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ORIGINAL ARTICLE

Cold Hardiness in 'Alphonse Lavallee' (*Vitis vinifera* L. *cv*) Grape Dormant Buds and Phloem Tissue: Seasonal Insights and Some Treatment Impacts

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Abstract
Grapes are highly susceptible to cold damage during critical developmental stages, impacting viticulture. Understanding the intricate dynamics of cold hardiness in grape dormant buds and phloem tissue is crucial for developing effective mitigation strategies. In this study, we investigated the LT_{50} values, representing the temperature at which 50% of buds are damaged, under different treatments and sampling times. In our research, we evaluated the effects of four treatments—potassium oxide (K_2O), calcium chloride ($CaCl_2$), seaweed (SW), and a control—on the cold hardiness of grapevine buds and phloem tissue. Primary bud LT_{50} values varied across seasons, with January at –22.46 °C, February at –22.35 °C, and March at –20.45 °C. K_2O treatment showed a trend toward improvement, although the difference from the control (–21.99 °C) was not statistically significant. Regarding LT_{50} values, $CaCl_2$ and SW applications did not significantly differ from the control. Tertiary buds, however, exhibited a substantial enhancement in cold hardiness with K_2O application, displaying significantly lower LT_{50} values compared to the control (–23.55 °C). Phloem tissue LT_{50} values did not significantly differ among treatments, showing less variability. Bud water content significantly increased with K_2O application in all sampling periods (January: 35.41% vs. 35.61%; February: 34.03% vs. 39.16%; March: 42.40% vs. 37.82%), while shoot water content remained stable. In conclusion, K_2O emerges as a key influencer, particularly in enhancing the cold hardiness of tertiary buds. These insights contribute to the knowledge base for targeted frost mitigation strategies in viticulture.

Keywords Calcium chloride · Potassium oxide · Seaweed · *Vitis* spp. · Dormant grape buds

Introduction

Grapes, belonging to the *Vitis* species hold immense global importance due to their widespread utilization in various industries, ranging from winemaking to fresh produce markets. Beyond their culinary significance, grapes are recognized for their nutritional richness, containing antioxidants, vitamins, and minerals that contribute to human health and well-being (Kaya 2020a, b; Incesu et al. 2022; Karakus et al. 2023). Despite their agricultural prominence, *Vitis vinifera* L. European grape varieties, the most predominant in cultivation, are particularly susceptible to winter cold, which can lead to various forms of injury, including bud damage and trunk splitting. The impact of low temperatures on plants extends beyond yield and quality, potentially jeopardizing the survival of grapevines (Kaya and Köse 2017; Köse and Kaya 2017; Köse et al. 2018). In addition, climate change exacerbates these challenges, causing frequent and unpredictable extreme winter cold events in wine and table grape regions worldwide, which leads to freezing in buds and shoots and consequently to economic losses (Buztepe et al. 2017; Jones et al. 2022). As global climate patterns undergo shifts with seasonal transitions and abrupt temperature fluctuations in both summer and win-

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ter (Droulia and Charalampopoulos 2021), the majority of *V. vinifera* cultivars face significant challenges in adapting to cold continental climates. In general, the vulnerability of *Vitis vinifera* cultivars to freeze-induced damage is typically manifested at low temperatures ranging from –15 to –25 °C, contingent upon seasonal dynamics and varietal distinctions (Fennell 2004; Kaya and Köse 2018). Conventional practices entail the subterranean burial of delicate shoots as a protective measure against the rigors of winter cold (Zabadal et al. 2007). However, the efficacy of snow cover, an additional natural safeguard, is diminishing in reliability due to erratic snowfall patterns attributed to global climate change in cold regions (Köse and Ateş 2017; Köse et al. 2018). The burial of vine shoots, whether beneath soil or snow, particularly in areas characterized by severe winter cold, presents formidable challenges, encompassing both labor-intensive endeavors and substantial financial implications (Rende et al. 2018; Kaya and Köse 2020; Yilmaz et al. 2020). This cultivation practice augments input expenditures and exacerbates the susceptibility of vines to soil-borne pathogens, with crown gall (*Agrobacterium vitis*) emerging as a potential ramification resulting from cold-induced damage or mechanical trauma associated with the burial process (Hamman et al. 1996).

