclimation and responded to tetralone-ABA treatment, categorized by VitisNet pathway system. E) Average expression difference between control and tetralone-ABA of the 'increase in deacclimation' genes. The greatest expression differences (highlighted) were observed on day 5 during the late winter deacclimation assay. F) Average expression difference between control and tetralone-ABA of the 'decrease in deacclimation' genes.

winter buds showed a rapid increase between day 3 and day 5 and generally plateaued at a higher value after 7 d (Fig. 4A and B). The same increase was observed in the samples collected from tetralone-ABA treated buds, but the increase was delayed (Fig. 4A and B). The WGCNA using all the genes in the final gene count matrix identified 11 gene co-expression modules, excluding module 'grey' containing noisy genes. The number of genes in each module ranged from 57 in module 'greenyellow' to 5,299 in module 'turquoise'. The MEs of all the gene co-expression modules are depicted in Figure S6. Notably, the MEs of the two largest modules, 'turquoise' (5,299 genes) and 'blue' (4,685 genes) displayed divergent behaviors during the experiment, and their behaviors are either nearly identical or opposite to PC1 from PCA (Fig. 4A and C). These genes represented nearly 2/3 of the active transcriptome identified in our experiment. In control, the expression of these eigengenes remained relatively unchanged in the first and second deacclimation assays conducted on early winter buds and mid-winter buds but showed significant increase/decrease in the third deacclimation assay with late winter buds when buds were rapidly deacclimating (Figs. 3 and 4C). In summary, there were significant differences in the transcriptomes of the buds under tetralone-ABA treatment and control, especially on day 3 and day 5 in the third deacclimation assay when the buds were rapidly deacclimating. Two gene clusters had expression patterns closely linked to deacclimation and are impacted by tetralone-ABA treatment.

3.3. Pathways impacted in deacclimation and in response to tetralone-ABA treatment in late winter buds

After gene filtering, we identified 1,461 genes of interest associated with deacclimation from gene co-expression modules 'turquoise' and 'blue'. Among these genes, 1,094 genes showed increasing expression during deacclimation (same trend as ME 'turquoise'), and 367 genes showed decreasing expression (same trend as ME 'blue'). The statistical results of the differential expression analysis and detailed information of these genes are available in Data S1 and Data S2. Enrichment analysis identified 20 pathways in VitisNet that were significantly enriched at FDR $\leq$ 0.001 (Fig. 4D). Genes that significantly increased during deacclimation were associated with several pathways including ribosome (vv23010), regulation of actin cytoskeleton (vv44810), cell cycle (vv44110), photosynthesis (vv10195), transport electron carriers (vv50105), oxidative phosphorylation (vv10190) and cell wall (vv40006) (Fig. 4D). The genes in these pathways showed limited expression change and differences between the two treatments in the first and second deacclimation assays with early winter and mid-winter buds, respectively (Fig. 4E). However, in the third deacclimation assay with late winter buds, significantly lower expression was identified in tetralone-ABA treatment on day 3 and day 5 in almost every gene in every enriched pathway (Fig. 4E). Genes which significantly decreased during deacclimation were associated with pathways including protein processing in endoplasmic reticulum (vv24141), ABA signaling (vv30001), ethylene signaling (vv30008), HSF (heat shock factor, vv60037) and galactose metabolism (vv10052). The genes in these pathways did not show consistent differential expression in the first and second deacclimation assays but showed consistently higher expression on day 3 and day 5 in tetralone-ABA treated buds in the third deacclimation assay (Fig. 4F).

3.4. Detailed investigation of enriched and functional pathways

In addition to these analyses, we manually chose and investigated pathways in detail that have been previously reported to be important during grapevine cold hardiness and deacclimation (Nishizawa et al., 2008; Rubio et al., 2019b; Kovaleski and Londo, 2019; Kutsuno et al., 2023; Ren et al., 2023).

