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Evaluation of Four Training Systems for Inducing Physiological and Biochemical Changes Related to Winter Cold Hardiness of Two Grapevine Cultivars

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ABSTRACT

The objectives of this study were to compare some physiological indices related to winter cold tolerance of two field-grown grapevine (*Vitis vinifera* L.) cultivars under different training systems. This experiment was conducted over two consecutive years (2020 and 2021) in a factorial arrangement based on a randomized block design, with three sampling dates, i.e., December, January, and February, for each year. The treatments included two factors: (1) four training systems (one non-trellis or creeping system (C) and three bilateral cordon trellis systems: I-shape, T-shape, and Y-shape), and (2) two grape cultivars ('Khalili' and 'Perlette'). In all three sampling phases across both years, bud malondialdehyde (MDA), hydrogen peroxide (H2O2), and water content (WC) were higher in the 'Perlette' cultivar under the creeping training system compared to the cordon trellis systems. However, the 'Khalili' cultivar under the Y-shape bilateral cordon training system had higher proline, soluble sugar, polyamine, and abscisic acid content in all three sampling stages compared to other treatments. In both years, the content of these compounds was lower in the 'Perlette' cultivar under the creeping training system compared to the bilateral cordon trellis systems in all three sampling stages. According to the results, the cordon training systems increased the cold tolerance of both cultivars compared to the creeping system, with the Y-shape cordon training system inducing greater cold tolerance in the vines compared to the T-shape and I-shape training systems.

Introduction

Grapes, as a temperate fruit, are perpetually vulnerable to low-temperature damage due to their wide distribution. Consequently, enhancing the cold tolerance of grapes is a primary objective for vine growers in colder climates. Among various environmental stresses, cold stress significantly impacts vineyard productivity. The severity of cold damage is influenced by factors such as minimum temperature, duration of freezing events, grape cultivar, and the specific tissues affected. Cold stress can lead to substantial yield reductions, trunk splitting, the development of trunk diseases, and even the death of the vine (Gonzalez et al., 2020).

As temperatures drop and day lengths shorten, grapevines undergo acclimation to low-temperature stress. Cold acclimation is a complex, genetically regulated process that triggers a series of molecular, biochemical, and physiological changes. The regulation of cold-resistant genes influences the production and abundance of various proteins, including antifreeze proteins, dehydrins, chaperones, metabolites, and hormones. The levels of soluble sugars, proteins, and hormones are closely correlated with the cold hardiness of grapevines (Karimi, 2017; Chai et al., 2019). These substances play a crucial role in preventing dehydration and mitigating cellular damage during exposure to low temperatures.

During cold acclimation, several events occur in grapes, including cessation of shoot growth, gradual senescence and abscission of leaves, and dormancy of overwintering buds. These changes are associated with an increase in abscisic acid (ABA) content, a decrease in water content, an increase in compatible osmolytes, and the remobilization of elements from leaves to the trunk and canes. Additionally, cane maturation progresses acropetally from the base to the apex, marked by the deposition of cork in the outer bark (periderm development; Karimi and Ershadi, 2015; Ren et al., 2023).

To mitigate cold stress damage over the long term, growers can employ strategies such as selecting cold-hardy cultivars, choosing suitable vineyard sites, implementing delayed and double pruning, applying chemical protectants, and adopting optimal trellising systems. In the short term, the severity of cold damage can be lessened through the use of wind machines, insulating covers, and over-vine sprinkling of water (Rahemi et al., 2022). Trellis systems significantly influence the leaf area exposed to light, canopy density, and leaf efficiency (Mota et al., 2011). The leaf surface area exposed to light affects critical physiological functions, including bud initiation and differentiation, fruit ripening timing, carbohydrate storage, wood maturation, natural leaf fall timing, and cold hardiness (Costa et al., 2023; Simonetti et al., 2021).

Cordon training systems effectively reduce cold stress damage by maintaining the phototropic orientation of the stem, preventing apical dominance, slowing the movement of materials within the vessels, limiting the carbon/nitrogen (C/N) ratio, and positioning the renewal and fruiting area at a height above the risk of cold damage (Mehrpour et al., 2022). Breeders are actively seeking to mitigate winter cold damage through the use of appropriate training systems. However, selecting a suitable training system is challenging, as it must align with the characteristics of the desired grape cultivar.