Previous studies have duly acknowledged the capacity of plant nutrition to bolster cold tolerance or/and hardiness by exerting influences on the physiological characteristics, dormancy, and overall nutritional status of vines. These investigations have concentrated on examining the prospect that supplementary nutrition can indeed heighten the cold hardiness of grapevines. These studies have unveiled correlations between cold hardiness and parameters such as dry matter and calcium (Ca) content, underscoring the role of Ca in its translocation to the root during the autumnal season (Domagała-Świątkiewicz and Błaszczyk 2007; Haghi et al. 2019). The Ca, indeed, an elemental requisite for cell division, developmental processes, and the permeability of cell walls, is renowned for its involvement in fortifying plant cell walls. This involves inhibiting enzymes responsible for cell wall degradation and mitigating damage induced by environmental stressors (Harandi et al. 2013). Specifically, the foliar application of 1% $CaCl_2$ has been documented to enhance freezing tolerance in grapevine dormant buds. On the other hand, potassium (K), another vital element for plants, plays integral roles in meristem growth, water status, photosynthesis, and assimilate transportation (Sarikhani et al. 2014). While grape berries often contain abundant K (Amiri and Fallahi 2007), the influence of K on cold hardiness has been explored by researchers, indicating that 3% K fertilizers and 2% doses of potassium sulfate (K_2SO_4) can enhance cold hardiness in grapevines (Nojavan et al. 2020). Moreover, seaweed extracts (SW) encompass a variety of substances that play a pivotal role in promoting plant growth. These include auxins, cytokinins, betaines, and gibberellins, along with organic compounds such as amino acids, macronutrients, and trace elements. The collective presence of these compounds in seaweed extracts has been recognized for its potential to enhance both crop yield and quality (Battacharyya et al. 2015). However, to the best of our knowledge, there is a paucity of studies examining the impact of seaweed application on the cold hardiness of grapevines.

Nevertheless, the application of these protective measures has predominantly been examined in a generalized context, leaving a notable void in our understanding of their efficacy within specific grape varieties. One such cultivar that has received limited scrutiny in the realm of cold damage management is the 'Alphonse Lavallee' (*Vitis vinifera* L.*cv*) grape cultivar. Renowned for its distinctive characteristics, the 'Alphonse Lavallee' cultivar represents an interesting subject for investigation, holding the promise of revealing nuanced responses to external substances in cold conditions. Despite the escalating significance of comprehending the cold hardiness of grape varieties for sustainable viticulture, there remains a scarcity of studies delving into the precise impacts of K_2O, $CaCl_2$, and seaweed extracts (SW) on the 'Alphonse Lavallee' grape cultivar. Therefore, this study aimed to bridge this research gap by undertaking a comprehensive examination into the effects of K_2O, $CaCl_2$, and SW on the cold hardiness of the 'Alphonse Lavallee' grape variety. Through a detailed examination of LT_{50} values in dormant buds and canes (shoots) of this grape cultivar, our research aspired to contribute insights into the overarching viticultural knowledge base, offering pragmatic recommendations for customized strategies in mitigating cold damage.

Materials and Methods

Plant Materials and Chemicals

This investigation examined the impact of combined calcium chloride ($CaCl_2$), potassium oxide (K_2O), and liquid seaweed (SW) applications on the cold hardiness of 'Alphonse Lavallee' (*Vitis vinifera* L.*cv*) grape cultivar. The field-based interventions were conducted at the Ondokuz Mayıs University, Bafra Research and Application Center Vineyard. The study focused on the 'Alphonse Lavallee' grape cultivar, selected for its characteristics of delayed bud burst, along with its hardiness to low temperatures and fungal diseases. This variety underwent a series of precise foliar chemical treatments from 15 June 2021 until the onset of veraison, with applications made at consistent 15-day intervals. Vines in the control group did not receive any foliar treatments. The vines used in the experiment were grafted

onto 1103 Paulsen rootstocks and were strategically planted at a spacing of 3 m × 1.5 m. These vines were 7 years old and were pruned following the double-armed cordon training system to ensure uniform growth and development. To provide the vines with the necessary hydration and to maintain soil moisture at optimal levels for growth, drip irrigation was employed throughout the growing season.

Foliar Applications in the Vineyard

Foliar applications were performed by incorporating distinct treatments targeting various nutritional aspects crucial to the cold hardiness of 'Alphonse Lavallee' grape cultivar.

Potassium Oxide (K_2O) Application

Following the protocol of Sarikhani et al. (2014), K_2O foliar fertilizer was administered at a concentration of 2% (w/v) to five vines per replication. The treatment commenced in the second week of June and continued until the onset of veraison, with a total of five applications administered at biweekly intervals. Vines designated as controls did not receive any treatment.

Seaweed Application

The seaweed employed in this study contained organic matter (10%), water-soluble potassium oxide (K_2O 2%), and alginic acid (0.8%). Liquid seaweed was prepared at a concentration of 25 cc per 10 L water and applied starting in the second week of June, coinciding with the beginning of veraison. This application was repeated five times, at 2-week intervals. Vines in the control group did not receive any such treatment.