3.4.1. ABA metabolomics and signaling pathways

The ABA signaling pathway, including the genes associated with ABA metabolism, ABA signaling and ABA-responsive genes, was significantly enriched for genes that decreased in expression during deacclimation (Fig. 4F). Most of the genes in the ABA metabolism portion did not reveal notable treatment effects, except for *VIT_03s0063g00380* encoding ABA 8′-hydroxylase and *VIT_17s0000g02680* encoding ABA glucosidase (Figs. S7 and S8). Some ABA signaling and ABA-responsive genes not only showed decreasing expression correlated with deacclimation but also revealed that the tetralone-ABA treatment delayed this decrease, especially in the third deacclimation assay with late winter buds (Fig. S8C). These genes include signaling genes such as one encoding AREB2, two for RCAR, one for PP2C and two for SnRK2, as well as six ABA responsive genes, including a highly expressed member of *EIN3* (Figure S8C).

3.4.2. Sugar metabolism pathways

A total of 16 genes in the galactose metabolism pathway were identified with correlated expression with deacclimation and tetralone-ABA treatment. Among these genes, three genes encoding beta-galactosidase were upregulated in the third deacclimation assay (late winter buds), and upregulation was delayed by tetralone-ABA treatment (Fig. S9). Four genes encoding galactinol synthase were downregulated in the third deacclimation assay, and downregulation was delayed by tetralone-ABA (Fig. S9). A total of 36 genes in starch and sucrose metabolism pathways (after excluding overlapping genes in the galactose metabolism pathway) were identified for their expression correlation with deacclimation and tetralone-ABA (Fig. S10). Among these genes, 13 genes associated with the proteins related to endo-1,3-beta-glucosidase and one gene encoding callose synthase catalytic subunit were upregulated in the third deacclimation assay (late winter bud), and the upregulation was delayed by tetralone-ABA in most of these genes (Figure S10).

3.4.3. Cell wall pathway

In the cell wall pathway, the expression of 42 genes correlated with deacclimation and showed response to tetralone-ABA. Of these, 39 genes were upregulated in the third deacclimation assay, and this upregulation was delayed by tetralone-ABA (Figure S11). These proteins can be categorized into two functional groups: cell wall proteins and cell wall substrate modification enzymes. The cell wall protein group includes unannotated hydroxyproline-rich glycoproteins (six genes), fasciclin-like arabinogalactan-protein (FLA, three genes) and leucine-rich repeat protein/extensin (LRX, four genes) (Fig. S11). The cell wall substrate modification enzyme group includes endo-1,4-beta-glucosidase (also known as cellulase, five genes), pectate lyase (PL, two genes), pectin-acetylesterase (PAE, six genes), polygalacturonase (PG, also known as pectinase, seven genes) and xyloglucan endo-transglucosylase/hydrolase (XTH, two genes) (Figure S11).

3.4.4. ICE, CBF and DREB

Among the 14 *Inducers of CBF Expression* (*ICE*) and *CBF/DREB* genes annotated in VitisNet, five genes passed the low count filtering, and none of these genes are *CBF* (Figure S12). One gene encoding DREB (*VIT_13s0067g01960*) was downregulated during deacclimation, and tetralone-ABA delayed this downregulation in the third deacclimation assay with late winter buds (Figure S12). One gene encoding ICE (*VIT_14s0068g01200*) was upregulated during deacclimation, and tetralone-ABA delayed this upregulation in the third deacclimation assay (Figure S12).

3.4.5. ABA-unresponsive deacclimation genes

We identified a total of 81 genes that exhibited significant changes in expression during deacclimation but remained unaffected by tetralone-ABA. This particular behavior indicates their role in deacclimation independently of ABA regulation (Data S3). Several of these ABA-

unresponsive deacclimation genes encode key regulatory proteins involved in ABA signaling (ABI2, *VIT_04s0008g01420*), ethylene signaling (CTR1, *VIT_18s0001g07700*), auxin signaling (ARF4, *VIT_06s0004g03130*), and flower development (TFL2, *VIT_15s0024g00620*) (Figure S13).

