

Low temperatures trigger jasmonic acid signaling in grapevine bud dormancy release

Las bajas temperaturas desencadenan la señalización del ácido jasmónico en la liberación de la latencia de las yemas de la vid

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ABSTRACT

Jasmonic acid (JA) is a signaling molecule that regulates plant development and defense processes. Several studies have shown that basal JA levels increase and stimulate plant protection systems when exposed to low temperatures. In grapevine, low temperatures contribute to both the onset and offset of bud dormancy. This study is the first to explore the levels and expressions of genes related to the JA synthesis pathway and signaling in dormant grapevine buds under the effects of accumulated exposure to cold temperatures. JA quantification was performed using HPLC, and gene expression analysis was performed using RT-qPCR after dormant buds were exposed to 600 and 900 chilling units (CUs). This work presents evidence that the accumulation of CUs stimulates the expression of genes involved in JA biosynthesis pathway (AOS) and signaling (*COI1*, *JAZ1*, and *VSP2*), which in turn increases JA levels in dormant grapevine buds. These findings enhance our understanding of dormancy regulation and open new possibilities for improving grapevine cultivation. It is concluded that the accumulation of 600 and 900 CUs activates the expression and signaling of JA-associated genes in the dormant grapevine buds, thereby shedding new light on the role of JA in grapevine dormancy under low temperature.

Keywords: Phytohormones, dormant buds, abiotic stress, chilling units.

RESUMEN

El ácido jasmónico (AJ) es una molécula señal clave en procesos de desarrollo y defensa en plantas. Diversos estudios han demostrado que los niveles de AJ aumentan al exponer las plantas a bajas temperaturas, activando mecanismos de defensa. En vid, el frío influye tanto en el inicio como la finalización de la latencia de las yemas. Este estudio es el primero en analizar niveles de AJ y la expresión de genes involucrados en su síntesis y señalización en yemas latentes de vid sometidas a frío. Se cuantificó el AJ mediante HPLC y se evaluó la expresión génica por RT-qPCR en yemas de vid expuestas a 600 y 900 unidades frío (CUs). Los resultados muestran que la acumulación de CUs estimula la expresión

de genes involucrados en la síntesis y señalización de AJ: AOS, *COI1*, *JAZ1* y *VSP2*, incrementando los niveles en las yemas. Estos hallazgos contribuyen al mejor entendimiento del papel del AJ en la regulación de la latencia inducida por frío. En conclusión, la acumulación de 600 y 900 CUs activa la expresión de genes asociados al AJ en yemas latentes, resaltando su participación en la respuesta de la vid a bajas temperaturas y su potencial aplicación en viticultura.

Palabras clave: fitohormonas, yemas latentes, estrés abiótico, unidades de enfriamiento.

INTRODUCTION

Temperature, as an environmental factor, significantly impacts the growth and development of plants. Low temperatures, a key stress factor, can drastically affect crop productivity (Kopecká *et al.*, 2023). These low temperatures include both non-freezing (2-6 °C) and freezing (< 0 °C) temperatures. Non-freezing temperatures are associated with cold stresses that influence plant development (Aslam *et al.*, 2022). Plants exhibit remarkable cold tolerance by activating various biochemical mechanisms. These adaptations include changes in membrane composition, increased activity of certain enzymes, and alterations in cell solute content and cryoprotectant compounds (Pauwels and Goossens, 2011; Raza *et al.*, 2023). While these mechanisms are triggered by individual signaling pathways, they are often mediated by hormones that play a crucial role in stress responses. Notable hormones involved include salicylic acid (SA), jasmonic acid (JA), abscisic acid (ABA), and ethylene (ET) (Orrantia-Araujo *et al.*, 2020; Raza *et al.*, 2023).