Calcium Chloride ($CaCl_2$) Application

Following the methodology outlined by Haghi et al. (2019), $CaCl_2$ applications were conducted three times through manual spraying at 2-week intervals from the second week of June to veraison, maintaining a 1% (w/v) $CaCl_2$ concentration. Control vines did not undergo any $CaCl_2$ application.

Frost Test in Laboratory

After the foliar applications, the susceptibility to cold injury was quantified through freezing tests in a controlled laboratory environment. The degree of frost injury in primary, secondary, and tertiary buds was systematically assessed using a programmable freezer (Utest-UTD 1440, Turkiye). In January, February, and March, canes were extracted from the vines, and single-bud cuttings were prepared, wrapped in foil, and subjected to a programmed freezing regimen initiated at 4 °C. The canes were maintained at this temperature for 24 h, after which the temperature was gradually reduced from 0 to –28 °C at a rate of 4 °C per hour. At each predetermined temperature of –16, –20, –24, and –28 °C, samples were taken out after 1 h (60 min) of exposure, following the methodology established by Karimi (2020). The cane samples, obtained from the field, underwent a thorough examination. This involved evaluating the survival rates of primary, secondary, and tertiary buds, as well as phloem tissue. The objective of this comprehensive approach was to understand the influence of chemical applications on the level of cold hardiness. This was achieved by studying bud mortality through cross-sectional analysis and observing the subsequent sprouting of single-bud cuttings in a controlled growth environment.

Quantification of Bud and Tissue Water Content (%)

Regular assessments of the weight when the material was fresh (FW) were performed, and the weights of both the cane tissue and buds were measured thereafter. The specimens were subjected to a drying process lasting 48 h at 65 °C This approach facilitated the calculation of water content, represented as a percentage, using the formula [% Water content = (FW – DW) × 100/FW].

Data Analysis

In this investigation, we examined a series of equations incorporating natural logarithmic functions ($ln(x)$). These functions were employed to characterize the complex interdependencies among various variables (primary-LT_{50}, secondary-LT_{50}, tertiary-LT_{50}, phloem-LT_{50} values) under diverse experimental conditions throughout three periods (January, February, March). The variables investigated included control, K_2O, $CaCl_2$, and SW each characterized by its own equation within each experimental period. The equations were formulated in the general logarithmic form:

$$yij = aijln(x) + bij \quad \text{(a)}$$

where i denotes the experimental period (1, 2, 3), and j represents the various variables (control, K_2O, $CaCl_2$, SW). The coefficients aij and bij are specific to each combination of experimental period and variable, reflecting the nuanced nature of the relationships observed (Neter et al. 1983).

For instance, to derive a formula for the control variable during the first period, the coefficients aij and bij were employed from the corresponding equation. This formulation was then adaptable to other periods and variables, ensuring a comprehensive representation of the observed logarithmic relationships. On the other hand, all descrip-

tive analyses were conducted utilizing the agricolae package in (Vienna, Austria, 2013) R Studio (Mendiburu 2023). The investigation into the influence of sampling time, treatments, and their interactions with LT_{50} values and water content employed analysis of variance (ANOVA) with the stats package in R Studio (R Core, 2013). A comprehensive model, encompassing all main effects and interaction effects, was subjected to testing for adherence to normality assumptions. Linear models (lm function) were employed to assess the main effects (sampling time and treatments) for LT_{50} values and water content. Subsequent post hoc analysis using the Tukey HSD (honestly significant difference) test was performed utilizing the agricolae package in R Studio (Mendiburu 2023). Principal component analyses (PCAs) for LT_{50} values and water content were undertaken using ggplot2 within R Studio (Wickham and Wickham 2016). We used PCA, a valuable analytical technique, to condense multidimensional data into a more interpretable format, facilitating the identification of underlying patterns and trends within complex datasets. The heatmap was generated employing the heatmap package in R Studio.

Results

The LT_{50} values, representing the temperature at which 50% of buds are damaged, varied across seasons and treatments. The LT_{50} values for primary buds in January were –22.46 °C, in February –22.35 °C, and in March –20.45 °C. Among treatments, K_2O showed a trend toward improvement, although not significantly different from the control (–21.99 °C). Tertiary buds, however, exhibited a significant enhancement in cold hardiness with K_2O application. For tertiary buds, the January LT_{50} was –24.35 °C, February –23.72 °C, and March –22.77 °C. Significantly lower LT_{50} values were observed with K_2O application compared to the control (–23.55 °C, $p = 0.0383$). Interestingly, the phloem tissue LT_{50} values did not show significant differences among treatments, i.e., phloem tissue LT_{50} values exhibited less variability. In January, it was –23.30 °C, February –23.64 °C, and March –23.45 °C. Bud water content increased significantly with K_2O application in January (35.41% vs. 35.61%, $p = 0.000329$), February (34.03% vs. 39.16%, $p = 0.7864$), and March (42.40% vs. 37.82%, $p =$ 7864). By contrast, shoot water content remained relatively stable across treatments, with no significant differences observed in January (48.67% vs. 49.88%), February (49.64% vs. 50.19%), and March (50.78% vs. 48.88%). In general, the influence of the applications on the cold hardiness of buds and phloem tissue was more discernible in February as opposed to other time periods (Table 1).