3.5. Budbreak phenology

In the 2022–2023 experiment, field phenology recording began on 04/19/2023. A significant delay in phenology was observed in tetralone-ABA treated vines for all three cultivars, starting from the initial data collection and continuing until late May when clear inflorescences developed (Fig. 5A). The dates of budbreak, defined as when >50 % of experimental vines reached the budbreak stage (Modified Shaulis Field Score 4), were 04/24/2023, for 'Riesling' and 'Aravelle,' and 05/01/2023, for 'Cayuga White' in the control vines (Fig. 5A). In the tetralone-ABA treated vines, budbreak occurred on 05/05/2023, for 'Riesling,' 05/03/2023, for 'Aravelle', and 05/09/2023, for 'Cayuga White,' representing delays of 11, 9, and 8 d, respectively, compared to the control vines (Fig. 5A). In the 2023–2024 experiment, field phenology recording began on 04/23/2024. Similarly, significant delay in phenology was observed in tetralone-ABA treated vines for all three cultivars until when clear inflorescences developed, but the difference in the numeric phenological stage between two treatments was smaller as compared to the previous year (Fig. 5A). The dates of budbreak were 05/01/2024, for 'Riesling' and 'Aravelle,' and 05/03/2024, for 'Cayuga White' in the control vines (Fig. 5A). In the tetralone-ABA treated vines, budbreak occurred on 05/05/2024, for 'Riesling and 'Aravelle,' and 05/07/2024, for 'Cayuga White,' representing a delay of 4 d, compared to the control vines in all three cultivars (Fig. 5A).

While the delay in days exhibited substantial differences between the two years, we also observed a significant difference in weather conditions: heat accumulation (calculated in GDH4) occurred much faster in the early spring of 2024 compared to 2023 (Fig. 5B). When transforming the delay (the gap in budbreak timing between control vines and tetralone-ABA treated vines) from days to GDH4, a more precise measurement of thermal units, the delay became much more consistent between the two years (Fig. 5B). The delays from tetralone-ABA treatment ranged narrowly between 812 GDH4 and 1,059 GDH4 across cultivars and years (Fig. 5B).

3.6. Growing season physiology and harvest yield

During the growing season in 2023, we measured photosynthetic efficiency and stomatal conductance on 06/28/2023, 07/11/2023 and 07/24/2023. Neither physiological parameter showed a difference between the two treatments in any of the three cultivars (Fig. 5C and D). During the growing season in 2024, we measured photosynthetic efficiency and stomatal conductance on 06/25/2024, 07/01/2024 and 07/08/2024. Except for the differences between the two treatments in g_{sw} of 'Riesling' on 07/08/2024 and *ETR* of 'Cayuga White' on 06/25/2024, neither physiological parameter showed a difference between the two treatments in any of the three cultivars (Fig. 5C and D).