Jasmonic acid is an endogenous growth-regulating substance and a signaling molecule that induces defense mechanisms and regulates developmental processes (Wang *et al.*, 2021). It is well established that plants exposed to biotic stresses, such as insect and herbivore damage, increase their basal levels of JA (Ghorbel *et al.*, 2021). Similarly, JA levels rise in response to abiotic stresses, such as ozone, drought, salinity, and low temperatures (Per *et al.*, 2018; Rehman *et al.*, 2023).

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Specifically, low temperatures stimulate JA synthesis through the octadecanoid pathway, which involves the enzymes lipoxygenase (LOX), allene oxide synthase (AOS), allene oxide cyclase (AOC), and oxyphydienic acid reductase 3 (OPR3), triggering a signaling cascade initiated by coronatine insensitive1 (*COI1*) gene, which represses the expression of the jasmonate zim gene (*JAZ1*), preventing that JAZ protein suppressing the expression of response genes such as vegetative storage proteins (*VSP2*) (Wang *et al.*, 2021).

Low temperatures play a crucial role in bud dormancy in perennial deciduous plants, in which the buds enter a state of deep dormancy induced by shorter photoperiods and/or low temperatures. Dormancy is a critical physiological characteristic that not only provides protection against environmental stress, but also affects the synchronization of essential developmental stages, such as bud break and flowering, which in turn have a significantly impact in fruit production and quality (Ding *et al.*, 2024).

The grapevine (*Vitis vinifera* L.), a standout among deciduous perennial plants, is renowned for its wide distribution and the scale of fruit production it supports (Monteiro *et al.*, 2021). Despite being an economically important crop globally, there is little knowledge about the JA content and signaling in the bud dormancy of this species. This is unexpected given that the JA signaling pathway plays a crucial role in cold acclimation, a process closely linked to dormancy (Xu *et al.*, 2016).

During dormancy, the buds accumulate chilling units (CUs) and remain inactive until their chilling requirement is satisfied (Rahemi *et al.*, 2021). In grapevine, buds must accumulate between 50 and 400 CUs to break dormancy (Dokoozlian, 1999). Given the importance of low temperatures in the development of grapevine bud dormancy, a wide variety of studies have been conducted to understand the biochemical events that occur in dormant buds during and after cold accumulation (Monteiro *et al.*, 2022; Chen *et al.*, 2025). However, a considerable gap remains in the literature concerning content and signaling of JA-related genes in dormant grapevine buds exposed to low temperatures. This study aimed to evaluate the effects of low temperatures on the content and signaling of JA in dormant grapevine buds, to advance our understanding of grapevine physiology and hormone signaling significantly. This knowledge is particularly relevant to the Pesqueira, the main table grape producing region in Sonora, Mexico, which stands out especially for the cultivation of the cv. 'Flame Seedless'. This region presents unique challenges in producing table grapes over a large area (Cebreros *et al.*, 2022); the warm and arid climate, with winter temperatures typically around 6 °C and summer temperatures around 38 °C, poses difficulties. The accumulation of CUs required to initiate budbreak is unfavorable, leading to a delayed and extended period of budbreak under natural conditions. The chilling requirement of the grapevine cv. 'Flame Seedless' buds is not well established; therefore, after the grapevine buds accumulate ~100 CUs, producers often apply chemical treatments to induce a timely and relatively uniform budbreak, thereby initiating the growing season.