Figure 1 displays the PCA biplots of LT_{50} values and water content, segmented by treatments. The biplots illustrate the relationships and variance among LT_{50} values such as for primary buds, tertiary buds, phloem tissue, bud water content, and shoot water content. Upon examination of the sampling times, a robust correlation among the primary buds, secondary buds, tertiary buds, phloem tissue, as well as the bud and shoot water content values was evident in January and February, diminishing in March (Fig. 1a). Furthermore, the impact of the applications on primary buds, secondary buds, tertiary buds, phloem tissue, as well as bud and shoot water content values was more pronounced than that of the control. Notably, the most effective applications were identified in the order of K_2O, $CaCl_2$, SW, and control (Fig. 1b). Regarding LT_{50} values,

Table 1 LT_{50} values (°C) for primary buds, secondary buds, tertiary buds, phloem tissue, bud and shoot water content (%) according to applications

Sampling time (S)[x]	Primary-LT_{50}	Secondary-LT_{50}	Tertiary-LT_{50}	Phloem-LT_{50}	Bud-WC	Shoot-WC
January	–22.46 ± 1.05b	–24.00 ± 0.53b	–24.35 ± 0.56b	–23.30 ± 1.02	35.41 ± 5.74b	48.67 ± 5.96
February	–22.35 ± 1.23ab	–23.18 ± 0.90ab	–23.72 ± 0.36ab	–23.64 ± 0.42	34.03 ± 8.86b	49.64 ± 1.48
March	–20.45 ± 3.21a	–22.08 ± 2.52a	–22.77 ± 2.75a	–23.45 ± 1.34	42.40 ± 3.91a	50.78 ± 1.17
Treatment (T)[y]						
Control	–21.99 ± 2.12	–23.19 ± 1.55	–23.55 ± 1.92	–23.52 ± 0.61	35.61 ± 11.61	49.88 ± 7.29
K_2O	–21.93 ± 2.25	–23.54 ± 0.99	–24.17 ± 0.49	–23.81 ± 0.58	39.16 ± 7.38	50.19 ± 1.21
$CaCl_2$	–21.53 ± 2.03	–22.38 ± 2.41	–23.09 ± 2.44	–23.70 ± 0.57	37.82 ± 3.90	48.88 ± 0.77
SW	–21.54 ± 2.73	–23.24 ± 1.69	–23.64 ± 1.49	–22.83 ± 1.59	36.52 ± 4.34	49.84 ± 0.58
Significance						
S	0.0275 *	0.00738 **	0.0383 *	0.6259	0.000329 ***	0.324
T	0.9490	0.3523	0.4796	0.0826	0.4482	0.8582
S x T	0.5703	0.7585	0.6255	0.6637	0.7864	0.9556

Data are expressed as means. *Primary-LT_{50}* LT_{50} values for primary buds, *Secondary-LT_{50}* LT_{50} values for secondary buds, *Tertiary-LT_{50}* LT_{50} values for tertiary buds, *Phloem-LT_{50}* LT_{50} values for phloem tissue, *Bud-WC* bud water content, *Shoot-WC* shoot water content
X mean separation in sampling time, *Y* mean separation in treatments, *S* sampling time, *T* treatment, *S* × *ST* interactions
For a given factor different letters within a column represent significant differences
*$p<0.05$, **$p<0.01$, ***$p<0.001$ (Tukey test)