In all training systems, various features must be considered when choosing the appropriate system based on the intended purpose, local climatic conditions, soil fertility, prevalence of diseases, vine growth strength, labor availability, and associated costs. In temperate regions with cold winters, weather conditions are particularly crucial in selecting a training system (Chatrabgoun et al., 2020; Mehrpour et al., 2022). Most studies on pruning systems have concentrated on the quality and quantity of vine yields, with limited research addressing the damage to buds and branches during the dormant season.

A study comparing trellised and non-trellised training systems on the cold tolerance of two grape cultivars, 'Bidaneh Sefid' and 'Bidaneh Ghermez,' identified the highest cold tolerance in the 'Bidaneh Ghermez' cultivar within the trellis system (Mehrpour et al., 2022). Although the height of the trellis did not significantly affect the cold tolerance of vine canes, it positively influenced the cold tolerance and maturity of buds (Costa et al., 2023; Simonetti et al., 2021). A three-year study on 12-year-old 'Karaerik' grapevines in both trellised and non-trellised training systems showed that the trellised system yielded the highest fruit quality, with no significant cold damage observed in dormant buds between the two systems (Küpe and Köse, 2018).

An examination of four grape cultivars across three training systems revealed that the Scott-Henry (SH) and vertical shoot positioning (VSP) trellis systems produced the highest and lowest yields, respectively (Wimmer et al., 2018). A two-year comparison between two VSP and the single high wire (SHW) trellis systems indicated a 30% and 11% increase in yield and dry matter, respectively, alongside a 25% decrease in stem growth in the SHW system (Salvi et al., 2021). Additionally, the highest sugar level and chlorophyll content were recorded in the single-curtain (SCT) training system compared to the pergola (PER) system (Du et al., 2023).

Damage from temperature drops poses a significant challenge for vine growers. For instance, a severe three-day temperature-drop in 2007 led to a 20% decrease in annual grape production in Iran, highlighting the economic and export repercussions of winter cold damage (Ershadi et al., 2016). Despite the critical importance of training systems on vine cold tolerance, research in this area remains scarce. Therefore, this study aimed to investigate the effects of different training systems on coldinduced physiological and biochemical indices in two grape cultivars with varying cold tolerances over two consecutive years.

Materials and Methods

Plant materials

The present research was carried out during the years 2020 and 2021 in a 6-year-old vineyard at the Grape and Raisin Research Institute of Malayer University (lat. 34°16'N, long. 48°51'E, alt. 1725 m). The vines were grown on their own roots in clay–loamy soil, with a pH of 7.8 and 8.1 at depths of 0–30 cm and 30–60 cm, respectively, at a planting density of 1.5 × 3 m under a drip irrigation system. Vineyard daily low temperatures from September through March of 2020 and 2021 are shown in Figure 1. Experimental treatments were applied factorially based on a randomized complete block design with three biological replications (two vines in each replication). Treatments included two factors: (1) four training systems (one non-trellis or creeping (C) and three bilateral cordon trellis (I-shape, T-shape, Y-shape) systems) and (2) grape cultivars of (i) 'Perlette' (P) as the least cold hardy and (ii) 'Khalili' (K) as a cold hardy cultivar (Karimi, 2020). The different cultivar/training system combination treatments were named as follows: P × C ('Perlette' (P) and non-trellis or creeping (C) systems); P × I ('Perlette' and I-shape bilateral cordon trellis systems); P × T ('Perlette' and T-shape bilateral cordon trellis systems); P × Y ('Perlette' and Y-shape bilateral cordon trellis systems); K × C ('Khalili' (K) and non-trellis or creeping (C) systems); K × I ('Khalili' and I-shape bilateral cordon trellis systems); K × T ('Khalili' and T-shape bilateral cordon trellis systems); K × Y ('Khalili' and Y-shape bilateral cordon trellis systems).