MATERIAL AND METHODS

Plant Material and Treatment

In a commercial vineyard of the Pesqueira table grape-producing region, canes with five dormant buds (floral and vegetative) from several older vines (*Vitis vinifera* L. cv. 'Flame Seedless'), without prior treatment, were collected at random after pruning on December 5th of 2018. The canes had accumulated an average of 100 natural CUs at harvest time. The Richardson model was used to calculate the chilling units accumulated in canes, based on data collected from an automatic weather station located approximately 12 km from the vineyard (Richardson *et al.*, 1974). The canes were coated with moist sawdust and immediately moved to the laboratory; before undergoing treatment, they were submerged in water, drained for a few minutes, arranged into bundles of 10 canes (each bundle was one experimental unit), and covered with paper towels. The experimental unit was randomized and assigned to one of three groups: two for treatment and one for control. The control group was placed in 2-liter plastic beakers filled with 7 to 10 cm of distilled water as a base and was weekly changed. The control unit was immediately placed in a growth chamber at 25 ± 1 °C, under a photoperiod of 16 h of light, eight hours of darkness, and the buds of each cane were excised for sampling at 0, 3, 6, 9, and 24 h (Kollist *et al.*, 2019). The remaining two groups were kept in a chamber at 4 ± 1 °C: one group until buds accumulated 600 CUs, and the second group for 34 days to reach 900 CUs. It was assumed that one hour spent below 7.2 °C corresponds to the accumulation of 1 CU (Dokoozlian, 1999). Once buds had accumulated 600 or 900 CUs, respectively, canes were placed in 2-liter plastic beakers filled with 7 to 10 cm of distilled water and transferred to a growth chamber at 25 ± 1 °C, and buds were handled identically to the control group (Andreini and Scalabrelli, 2009). Three replicates of each treatment were included. The dormancy status of the buds with 900, 600, or 100 CUs was considered endodormant, according to their metabolic heat values (*R_q*) of 1.7, 1.8, or 2.2 $\mu\text{W mg}^{-1} \text{dw}^{-1}$, respectively (Orrantia-Araujo *et al.*, 2020). The *R_q* values were determined by isothermal scanning calorimetry following the methodology by Gardea *et al.* (2000).

Quantification of jasmonic acid content of grapevine buds

The extraction of JA was carried out on excised buds from canes after the cold treatment and on the control, sampling at 0, 3, 6, 9, and 24 h. Samples of 2.5 g of buds per treatment were ground using liquid nitrogen until a fine powder was obtained, and then resuspended with 10 mL of ultrapure water and mixed with an Ultra Turrax T25 (Janke and Kunkel, IKA-Labortechnik, USA). The mixture was centrifuged at $10,000 \times g$ for 15 min (RC 5C plus centrifuge; Sorvall, USA). The supernatant was adjusted to a pH of 2.8 using 15 % acetic acid, and subsequently, a double liquid-liquid extraction was performed using equal amounts of cold diethyl ether. The aqueous phase was removed using a phase separator filter paper (Whatman; GE Healthcare Life Sciences, USA),

and the two organic phases were combined and dried using a gaseous nitrogen stream. The solid residue was dissolved in 500 μL of 50 % methanol/water (v/v) and filtered for HPLC analysis. The remaining liquid was called bud extract.

The JA content was quantified using high-performance liquid chromatography (HPLC) according to the techniques established by Orrantia-Araujo *et al.* (2020). The HPLC instrument (Agilent Technologies Infinity 1260, Palo Alto, CA, USA) was equipped with a quaternary pump connected to a diode array detector (DAD) (Agilent Technologies Inc., Germany). A volume of 100 μL of the bud extracts was injected into an Eclipse plus C-18 column (4.6 x 100 mm length), and a particle diameter of 3.5 μm . The column was maintained at room temperature and operated at a flow rate of 0.3 mL per minute. The mobile phases were made up of A (100 % methanol) and B (0.2 % glacial acetic acid in water), which were combined in a linear gradient. This gradient began with a ratio of 10/90 (A/B) for 10 min, then changed to 50/50 (A/B) for 5 min, followed by 100 % (A) for 5 min. These conditions were maintained for a total of 25 min. Subsequently, the mobile phase was restored to its initial condition. The JA was monitored at a wavelength of 303 nm using a DAD detector. For the quantification of endogenous concentrations of JA, two standard calibration curves were performed using diluted solutions of JA standard with a purity of 98 % (Sigma-Aldrich, USA). The first curve was in the range of 0.005 to 0.02 mg mL^{-1} , the second curve was from 0.05 to 0.2 mg mL^{-1} . The coefficient of determination (R^2) of the standard calibration curves was greater than 0.98.