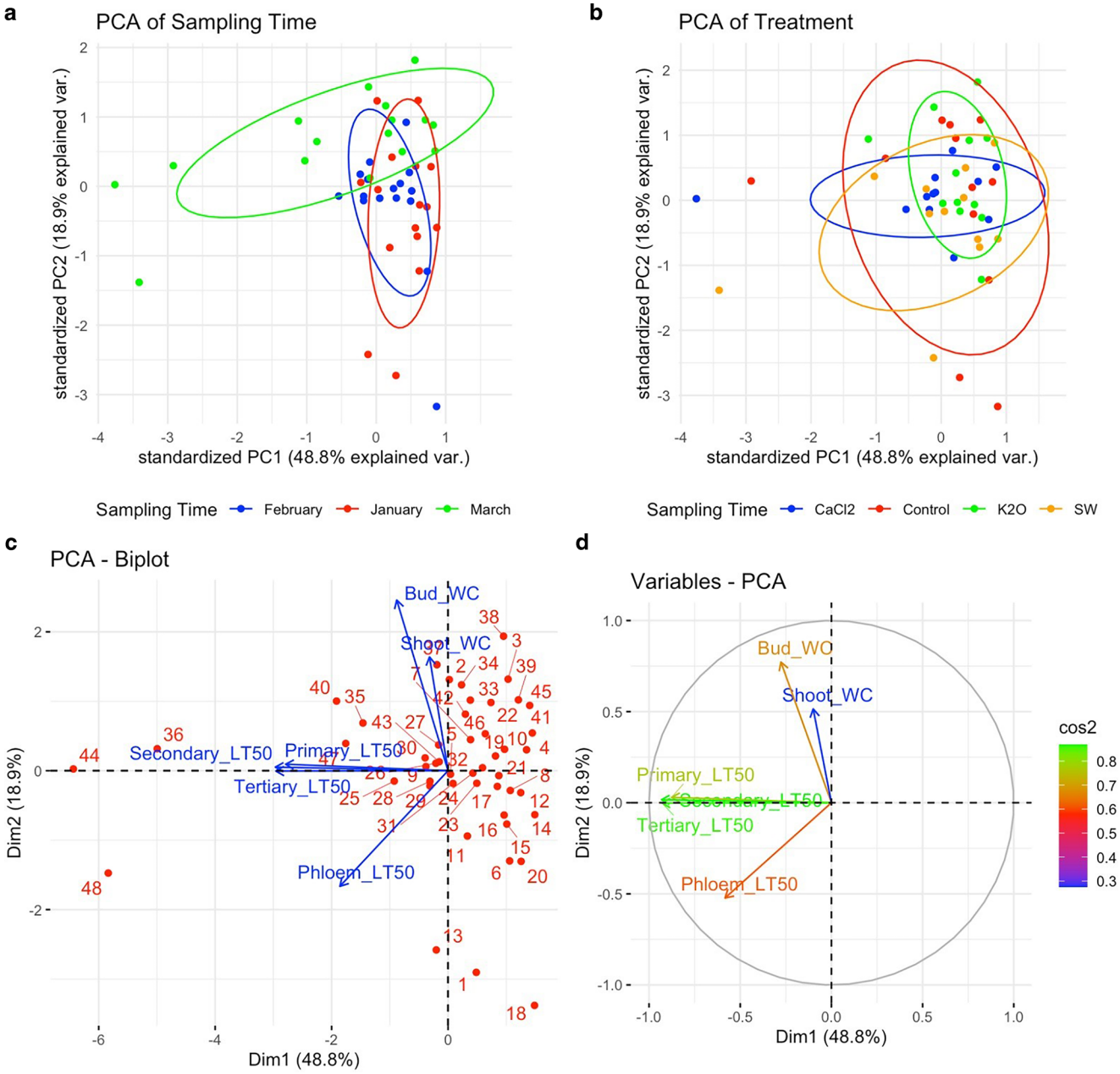

Fig. 1 Principal component analysis (*PCA*) biplots of berries colored by treatments: sampling times (**a**), treatments (**b**), primary buds, secondary buds, tertiary buds, phloem tissue LT values (**c**, **d**)

the first principal component (Dim1) accounts for 48.8% of the variance, and the second (Dim2) accounts for 18.9%. Tertiary buds and phloem LT_{50} values are closely aligned with the negative end of Dim1. Their cos2 values are high, indicating a strong correlation with this principal component. The cos2 values, particularly for the LT_{50} values of primary, secondary, and tertiary buds, were notably high and centrally located within Dim2. By contrast, the shoot and bud WC were close to Dim2, with the shoot exhibiting a considerably low cos2 value (Fig. 1c, d). Figure 2a, b illustrates the hierarchical clustering heatmap of LT_{50} values for primary buds, tertiary buds, phloem tissue, bud, and shoot water, across various treatments and periods. This heatmap demonstrates the relative concentrations of specific phytochemicals in the treatments and sampling time. The parameters determined (x axis) were clustered at the bottom of the heatmap. Distinct clusters emerged, suggesting some similarities and dissimilarities between these parameters. All LT_{50} values appeared closely related, as did sampling times, implying similar patterns of concentration across samples. The samples, labeled with their corresponding sampling times and treatments, were vertically clustered