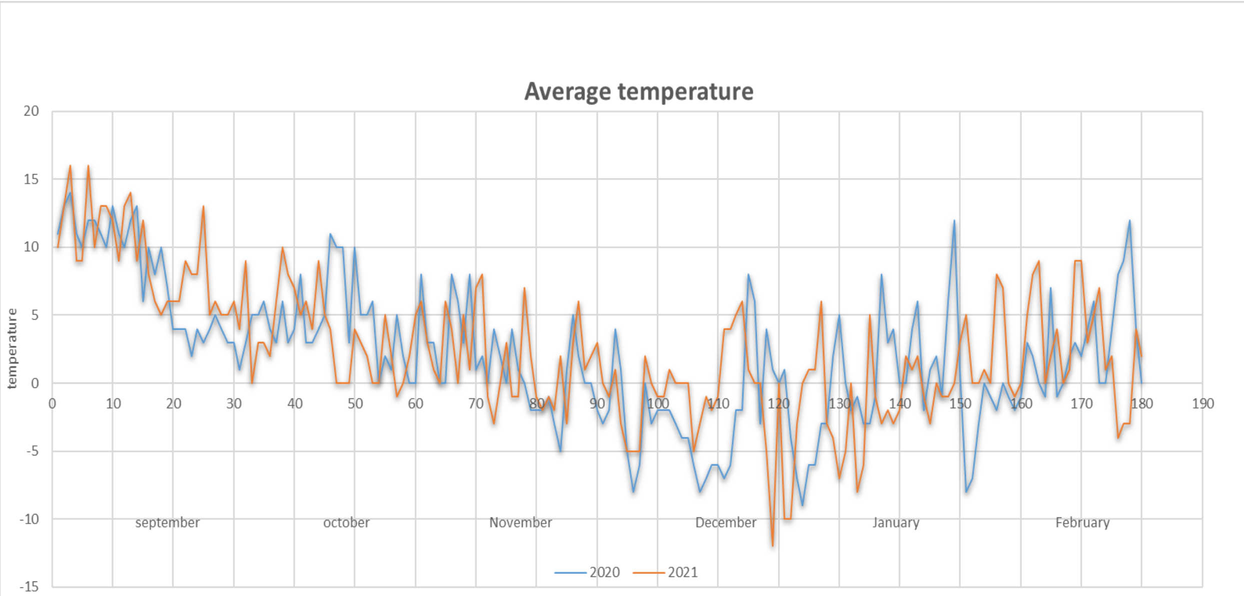

Fig. 1. Vineyard minimum daily temperatures (ºC) recorded from September through March of 2020 and 2021.

Controlled freezing tests

Shoot samples (at least five representative cuttings, 20–30 cm in length, from node positions 4–7 of mature canes) from each cultivar-training system (six vines in each treatment) were collected randomly on each sampling date. The collected canes were wrapped in moist paper towels and immediately transferred to the laboratory on ice in Styrofoam boxes for further evaluation. Cane segments were rinsed under cold running distilled water to remove surface contaminants. Samples were wrapped in moist paper towels to ensure ice nucleation, placed in polyethylene bags, and randomly assigned to predetermined test temperatures. Five replicates were assigned for each cultivar/training system/test temperature. Freezing was accomplished in a programmable freezing chamber (Rad Electronic, Tehran, Iran) according to a stepwise lowering freezing program, starting from the prevailing outdoor temperature. Cane segments were subjected to five different freeze test temperatures (-6, -10, -14, -18, and -22 ºC in December and February; -10, -14, -18, -22, and -26 ºC in January). Freeze test temperatures were determined following preliminary tests on canes collected two days before each sampling date. The freezing rate was 2 ºC h^{-1}, and samples were held at each test temperature for 75 min before being removed from the freezing chamber (Ershadi et al., 2016). Cold tolerance was measured in three stages on December 14, January 18, and February 20, over two consecutive years in Malayer, Iran, using the electrolyte leakage (EL) method. Moreover, the following bud physiological and biochemical indices related to cold acclimation were assayed in all vines.

Electrolyte leakage assay
To measure electrolyte leakage (EL), five buds from the sampled canes of each treatment were separated with a sharp knife and immersed in tubes containing 40 mL of distilled water. The tubes were placed on a shaker at room temperature for 24 hours. After this period, the solution's electrical conductivity (EC) was recorded using an electrical conductivity meter (EC1). The samples were autoclaved for 15 min at 121 ºC. After gradual cooling, their EC was recorded again (EC2), and finally, the bud EL was calculated using the following equation (Ershadi et al., 2016):

$$EL\ (\%) = [\frac{EC1}{EC2}] \times 100$$

Malondialdehyde (MDA)
Membrane lipid peroxidation was assayed by the thiobarbituric acid (TBA) test through measuring the amount of MDA. After extraction with TBA, the absorbance of the samples was read at two wavelengths of 600 and 532 nm and expressed as µmol g^{-1} FW (Heath and Packer, 1968).

Hydrogen peroxide (H_2O_2)
Bud H_2O_2 was measured by Velikova and Loreto (2001) method. The H_2O_2 content of the samples was calculated by comparing their absorbance at the wavelength of 390 nm and expressed as µmol g^{-1} FW.