In 2023, the experimental vines were harvested on 10/10/2023 for

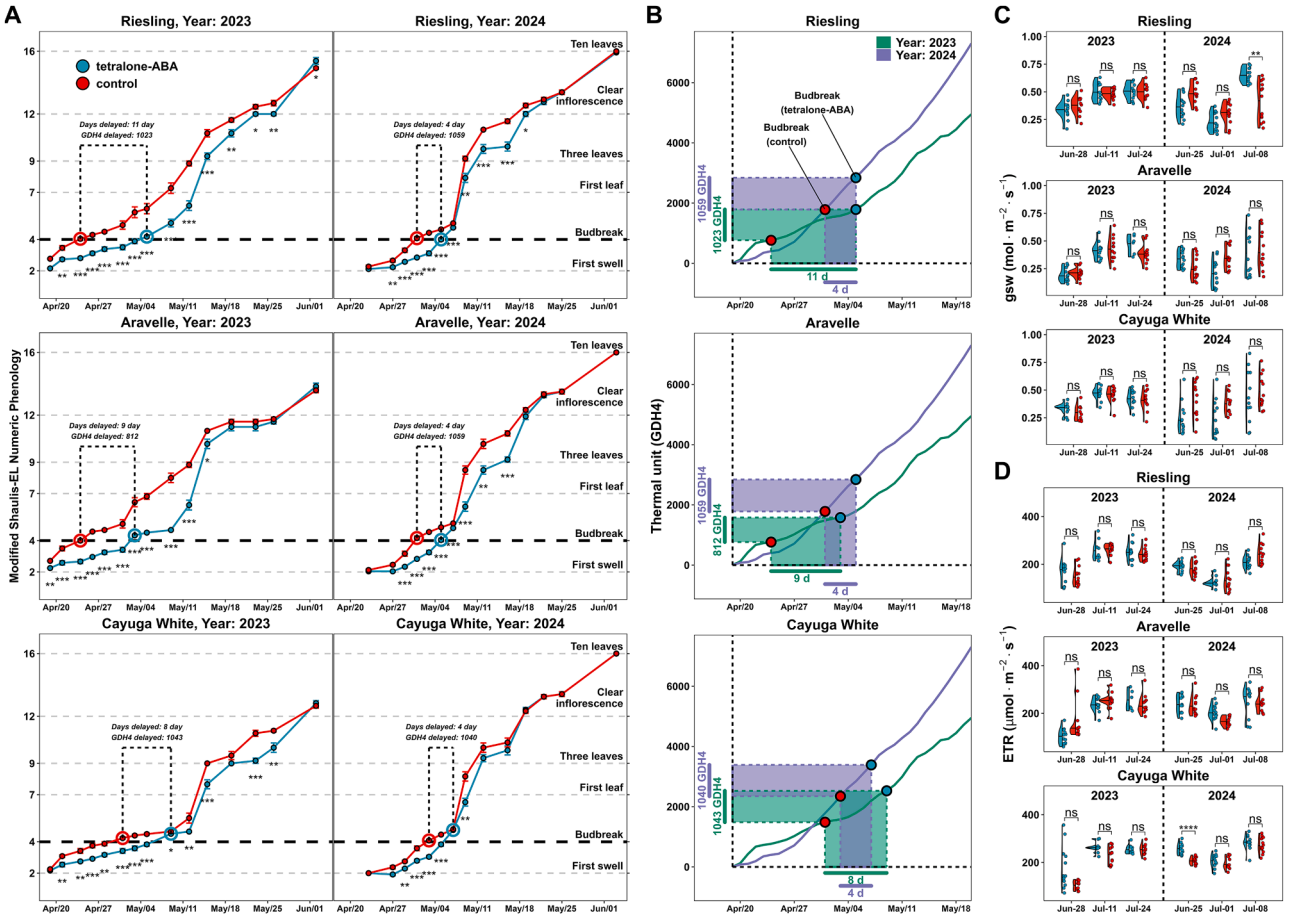

Fig. 5. The impact of tetralone-ABA on grapevine phenology and growing season physiology. A). The phenology of the three cultivars during the early growing season in 2023 and 2024. Error bar represents standard error ($n = 12$). All the observations after the last observation shown in the figure showed no difference between the two treatments. B) Comparison between day and GDH4 (growing degree hours using 4 °C as the base temperature) as a measurement for tetralone-ABA induced delay of budbreak. C) Stomatal conductance expressed as g_{sw} (mol·m^{-2}·s^{-1}). D) Relative photosynthesis efficiency expressed as *ETR* (µmol·m^{-2}·s^{-1}).

'Riesling' and 09/26/2023 for 'Aravelle'. In 2024, the experimental vines were harvested on 10/06/2024 for 'Riesling' and 09/16/2024 for 'Aravelle' and 'Cayuga White'. Harvest data, including clusters per vine and yield per vine, were collected to evaluate the treatment effect. The average cluster weight per vine was calculated based on clusters per vine and yield per vine. No differences were identified between the two treatments across the cultivars in both years (Figure S14).

4. Discussion

With a changing climate and shifts in frost risk, it is critical to develop and evaluate potential mitigation methods for reducing deacclimation risk and delaying budbreak phenology in perennial fruit crops. This research represents a detailed exploration of the effects of tetralone-ABA treatment as a novel sprayable product to enhance grapevine resilience in the dormant season in three grapevine cultivars: 'Riesling', 'Aravelle', and 'Cayuga White'. By analyzing the nuances of how such a treatment affects grapevine at both the molecular and physiological levels, the results of this study contribute to a more comprehensive understanding of grapevine dormant season biology, offering insights for enhancing grapevine resilience and informing sustainable viticultural strategies in an era of increasing environmental challenges under climate change.