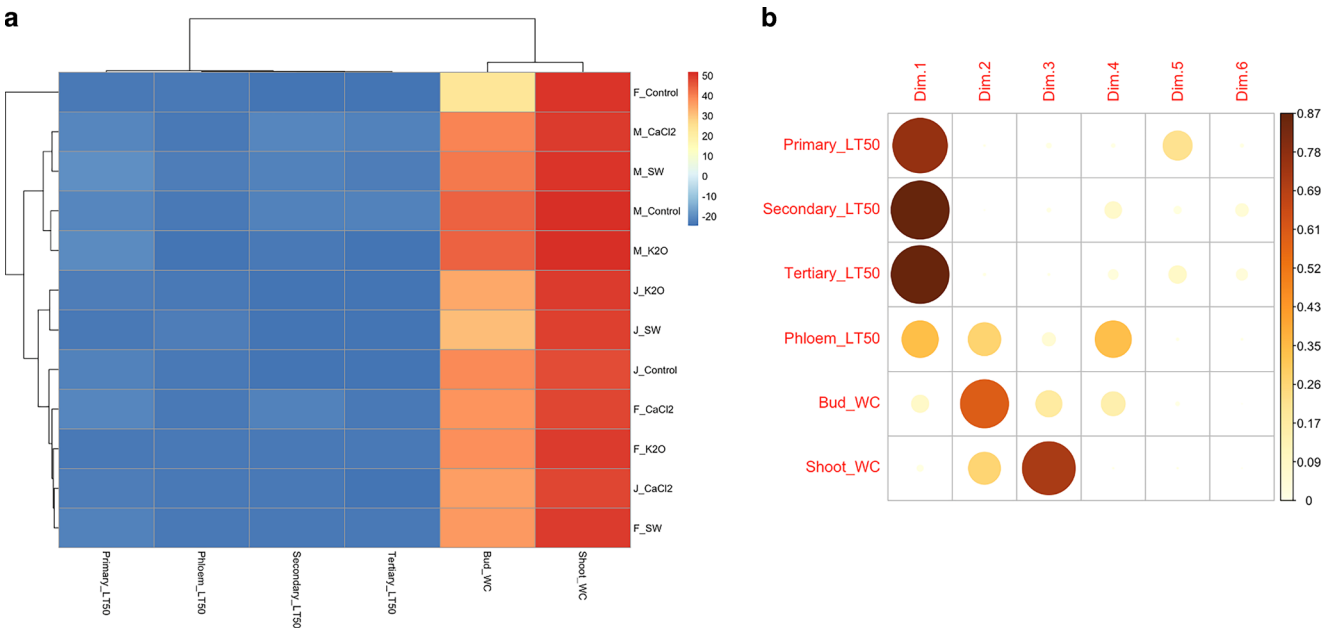

Fig. 2 Heatmap analysis (**a**) and dimension distribution (**b**) of numerous components, from primary buds, secondary buds, tertiary buds, phloem tissue, sampling times, and treatments

on the right, while primary buds, tertiary buds, phloem tissue, bud, and shoot water were horizontally clustered on the left. The parameters measured were divided into two main groups. The first group included primary buds, secondary buds, tertiary buds, and phloem LT_{50} values. The second group comprised bud and shoot water content values. Sampling time and applications were divided into two main groups, with the second main group further divided into two subgroups. Notably, excluding bud water content, a robust relationship was observed among all applications, sampling times, and the measured parameters, including primary buds, secondary buds, tertiary buds, phloem tissue, and shoot water content values.

Discussion

The determination of the lethal temperature for 50% of buds (LT_{50}), derived from controlled freezing experiments, establishes a crucial linkage with the ultimate survival of buds in natural vineyard conditions (Wolf and Cook 1994). This quantitative metric serves as a pivotal indicator, encapsulating the thermal threshold at which half of the bud population succumbs to the deleterious effects of freezing temperatures (Wolpert and Howell 1986). The application of controlled freezing experiments provides a controlled and replicable framework, allowing for the precise calculation of LT_{50} values and, consequently, a nuanced understanding of the critical temperature range governing bud survival (Wolf and Pool 1987). In particular, the supercooling capacity inherent in supercooled water resulting from ice nucleation events and lethal intracellular frost constitutes a distinctive feature in the context of cold damage detection within dormant buds. This phenomenon accentuates the proactive nature of frost damage detection, enabling the identification of impending injury events before their manifestation in visible field symptoms (Schnabel and Wample 1987). Shortly, the integration of bud or shoot LT_{50} value determination through controlled freezing experiments, alongside an exploration of the supercooling dynamics, contributes to a refined comprehension of the complex interplay between freezing events and bud survival in grapevines (Quamme 1991). Our investigation, in this regard, delved into the nuanced dynamics governing the cold hardiness of buds and phloem tissue in response to various treatments and sampling times. The LT_{50} values, serving as a key metric for the temperature causing damage to 50% of buds, exhibited discernible variability across both the seasons and the treatment modalities (Table 1). Our findings align with the established knowledge within the scientific community, as previous researchers have similarly affirmed the variability in cold hardiness exhibited by dormant buds of grapes across different seasons (Quamme 1991; Fennell 2004; Kaya and Köse 2017, 2020). This consistency between our results and prior research indicates the robustness of the observed seasonal fluctuations in the cold hardiness of grape buds during dormancy. The acknowledged variation in cold hardiness across seasons contributes to the broader understanding of the dynamic and context-dependent nature of vines responses to environmental conditions, particularly during