Water content (WC)
To determine the WC, first the fresh weight (FW) of the buds was weighed. Then, the dry weight (DW) of the samples was determined after 72 h at 72 ºC in oven (Karimi, 2020). Bud WC was calculated using the formula:

$$WC = [\frac{(FW - DW)}{DW}] \times 100$$

Proline assay
To measure proline, extraction was done with the help of sulfosalicylic acid and measurement was done in the presence of acetic acid and ninhydrin reagent. The absorbance of the samples was measured at a wavelength of 518 nm and the bud proline content was determined as µmol g^{-1} FW (Bates et al., 1973).

Abscisic acid
Endogenous ABA analyzed using a Crystal 200 series HPLC pomp (ATI Unicam, Cambridge, UK) equipped with a UV-Vis detector (SPD Philips, Cambridge, UK) and a Diamonsic-C_{18} Column (5 µm, 250 mm × 4.6 mm i.d.; Berkshire, UK) with 20–75% methanol in 1% acetic acid (v/v) as mobile phase and a flow rate of 1.2 mL min^{-1} (Li et al., 2010).

Soluble sugars
The bud samples (0.5 g) were powdered, dissolved in 10 mL ethanol (80%), and centrifuged at 8000 rpm for 15 min at 4 °C. After filtration, ethanolic extract (10 µL) was injected to HPLC pump (ATI Unicam, UK) equipped with a SPD UV–Vis detector and a Spherisorb ODS-2 Column (0.3 µm, 150 mm × 4.6 mm i.d.) with 500 mM sodium citrate (pH 5.5) and ultrapure acetonitrile (1:99, V/V) as the mobile phase at a flow rate of 0.1 mL min^{-1} (Shin et al., 2002). The concentrations of each soluble sugar were determined based on standard solution calibration and expressed as µmol g^{-1} FW.

Free polyamines
The free polyamines (putrescine, spermidine, and spermine) were detected using direct dansylation followed by high-performance liquid chromatography (HPLC) according to a method described by Walter and Geuns (1987). In brief, 250 mg of frozen bud samples were dissolved in 2 mL of an internal standard solution consisting of 4% $HClO_4$ containing 1,7-diaminoheptane-2HCl. The mixture was homogenized for 1 h at 4 °C and then filtered carefully using a 0.45 µm filter.
A volume of 0.2 mL of the resulting supernatant was mixed with 1 mL of carbonate buffer (pH 9) and 1 mL of a dansyl chloride solution (10 mg mL^{-1} in acetone). The solution was then heated at 60 °C for 1 h. After heating, 3 mL of toluene was added to extract the dansylated polyamines.
The extracted bud dansylated polyamines were loaded onto 0.5 g of silica gel and washed with 3 mL of toluene. Subsequently, 5 mL of a toluene-triethylamine solution (10.3 v/v) was used for elution, and the samples were reduced under

nitrogen after elution with 3 mL of ethyl acetate. For isocratic HPLC analysis, a 10 cm long octadecyl silica column was employed, utilizing an acetonitrile/H_2O mixture (72/28, v/v) as the mobile phase for 7 min, at a solvent flow rate of 2 mL min^{-1}, with the dansylated polyamines injected as references.

Data analysis
Cold tolerance was determined based on the LT50 index (lethal temperature at which 50% of the total ion leakage occurs) by fitting response curves (temperature in horizontal axis and EL percentage in vertical axis) in Microsoft Excel (Ershadi et al., 2016). Data analysis was done with SAS 9.1 (SAS Institute, Cary, NC) statistical software (GLM instruction) and mean values were compared using Duncan's multiple range test at 1% probability level.

Results
Cold hardiness
In both years (2020 and 2021), the cold hardiness of both cultivars across the four training systems showed an increase from December to January, followed by a decrease from January to February (Table 1). Throughout the three sampling stages, the highest cold tolerance was observed in the 'Khalili' cultivar under the Y-shaped and T-shaped training systems, while the lowest cold tolerance was recorded for the 'Perlette' cultivar under the non-trellis (creeping) training system (Table 1).

Table 1. Effect of four training systems (one non-trellis or creeping (C) and three bilateral cordon trellis (I-shape, T-shape, Y-shape) systems) on cold hardiness (bud LT50 value; ºC) of 'Perlette' (P) and 'Khalili' (K) grape cultivars from Dec. to Feb. of 2020 and 2021.