In our study, we evaluated the efficacy of a new tetralone-ABA product, ABA-1102, in enhancing cold hardiness and delaying spring budbreak of three grapevine cultivars: 'Riesling', 'Cayuga White', and their progeny, 'Aravelle'. Shortly after tetralone-ABA application, we observed accelerated chlorophyll degradation and early defoliation in tetralone-ABA treated vines; however, the effect varies between different cultivars (Fig. 2A). This result contrasts with previous studies that reported no impact of tetralone-ABA on leaf senescence (Willwerth and Abrams, 2019). Effects were strongest for 'Riesling' while 'Cayuga White' had a much more limited reduction in SPAD readings (Fig. 2A). 'Aravelle', the offspring of 'Riesling' and 'Cayuga White', exhibited intermediate treatment effect compared to its parents (Fig. 2A). This cultivar specific response is intriguing as it suggests there may be differences in sensitivity to tetralone ABA between the *V. vinifera* and *V. hybrid* genetic backgrounds. The intermediate phenotype in 'Aravelle' supports this hypothesis, though many more cultivars and pedigrees would need to be evaluated to fully explore this response.

For two dormant seasons, tetralone-ABA significantly enhanced the bud cold hardiness of 'Riesling' during early winter in the process of initial cold acclimation but had no effect on 'Cayuga White' in the same period (Fig. 2C). In contrast, tetralone-ABA significantly enhanced the bud cold hardiness of 'Cayuga White' in late winter and during deacclimation, while the enhancement was less intensive in 'Riesling' (Fig. 2C). Much like the senescence response, tetralone-ABA's effect on enhancing bud cold hardiness in 'Aravelle' was intermediate to its effect on its parents (Fig. 2C). The intermediate response to tetralone-ABA in 'Aravelle', compared to its parents, potentially indicates that tetralone-ABA's impact on bud cold hardiness is also an inheritable quantitative trait. Differences in cultivar responses may also be explained by cultivar variation in endogenous ABA levels, as ABA metabolism dynamics have been identified as key regulators of dormancy transitions and cold hardiness in grapevines (Zheng et al., 2015, 2018; Wang et al., 2022b). Baseline endogenous ABA level may itself be an inheritable quantitative trait, yet no comprehensive phenotyping of this trait has been conducted. Our key finding is that tetralone-ABA induced similar physiological effects across all three cultivars. While cultivar-specific ABA metabolism may influence the magnitude or timing of responses, the overall trends observed remain consistent suggesting that its mode of action supersedes initial endogenous ABA concentrations. Enhanced bud cold hardiness during deacclimation as a response to tetralone-ABA has been reported in previous studies (Bowen et al., 2016; Willwerth and Abrams, 2019; Gunn, 2023), but this is the first time that enhanced bud cold hardiness during cold acclimation has been observed.

Tetralone-ABA had a clear effect on reducing the deacclimation rate in the deacclimation assays conducted on the buds with >1,000 chilling units (Fig. 3). This result supports observations that tetralone-ABA-induced slower deacclimation under field conditions in this study and others (Willwerth and Abrams, 2019; Gunn, 2023). Detailed examination of the bud transcriptome during the deacclimation assays indicates diverse transcriptomic regulation differences in control and tetralone-ABA treated vines. No differentially expressed genes were observed in the first deacclimation assay on early winter buds. In the second assay on mid-winter buds, only 108 genes were differentially expressed between treatments. The differential expression of these genes, especially under field condition, could be associated with the underlying mechanism of the decreased deacclimation rate observed in the second deacclimation assay. Although no pathway was significantly enriched among these genes, we noticed several genes in the pathways previously reported to have crucial biological functions in the dormancy or cold hardiness of grapevines. These genes included three genes encoding enzymes/proteins in ABA signaling (*VIT_14s0030g00690* encoding for ABI3, *VIT_15s0046g01050* encoding for RCAR1 and *VIT_13s0067g02130* encoding for ERD15), one gene in ethylene signaling (*VIT_05s0020g04180* encoding for CTR1), two genes in flower development (*VIT_01s0011g02120* encoding for HUA2 and *VIT_02s0025g04390* encoding for SEU1), five genes encoding for ribosome subunits, three highly expressed genes encoding for heat shock proteins, two genes encoding for histone metabolism, nine genes encoding for various transcription factors and six genes encoding for cell membrane transporters or channels (Figure S4).