RNA extraction and cDNA synthesis

The RNA extraction was performed following the methodology by García-Baldenegro *et al.* (2015). Total RNA for each treatment was extracted from 12 buds per sample, with three replicates. Each sample containing five μg of total RNA was treated with RNase-free DNase I (3U for 10 μg^{-1} RNA) following the manufacturer's instructions (QIAGEN, USA). The quality and concentration of RNA were assessed by measuring the absorbance ratios of A_{260}/A_{280} and A_{260}/A_{230} with a NanoDrop 2000 (Thermo Scientific NanoDrop, USA). The RNA integrity was evaluated by electrophoresis on a 0.1 % formaldehyde

agarose gel. The first-strand complementary DNA (cDNA) was synthesized using two μg of total RNA with reverse transcriptase employing oligo dT from the Superscript™ II First-Strand Synthesis System kit, ideal for RT-qPCR (Invitrogen, USA).

Quantitative real-time PCR

The genes allene oxide synthase (AOS), coronatine insensitive1 (*COI1*), jasmonate zim-domain repressors (*JAZ1*), and vegetative storage protein 2 (*VSP2*) were analyzed using RT-qPCR. The sequences corresponding to each gene studied were taken from the Genoscope database, within the Grape Genome Browser (Genoscope, 2018). Specific primers were created (Table 1) by the online interface of the PRIMER3 plus software (<http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plusAbout.cgi>) (Rozen and Skaletsky, 2000).

Each PCR reaction was conducted in a total volume of 20 μL , which contained 5 μL of cDNA (4 ng), 10 μL of iTaq Universal Sybr Green Supermix (Bio-Rad Laboratories, USA), 1 μL of forward and reverse primers 5 μM each, along with ultrapure water obtained from the Milli-Q system. The amplification was conducted with the StepOne™ Real-Time PCR System (Applied Biosystem Inc., USA). The conditions for the PCR were set at 95 °C for 10 min, followed by 40 cycles consisting of 95 °C for 15 s and 60 °C for 1 min. Serial dilutions of cDNA were performed for each set of primers, and specific calibration curves were obtained for each gene. The calculations of mean values and standard deviations were based on three biological samples, each comprising three technical replicates. The normalization of data for each gene of interest was carried out using actin (*ACT*) as the reference gene (Tashiro *et al.*, 2016). To determine the relative expression levels of the genes, the $2^{-\Delta\Delta CT}$ method was followed (Livak and Schmittgen, 2001).

Statistical Analyses

The experiment was conducted using a completely randomized design, and the data on endogenous JA levels and gene expression levels at different times were examined using a one-way analysis of variance (ANOVA). Additionally, the Tukey-Kramer test ($\alpha = 0.05$) was used to test the significance of differences in means. Both statistical analyses were

Table 1. Primers used for qPCR.

Tabla 1. Primers utilizados para qPCR.

Gene	Forward primer (3'-5')	Reverse primer (5'-3')
VvAOS (GSVIVT01031885001)	GCGGTGGAGGGCATGTATGT	GCCACCTGGTCTTTCATGG
VvCOI1 (GSVIVT01016368001)	TACCTGCAAGACCGACCCACAT	CGTGTGCTCCTGGTGCTT
VvJAZ1 (GSVIVT01015042001)	TTCACCGTTCTTGAGAAG	GTGTCTCTTCAGGCTTTGG
VvVSP2 (GSVIVT01015277001)	TGTGACATTCCTCTGGGGTTC	GAATGGGTGAATTGGCTGAGG
VvACT (GSVIVT01026580001)	GCTGAGAGATTCCGTTGTCC	GCCACCACCTTGATCTTCAT

The codes in parentheses indicate the identifier of the genoscope annotation region of each gene.

performed with the NCSS software, version 2007 (Kaysville, USA) (Hintze, 2007).