the dormant phase. For primary buds, a diminishing trend in LT_{50} values was observed from January to March, with K_2O manifesting a noteworthy, albeit statistically nonsignificant, improvement compared to the control. Our results are in line with those reported by previous authors, affirming the role played by K in cold hardiness. Specifically, prior studies, such as the work by Nojavan et al. (2020), have indicated that the application of 3% K fertilizers and 2% doses of K_2SO_4 holds the potential to enhance cold hardiness in grapevines. This consistency in findings underscores the cumulative evidence supporting the positive influence of K on bolstering the cold hardiness of grapevines, providing a valuable contribution to the existing body of knowledge in viticulture. Building on this foundation, the implications of these findings extend beyond the immediate practical applications for vineyard management. They show the necessity for ongoing research into the mechanisms by which K and other nutrients influence plant physiology, especially under stressful conditions such as cold temperatures. This understanding could pave the way for the development of more targeted and efficient fertilization strategies that optimize plant health and resilience, thereby enhancing yield and quality in grape production.

Conversely, notable distinctions were evident among the LT_{50} values of primary, secondary, and tertiary buds, aligning with anticipated patterns. The observed order of cold hardiness, with tertiary buds exhibiting the highest tolerance followed by secondary and primary buds, substantiates previous findings (Table 1). Authors in prior studies similarly reported a gradient in frost sensitivity, where primary buds were the most susceptible, followed by secondary and tertiary buds, respectively (Quamme 1991; Fennell 2004). This corroborates the established understanding of the hierarchical vulnerability of grapevine buds to frost events, providing additional support for the robustness of the trends observed in our investigation. Besides, tertiary buds displayed a substantial boost in cold hardiness with K_2O application, evidenced by significantly lower LT_{50} values across all sampling times compared to the control. This underscored the specific efficacy of K_2O in fortifying the cold hardiness of tertiary buds. It is, however, plausible that cold damage may selectively affect only the primary bud or specific buds on the vine, allowing the vine an opportunity to recover, undergo development, and eventually yield fruit. Consequently, in the assessment of the cold hardiness of grape varieties cultivated within a particular region, the foundational criterion is often the ability of primary buds to endure the lowest temperatures. This approach aligns with the perspective put forth by Kaya (2020a, b), recognizing the pivotal role of primary buds as indicative markers of cold resistance in grape varieties. The results of our study were validated through comprehensive PCAs and heatmap data, both of which consistently underscored the hierarchical impact of different applications. Notably, the most influential applications, ranked in descending order, were K_2O, $CaCl_2$, SW, and control vines, as visually represented in Figs. 1 and 2. In the context of LT_{50} values, the principal components, particularly Dim1 and Dim2, played a pivotal role in elucidating the variance within the dataset. Dim1, as the first principal component, accounted significantly for 48.8% of the overall variance, reflecting a substantial influence on the observed outcomes. Similarly, Dim2 contributed an additional 18.9%, further substantiating its relevance in capturing nuanced patterns among the variables. The alignment of tertiary buds and phloem LT_{50} values with the negative end of Dim1, coupled with elevated cos2 values, signified a robust correlation with this principal component. Moreover, the cos2 values for the LT_{50} values of primary, secondary, and tertiary buds were conspicuously heightened and centrally situated within Dim2. This concentration of influence within Dim2 underscores its discriminatory power in delineating distinct patterns among the LT_{50} values, reinforcing its significance in capturing the complex interrelationships among the measured parameters.