Two large groups of genes demonstrated expression patterns that correlated with deacclimation and responded to tetralone-ABA in the third deacclimation assay. The behavior of these genes in our experiment suggests that the buds were either under endodormancy or transitioning from endodormancy to ecodormancy in the first two deacclimation assays, while by the third assay, they had fully transitioned to ecodormancy (Fennell et al., 2015; Kovaleski et al., 2018; Kovaleski, 2022; Noriega et al., 2024; Londo and Kovaleski, 2025). With tetralone-ABA treatment, the change of gene expression observed during deacclimation, whether increased or decreased, was significantly delayed (Fig. 4). Most of the enriched pathways among the genes that showed increasing expression during deacclimation are fundamental for plant growth, such as ribosome pathway, regulation of actin cytoskeleton pathway, photosynthesis-related pathway and cell cycle pathway (Fig. 4D) (Johnson and Lenhard, 2011). This finding aligns with previous research demonstrating that deacclimation is closely associated with the physiological, transcriptomic, and metabolomic changes to facilitate growth resumption in Arabidopsis (Pagter et al., 2017; Kutsuno et al., 2023), peach (Yu et al., 2020; Kwon et al., 2022), cherry (Fouché et al., 2023), apple (Palonen et al., 2021), grape (Kovaleski and Londo, 2019; Wang et al., 2022a), and other deciduous tree species (Rathore et al., 2022; Zuther et al., 2024; Walde et al., 2024). The genes with decreasing expression during deacclimation are enriched in the pathways such as ABA signaling, ethylene signaling, galactose metabolism, protein processing in the endoplasmic reticulum and HSF. ABA signaling, ethylene signaling, and galactose metabolism have been recognized or suggested to play significant roles in the regulation and development of grapevine cold hardiness (Nishizawa et al., 2008; Londo et al., 2018; Kovaleski and Londo, 2019; Rubio and Pérez, 2019; Wang et al., 2020, 2022a). Regarding the ABA-related pathways, most of the genes involved in ABA metabolism did not show significant response to tetralone-ABA (Figure S7). The only two genes that exhibited changes in expression in response to tetralone-ABA are *VIT_03s0063g00380*, encoding for ABA 8′-hydroxylase that degrades ABA, and *VIT_17s0000g02680*, encoding for ABA glucosidase that transforms ABA to its inactive form (Abrams and Loewen, 2019). The higher expression of *VIT_03s0063g00380* in the tetralone-ABA treated buds under field condition may suggest that tetralone-ABA treatment stimulates a response to degrade excessive active ABA-like metabolites as seen in the overexpression lines

(Figure S8A) (Thompson et al., 2007). This finding supports, although indirectly, that tetralone-ABA persists throughout the dormant season in grapevine and at the gene expression level performs as if ABA is being continuously applied to the bud tissue. This explanation also agrees with a previous study that reported detectable tetralone-ABA in grapevine buds by mid-April after the foliar application in the previous fall (Gunn, 2023). Assuming unchanged translation efficiency, the delayed upregulation of *VIT_17s0000g02680* in tetralone-ABA treated buds in the third deacclimation assay suggests the treatment may lead to a higher content of free ABA (Figure S8B), though direct measurements of endogenous ABA are needed to test this hypothesis. Downstream of ABA synthesis in the genes associated with ABA signaling, many genes encoding for the key proteins for ABA reception and signal transduction were found to be decreasing with deacclimation and the decreasing trend was delayed by tetralone-ABA (Figure S8C). This result indicates that, without tetralone-ABA, grapevine buds tend to downregulate ABA signaling pathway during deacclimation to facilitate the activation of growth-relative pathways, which aligns with previous findings (Kovaleski and Londo, 2019; Wang et al., 2022a; Liu et al., 2022a). With tetralone-ABA treatment, the downregulation of ABA signaling was delayed, thus likely promoting the expression of ABA responsive genes that contribute to prolonged dormancy. In the protein processing in the endoplasmic reticulum pathway, 17 out of 19 genes encode heat shock proteins. Coupled with the enrichment of HSF, these findings suggest the involvement of heat shock protein biosynthesis downregulation in temperature-regulated deacclimation, aligning with previous findings (Kovaleski and Londo, 2019; Majee et al., 2023).