RESULTS

Jasmonic acid content in dormant buds of grapevine

The endogenous JA content of cold-treated and control buds is shown in Figure 1. The buds that accumulated 600 or 900 CUs and were subsequently transferred at 25 °C (forcing condition to budbreak), showed a significant increase in JA contents and maintained this high level for the first three hours under forcing conditions, with maximum concentrations of 37 $\mu\text{g g}^{-1}\text{FW}^{-1}$ in buds treated with 900 CUs. This value was 1.3 times higher than the one displayed by the bud with 600 CUs at the same time. In both groups of cold-treated buds, the JA content showed a sharp decrease from 3 to 6 h, where the drop was most pronounced ($p < 0.05$) in the buds with 900 CUs, which maintained low JA levels from then on. Meanwhile, in the control buds with 100 CUs, JA content was undetectable throughout the assessment period. These findings suggest that in dormant grapevine buds, JA content increases in response to low temperatures.

Expression pattern of jasmonic acid signaling genes in dormant grapevine buds

The analysis of cold-treated and control buds using Real-time PCR (Figure 2) showed that the transcript level of the JA synthesis gene AOS presented an increased expression in buds that accumulated 600 CUs, with the highest transcript

level reached at three hours under forcing conditions, with a five-fold increase of the initial value. The level decreased slightly from 3 to 6 h but remained steady until the end of the assessment period (Figure 2A). The buds that accumulated 900 CUs kept low AOS transcript levels during the evaluation, with a pattern like the control, except that the extent of the transcription levels differed from the beginning of the assessment, where the levels of transcripts were more pronounced ($p < 0.05$) in the buds with 900 CUs. The control buds showed the lowest AOS transcription level ($p < 0.05$), only detected at the beginning of the experiment and at 9 hours (Figure 2A).

The expression level of the JA *COI1* receptor gene in buds with 600 CUs showed two peaks at 3 and 24 h, compared to the buds with 900 CUs, in which levels of expression were low and remained almost stable throughout the assessment period (Figure 2B). In the control buds, the expression of the *COI1* gene exhibited a significant decline within the initial six hours and remained low (Figure 2B).

The expression pattern of the *JAZ1* gene in buds of both cold treatments, 600 and 900 CUs, showed similar trends with low transcript levels compared to the control. Even when the buds treated with 600 CUs presented twice the transcript level ($p < 0.05$) than buds treated with 900 CUs (Figure 2C), the *JAZ1* transcript level was low. In contrast, *JAZ1* gene expression was evident from the first hour in control buds, presenting the highest level of expression with a twelve-fold increase of the initial value at six hours, then decreasing significantly until nine hours ($p < 0.05$), reaching levels similar to those at the beginning of the experiment at 24 h.

The expression of the response gene *VSP2* in the buds treated with 600 CUs showed the highest values ($p < 0.05$), with a gradual increase during the first nine hours, and it reached the highest transcript level after this time, presenting a value eight times greater than the original value. In buds with 900 CUs, the *VSP2* transcript level increased slightly from 6 to 9 h, with a subsequent decrease at 24 h, returning to the initial value. The control buds presented a slight increase in the expression levels at six hours (Figure 2D). Its value subsequently decreased for nine hours, then offered a second slight increase observed at 24 h, reaching a value lower than the initial one.