Reports indicate that the cold hardiness tolerance of phloem tissue exhibits variability within the range of –15 °C to –32 °C during the winter period (Wolpert and Howell 1986). This variance is attributed to distinctions among grape varieties and is influenced by the sub-zero temperatures to which the phloem tissue has been previously exposed (Slater et al. 1991). Our results, which align with these reported findings, reveal that phloem tissue exhibited LT_{50} values ranging between –23.30 and 23.63 °C. The temporal analysis unveiled a more conspicuous impact of the applications on the cold hardiness of buds and phloem tissue in February compared to other months. This temporal specificity underscored the intricate interplay between physiological processes, developmental stages, and the response of buds and phloem tissue to substances applied during distinct climatic periods. This consistency with prior research reinforces the understanding that phloem tissue responses to cold stress are indeed dynamic. The variations observed in LT_{50} values underscore the adaptability and acclimation of phloem tissue to preceding environmental and regional conditions, emphasizing the complex interplay between environmental influences on the cold hardiness of cane phloem tissue. Remarkably, LT_{50} values for phloem tissue demonstrated no significant variance among treatments, implying a consistent response across the substances applied. These findings, however, aligned with existing literature emphasizing the inherent hardiness of phloem tissue to fluctuations in cold temperatures. Indeed, the positioning of the phloem LT_{50} value at a distance from the cos2 region within quadrant III (Fig. 1b, c) and its presence in the Dim1, Dim2, and Dim4 regions (Fig. 2b) substantiate and align with the reported findings. This spatial arrange-

ment further reinforces the notion of the dynamic nature of phloem tissue responses to cold stress, signifying its distinct characteristics and adaptability within the multivariate context of the PCA. In our findings, the buds and shoots water level exhibited a gradual increase as spring progressed and bud water content, a crucial factor in determining cold hardiness, demonstrated significant augmentation with K_2O application throughout the sampling period. This indicates that K_2O positively influenced water retention in buds, thereby enhancing their hardiness to colder temperatures. Conversely, shoot water content remained relatively invariant across treatments, implying that the substances applied here exerted minimal influence on this parameter. The rapid hydration of tributaries during the spring season led to a limited correlation between water content and cold hardiness in our study. This observation aligns with previous reports indicating that cold hardiness diminishes at a slower rate than changes in water content. Such findings provide a coherent explanation for our results, suggesting that factors beyond water content contribute significantly to the nuanced dynamics of cold hardiness in buds during the spring period. However, it was observed that cold hardiness dissipated more gradually, providing an explanation for our results. This suggests that factors beyond water content contribute to the sustained cold hardiness of buds even as hydration levels fluctuate during the spring season. Our findings, substantiated by the comprehensive heatmap and PCAs, demonstrated a robust relationship among all treatments, sampling times, and measured parameters (Figs. 1 and 2). This encompassed primary buds, secondary buds, tertiary buds, phloem tissue, and shoot water content values, except for bud water content. The concordance observed in the results, especially when considering the intricate interplay between these variables, underscores the coherence and reliability of our experimental outcomes.

Conclusion

Our study provided a comprehensive assessment of the cold hardiness of grape dormant buds and phloem tissue under various treatments and sampling times. The LT_{50} values, representing the temperature at which 50% of buds are damaged, exhibited notable variations across seasons and treatments. While K_2O showed a trend toward improvement in primary buds, tertiary buds displayed a significant enhancement in cold hardiness with K_2O application, demonstrating lower LT_{50} values compared to the control. Conversely, phloem tissue LT_{50} values did not show significant differences among treatments. Bud water content, a critical determinant of cold hardiness, increased significantly with K_2O application across all sampling periods. By contrast, shoot water content remained relatively stable across treatments. The influence of the applications on the cold hardiness of buds and phloem tissue was more pronounced in February compared to other time periods. The PCA and hierarchical clustering heatmap analysis further elucidated the relationships and variances among LT_{50} values and water content. Robust correlations among primary buds, secondary buds, tertiary buds, phloem tissue, and bud and shoot water content values were evident in January and February, diminishing in March. The impact of the applications on these parameters was more pronounced than that of the control, with K_2O identified as the most effective treatment. The discernible impact of K_2O on enhancing cold hardiness, particularly in tertiary buds, has implications for horticultural practices. Future research should delve deeper into the physiological mechanisms underpinning the patterns observed to inform more targeted and effective strategies for mitigating cold damage in grapevines.

Author Contribution B.K. and O.K. played a pivotal role in this study, contributing to conceptualization, funding acquisition, investigation, project administration, data curation, formal analysis, methodology, software, resources, supervision, validation, visualization, and both the original and review & editing of the manuscript. O.K. wrote the manuscript. B.K., O.K., and T.Y. participated in data curation, formal analysis, methodology, software, and contributed to the review & editing of the manuscript. B.K., Y.U, and K.B conducted field and laboratory work, with a focus on analyzing samples in the laboratory.

Data Availability Data from this study will be made available upon request.

Conflict of interest B. Kose, Y. Uray, K. Bayram, T. Yilmaz and O. Kaya declare that they have no competing interests.

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