We also manually evaluated several pathways or genes that were previously reported to be important for grapevine dormant season physiology to further evaluate the impact of tetralone-ABA on grapevine transcriptome. These include sugar metabolism-related pathways, such as galactose metabolism pathway, starch and sucrose metabolism pathway, cell wall pathways, ICE, CBF and DREB genes. In the sugar metabolism pathways and the cell wall pathway, we noticed that many genes that showed delayed upregulation under tetralone-ABA only encode for a short list of proteins (Figure S9, S10 and S11). All these proteins are involved in plant cell wall modification, and their detailed functions and involvement in plant dormancy release are briefed in Note S1. Synthesizing our findings and previous studies validates that cell wall remodification is a key process that occurs during deacclimation and dormancy release. Assuming the observed alteration in transcriptome reflects changes in metabolome, our results support a theoretical model of the remodification of cell wall during deacclimation and tetralone-ABA's impact during this process (Figure S15). Similarly, cell wall loosening has been proposed to be a critical factor in the dormancy release of woody perennials (Marowa et al., 2016; Beauvieux et al., 2018), and the application of hydrogen cyanamide induces dormancy release on dormant grapevine buds through reactive oxygen species (ROS) and reactive nitrogen species (RNS)-mediated cell wall autodegradation (Sudawan et al., 2016; Liang et al., 2019; Zhang et al., 2021; Pérez et al., 2021). The tetralone-ABA treatments in this study resulted in a delay in the synthesis of cell proteins and cell wall substrate degradation enzymes, potentially delaying the loosening and activation of the cell wall and, therefore, contributing to delayed budbreak (Figure S15).

The *ICE-CBF/DREB-COR* (*COLD REGULATED*) cascade for the genetic control of plant cold stress response is activated with the upregulation of *ICE*, followed by the *ICE*-induced upregulation of *CBF/DREB* that stimulates the expression of *COR*s that encode functional metabolites to improve cold hardiness (Hwarari et al., 2022). The upregulation of this cascade has been reported to function in the enhancement of grapevine cold hardiness, however, in non-overwintering tissues such as shoot, leaves and petioles (Tillett et al., 2012; Dong et al., 2013; Li et al., 2014; Karimi et al., 2015). In this study, none of the four copies of *CBF* in *V. vinifera* 12X.v2 genome showed detectable expression in control or tetralone-ABA treated buds in our study. This result disagrees with a previous study that reported thousand-fold of increase in the expression of *CBF*s after ABA treatment (Rubio et al., 2019a). Among the three *ICE*s and two *DREB*s with detectable expression, *VIT_13s0067g01960* and *VIT_14s0068g01200* revealed significant but divergent expression in response to deacclimation and tetralone-ABA (Figure S12). The undetectable expression of *CBF*s and the opposite expression patterns of *ICE* and *DREB* potential indicate that the function of CBF-ICE-DREB genes in dormant grapevine buds requires further investigation (Wang et al., 2024).

In our analysis of tetralone-ABA unresponsive deacclimation genes (Data S3), we noted that several of these genes encode key regulatory proteins in plant hormone signaling pathways (Figure S13). These genes have been previously reported to play pivotal roles in plant temperature sensing, abiotic stress responses or flowering timing (Ju et al., 2012; Wang et al., 2018; Bouzroud et al., 2020). Regardless of tetralone-ABA's impact on the transcriptome, these results indicate that, while the activation of growth-related pathways during deacclimation is largely influenced by ABA, which aligns with previous findings (Kovaleski and Londo, 2019), there exists an additional ABA-independent regulatory component during deacclimation. Previous findings suggest that plant deacclimation is a complex, multifaceted process regulated not only by ABA but also by a variety of other mechanisms. These include ethylene and auxin signaling pathways that contribute to growth resumption and stress responses (Pagter et al., 2017; Kovaleski and Londo, 2019), temperature-responsive gene networks that sense and integrate environmental cues (Vyse et al., 2022), circadian rhythm and photoperiodic control that help synchronize physiological changes with seasonal transitions (Liu et al., 2022b), and dormancy-associated MADS-box genes along with hormonal cross-talk that coordinate the release from dormancy (Voogd et al., 2022). Future research is needed to evaluate the stand-alone effect of ABA and its interplay with other regulatory factors during deacclimation.