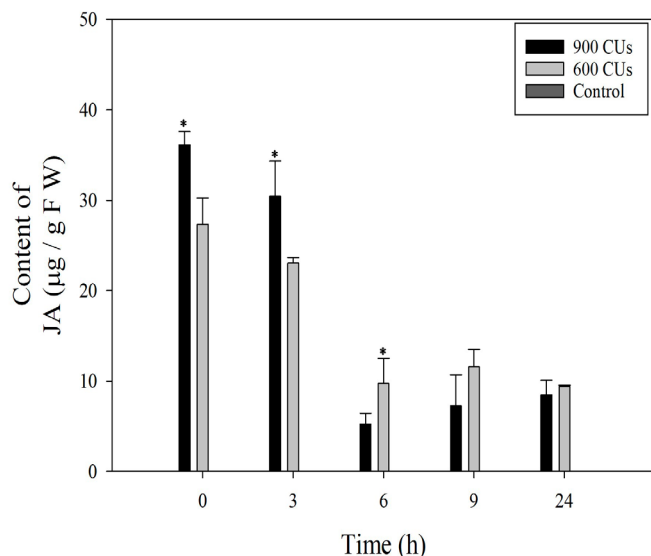


Figure 1. Jasmonic acid content of dormant buds of cv. 'Flame Seedless' grapevines induced by cold (chill units, CUs). Bars represent the average values of three biological replicates, and error bars indicate the standard error. Asterisks indicate mean values that are significantly different as determined by a Tukey test ($p < 0.05$).

Figura 1. Contenido de ácido jasmónico en yemas latentes de vid del cv. 'Flame Seedless' inducidas por frío (unidades de frío, CUs). Las barras indican los valores promedios de tres réplicas biológicas y las barras de error, indican los errores estándar. Los asteriscos indican valores medios significativamente diferentes, determinados mediante la prueba de Tukey ($p < 0,05$).

DISCUSSION

Dormancy is a complex and highly regulated process that allows deciduous perennial plants to survive adverse environmental conditions during winter (Rahemi *et al.*, 2021). This process is influenced by various internal and external factors. Certain phytohormones have been identified as key internal molecules that participate as regulators of dormancy in perennial plants (Singh *et al.*, 2018; Yamane *et al.*, 2019; Li *et al.*, 2020; Orrantia-Araujo *et al.*, 2020). Although JA is recognized as actively involved in acclimation to low temperature (Raza *et al.*, 2021), its specific involvement in the transition from dormancy to active bud break in grapevines has not been extensively studied. To investigate the role of JA in breaking