Under field conditions, there was a significant delay in budbreak on tetralone-ABA treated vines across three experimental cultivars over two years. While we observed variability in the delay between cultivars and years when measured in days, when converting Julian days to thermal units using GDH4, the phenology delay was observed consistently around 1,000 GDH4 across cultivars and years (Fig. 5A and B). While endogenous ABA and tetralone-ABA levels were not measured in plant tissues, this consistent delay suggests that tetralone-ABA may have a functional catabolic threshold of around 1,000 GDH4. This hypothesis remains to be tested across additional growing seasons and in different regions but also offers the potential to model the potential budbreak delay using historical temperature datasets. The results of this study suggest three key points: 1) nearly six months after tetralone-ABA application on the foliar, there is sufficient active tetralone-ABA in the vines to result in significant and consistent delay in budbreak; 2) the delay is likely associated with resistance to growth when exposed to accumulated heat, and the degree of resistance is likely consistent across cultivars and years; 3) for future studies, GDH4 or other measurements of thermal units or chilling units are better metrics for quantifying the delay or advancement of phenology resulting from treatment. After budbreak, the delaying effect on grapevine growth varied between different cultivars, but no phenological difference could be observed after clear inflorescence is developed. Physiological measurements during the growing season and harvest data showed minimal difference between tetralone-ABA treated vines and control vines (Fig. 5C and D). Although we did not conduct fruit quality and wine chemistry analysis, our results indicate that tetralone-ABA treatment effectively induces budbreak delay without negatively impacting growing season physiology and yield (Fig. S14).

Overall, what underlying mechanism drives the transcriptomic and physiological changes observed in this study? Our findings support previous studies that show that deacclimation in grapevine is closely correlated with the activation of growth-related pathways and genes (Kovaleski and Londo, 2019), and tetralone-ABA application delays this

process. We assume that due to its structural differences from natural ABA, remains active without undergoing degradation through the ABA catabolism pathway (Diddi et al., 2023). Because tetralone-ABA is functionally analogous to ABA, it presumably continuously activates the ABA signaling pathway throughout the dormant season, effectively prolonging ABA-mediated dormancy responses and delaying deacclimation. The observed transcriptomic changes induced by tetralone-ABA reveal a broad and persistent ABA response, which masks the natural progression of dormant season physiology. This further suggests that the genes we identified as tetralone-ABA-responsive represent a significant portion of the ABA-regulated transcriptome under natural conditions.

The findings of this study indicate tetralone-ABA is a promising viticultural tool for enhancing dormant season resilience in grapevines. Its potential integration into vineyard management could be beneficial in the regions where unpredictable temperature fluctuations pose risks to grapevine survival and productivity. Future research should focus on optimizing the application of tetralone-ABA, including its concentration, timing, and frequency, to maximize its efficacy across diverse grape cultivars. Further exploration of the genetic and metabolic mechanisms underlying cultivar-specific responses to tetralone-ABA will provide valuable insights into the adaptive strategies of different *Vitis* genotypes for winter survival. Expanding this research to other perennial crops could also contribute to broader agricultural applications to mitigate climate-induced risks in fruit production.

5. Conclusion

In conclusion, post-harvest foliar application of tetralone-ABA on grapevines induced faster leaf chlorophyll degradation, earlier defoliation, enhanced cold acclimation, slower deacclimation, and delayed budbreak without affecting growing season physiology or harvest yield. Transcriptome analysis of grapevine buds during deacclimation indicates that multiple fundamental pathways for growth restoration are upregulated during deacclimation, and tetralone-ABA treatment delayed this upregulation. These results align with our hypotheses. The slower deacclimation in tetralone-ABA treated buds observed in both field and controlled environment conditions is likely a consequence of tetralone-ABA's delaying effect on the regulation of key functional pathways during deacclimation. The results presented in this study not only indicate the feasibility of using tetralone-ABA as a sprayable product to enhance viticultural resilience in dormant season but also contribute to our understanding of the genetic control of grapevine deacclimation. Optimization of treatment application remains to be conducted. Additionally, since ABA has similar functions in other plant species, further testing of tetralone-ABA's effectiveness in other perennial fruit crops could help develop novel methods to combat climate change.

CRediT authorship contribution statement

Hongrui Wang: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Yue Pan:** Investigation, Data curation. **Jason P. Londo:** Writing – review & editing, Writing – original draft, Supervision, Resources, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.stress.2025.100861.

Data availability

All RNA-seq raw data along with processed gene count matrix and sample metadata are available in NCBI-GEO (accession: GSE273240).

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