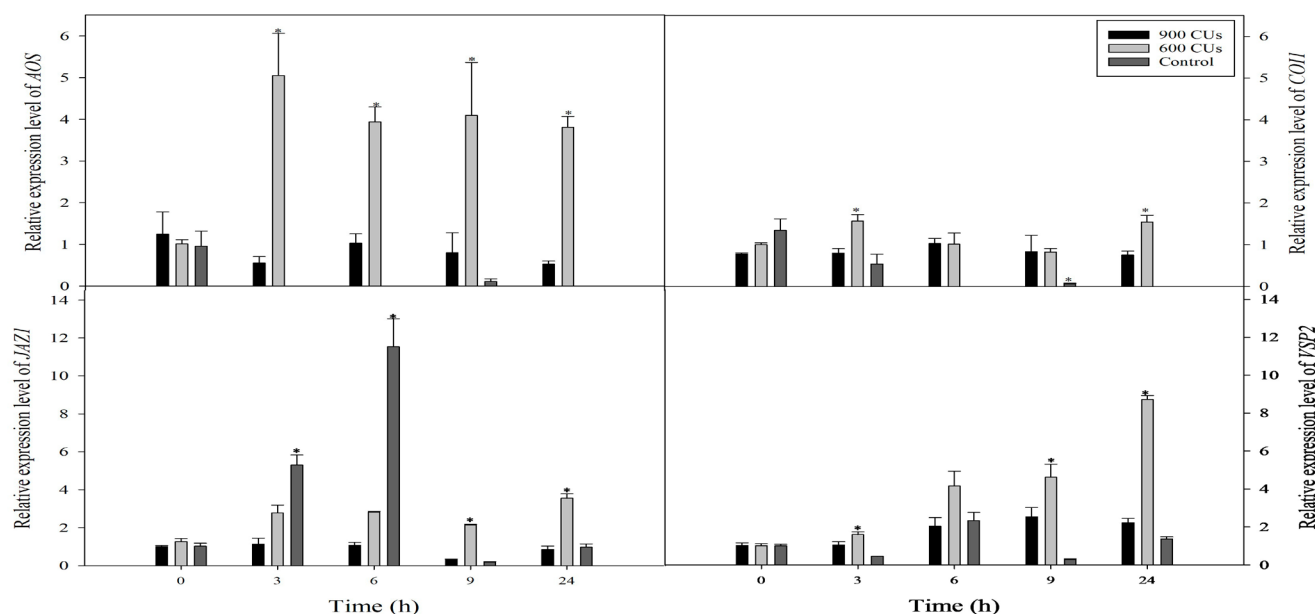


Figure 2. Relative expression levels of the *AOS*(A), *COI1*(B), *JAZ1*(C) and *VSP2*(D) genes in the dormant cv. 'Flame Seedless' grapevine buds induced by chilling (chill units, CUs). Data are presented as the averages of three biological replicates, each with three technical replicates, and error bars indicate the standard error. The asterisks indicate significant differences (Tukey test ($p < 0.05$)).

Figura 2. Niveles relativos de expresión de los genes *AOS*(A), *COI1*(B), *JAZ1*(C) y *VSP2*(D) en yemas latentes de vid del cv. 'Flame Seedless' inducidas por frío (unidades de frío, CUs). Los datos se presentan como promedios de tres réplicas biológicas, cada una con tres réplicas técnicas y las barras de error, indican los errores estándar. Los asteriscos indican diferencias significativas (prueba de Tukey ($p < 0,05$)).

dormancy in grapevines, the dormant buds of the cv. 'Flame Seedless' was subjected to artificial cold treatments and the JA content and the expression of genes involved in the JA synthesis pathway and signaling processes, were measured.

The accumulation of chilling units (CUs) plays a crucial role in stimulating the production of JA. Buds that accumulated 600 CUs maintained a JA content above $10 \mu\text{g g}^{-1} \text{FW}^{-1}$, and buds that accumulated 900 CUs maintained high levels in the first three hours, showing very low values after this time. The control buds, which were not subjected to any cold treatment, showed no detectable JA content. These results confirm that low temperatures induced JA accumulation in grapevine buds. The low temperatures induce de-esterification of galactolipids from chloroplast membranes (Ullah *et al.*, 2025), releasing α -linolenic acid (α -LeA), a substrate for the synthesis of JA (Ullah *et al.*, 2025). Studies with rice and *Arabidopsis* have reported that as cold stress rises, JA content increases, triggering the regulation of the expression levels of the JA-associated genes (Hu *et al.*, 2013; Kim *et al.*, 2021), which stimulate the budbreak in perennial plants (Juvany *et al.*, 2015), showcasing the complexity of dormancy. The accumulation of 600 or 900 CU in grapevine buds (cv. 'Flame Seedless') also leads to budbreak, when buds satisfy the chilling requirements for the transition from dormant to eco-dormant buds (Rahemi *et al.*, 2021), which budbreak when transferring to favorable climatic conditions (25°C) (Orrantia-Araujo *et al.*, 2020).

Even though this study did not quantify α -linolenic acid (α -LeA) content in cold-treated buds, the expression of the JA synthesis gene (*AOS*) was quantified, reaching an expression

level of four-fold higher in buds that accumulate 600 CUs than buds with 900 CUs. The JA endogenous content could be the reason why the synthesis gene *AOS* was expressed in buds with 600 CUs (Kim *et al.*, 2021), and not in buds with 900 CUs (Figure 2A), since it has been reported that there is a correlation between the increase in endogenous JA concentrations and the release of dormancy, reported in apple seeds (Sapkota *et al.*, 2023). Values below $5 \mu\text{g g}^{-1} \text{FW}^{-1}$ of JA failed to release dormancy. The JA content in the 900 CUs treatment buds could induce one of the other JA synthesis genes, perhaps *LOX*, *AOC*, *OPR3*, but not *AOS* (Kim *et al.*, 2021). This finding could be clarified in future research. Even with the disproportionate responses of JA content and *AOS* expression, the results indicate that both cold treatments induced the accumulation of JA in grapevine buds. The control buds, which were not subjected to any cold treatment, showed no detectable JA content (Figure 1) or expression of the *AOS* gene (Figure 2A).

Several reports on the bioactivity of JA (Ghorbel *et al.*, 2021; Kim *et al.*, 2021), found that JA content induces the expression of JA-associated genes, such as the *COI1* receptor gene. This finding has significant implications for our understanding of plant dormancy and signaling pathways. When JA levels increase, the *COI1* receptor initiates signaling by promoting the degradation of JAZ proteins and inducing the expression of response genes from JA (Wasternack and Strnad, 2016). In buds with 600 CUs, we observed higher levels of *COI1* gene expression in the first six hours, with a lower expression in buds with 900 CUs (Figure 2B). This pattern of expression in the initial hours of low temperature

has been reported for rice (Du *et al.*, 2013) and dormant buds of *Euphorbia esula* L. (Chao *et al.*, 2016). The level of *COI1* expression was sufficient to cause degradation of the JAZ proteins, as evidenced in the cold-treated buds, where *JAZ1* expression levels were lower than in the control buds (Figure 2C). The high levels of *JAZ1* expression observed in control buds suggest an abundance of JAZ proteins, leading to the repression of *VSP2* gene expression (Ghorbel *et al.*, 2021). The level of *JAZ1* expression in buds treated with 600 and 900 CUs was insufficient to halt the JA response signaling, as evidenced by the detected expression of the *VSP2* gene (Figure 2D) (Wasternack and Strnad, 2016).

In perennials such as grapes, increases in JAZ proteins repress the expression of *Flowering Locus T* genes (*FT*) involved in flowering and in the development of dormancy of vegetative buds (Sreekantan *et al.*, 2010; Zhai *et al.*, 2015). Therefore, the lower expression of *JAZ1* observed in our cold-treated buds could positively affect dormancy release. However, further studies are required to fully understand and test this idea.

The activation of JA signaling, ultimately reflected in increased expression of the response gene *VSP2* (Ghorbel *et al.*, 2021), was observed in the cold-treated buds (Figure 2D). The expression of *VSP2* in *Arabidopsis* depends on *COI1*, and its expression occurs preferentially in growing organs such as buds and flowers (Utsugi *et al.*, 1998).

The high levels of expression of *VSP2* observed in the cold-treated buds were congruent with *COI1* level expression, mainly observed in buds with 600 CUs, in which higher expression of the *VSP2* gene occurred (Figure 2D). The *VSP2* gene expression level in grapevine buds can contribute to the pool of nutrients needed for plant growth and maintenance, since the vegetative storage proteins (VSP) have been reported to function in the temporary storage of amino acids, participating in the redistribution of nitrogen (Xiong *et al.*, 2025). Therefore, once the buds have accumulated the cold requirement and had the appropriate growth conditions, the degradation of the VSP possibly begins, providing the substrates required for respiration and other important metabolic events for the budbreak and early stages of growth (Monteiro *et al.*, 2022). Meanwhile, in the control buds, the low expression of *VSP2* could affect defense against future stress situations or the establishment of later development events.

Changes in the expression levels of *AOS*, *JAZ1*, *COI1*, and *VSP2* in dormant grapevine buds may indicate activation of JA-associated genes upon exposure to cold temperatures. Nonetheless, how plant hormones control dormancy is poorly understood, mainly due to the extensive interdependent interactions among plant hormones. Therefore, validating whether JA is closely related to regulating cold acclimation and dormancy release in grapevine buds is necessary. Generating this knowledge is relevant to improve growth management and stress adaptation in perennial crops such as grapevine.

CONCLUSIONS

The accumulation of 600 or 900 CUs in dormant grapevine buds activates the expression of JA pathway-associated genes, such as *AOS*, *JAZ1*, *COI1*, and *VSP2*. This activation could be a key player in cold acclimation and dormancy release in grapevine buds. However, due to the complex interplay between plant hormones, further validation is still required to confirm the specific role of JA in these physiological processes.

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CONFLICTS OF INTEREST

The authors state that they have no personal or financial conflicts of interest to disclose.

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