Cultivar × Training systems	14 Dec.		18 Jan.		20 Feb.	
	2020	2021	2020	2021	2020	2021
P × C	-5.6 ± 0.50^{e}	-7.0 ± 0.0^{e}	-10.6 ± 0.5^{d}	-11.6 ± 0.19^{e}	-7.0 ± 0^{d}	-7.8 ± 0.38^{e}
P × I	-12.0 ± 0.0^{c}	-13.0 ± 0.0^{c}	-15.0 ± 0.47^{c}	-15.7 ± 0.47^{c}	-10.5 ± 1.1^{c}	-11.5 ± 0.7^{d}
P × T	-13 ± 0.38^{bc}	-15.6 ± 0.09^{b}	-17.6 ± 0.3^{b}	-17.5 ± 0.33^{b}	-14.3 ± 0.69^{b}	-15.0 ± 0.38^{b}
P × Y	-13.3 ± 0.19^{bc}	-15.8 ± 0.96^{b}	-18 ± 0.33^{a}	-17.6 ± 0.19^{b}	-14.1 ±0.34^{b}	-15.3 ± 0.6^{b}
K × C	-9.6 ± 0.19^{d}	-11.0 ± 0.33^{d}	-13.3 ± 0.19^{c}	-14.3 ± 0.19^{d}	-11.6 ± 0.1^{b}	-12.6 ± 0.1^{cd}
K × I	-13.3 ± 0.5^{bc}	-15.0 ± 0.57^{b}	-16.8 ± 0.1^{b}	-17.3 ± 0.69^{b}	-13.8 ± 0.69^{b}	-14.6 ± 0.5^{bc}
K × T	-14.6 ± 0.5^{ab}	-17.3 ± 0.19^{a}	-19.3 ± 0.1^{a}	-21.3 ± 0.19^{a}	-16.0 ± 0.88^{a}	-17.0 ± 0.5^{a}
K × Y	-15.3 ± 0.38^{a}	-16.3 ± 0.5^{ab}	-19.6 ± 0.6^{a}	-21.2 ± 0.19^{a}	-16.5 ± 1.2^{a}	-18.6 ± 0.88^{a}

ǂ Means with the same letters are not significantly different according to Duncan's test at 5% level of probability.

Malondialdehyde
The MDA content in the buds of all vines exhibited a significant increase from the onset of cold acclimation in December to the full cold acclimation stage in January, followed by a decrease until the beginning of the cold de-acclimation stage in February (Table 2). In the December sampling phase for both years (2020-2021), the MDA content was higher in the buds of the 'Perlette' cultivar under the non-trellised (creeping) training system compared to other treatments (Table 2). This trend was consistent in January and February for the 'Perlette' cultivar under the creeping training system.
Conversely, the lowest MDA content across all three sampling stages was observed in the 'Khalili' cultivar under the Y-shaped bilateral cordon training system. However, in some stages, there was no statistically significant difference in MDA content between the 'Khalili' cultivar under the Y-shaped system and those under the T-shaped cordon system (Table 2).

Hydrogen peroxide
In both sampling years, the H_2O_2 content of vine buds was changed under the influence of cultivar and training system in all three sampling stages from December to February. In general, the vines trained in both cultivars under bilateral cordon training system had lower H_2O_2 content compared to the vines trained under the creeping systems. Also, there was a difference between bilateral cordon training systems in terms of bud H_2O_2 content (Table 3). In all sampling stages, the 'Khalili' cultivar especially under the Y-shape training system, showed lower H_2O_2 content in their buds. These changes in the content of H_2O_2 were also observed in 'Perlette' cultivar and the vines of this cultivar under the Y-shape system had less H_2O_2 content. The amount of H_2O_2 accumulation in the vines of both cultivars under the Y-shape training system was significantly lower than the content of H_2O_2 in the vines of the same cultivars under the creeping training system. H_2O_2 content measured in 'Khalili'

cultivar in both years in all training systems was lower than 'Perlette' cultivar (Table 3). In the deep acclimation stage in Jan., no statistical difference was observed between the content of H_2O_2 measured in the bud of 'Perlette' cultivar under creeping system and T-shape cordon system. Moreover, in 2021 (Jan. stage) there was no statistical difference between the H_2O_2 content of 'Perlette' cultivar under creeping system and the H_2O_2 content of 'Khalili' cultivar under creeping system and the same cultivar under I-shape cordon system (Table 3).

During the February sampling in 2020, no significant difference was observed between the H_2O_2 content of the vine bud of 'Perlette' cultivar under the creeping system (P × C), 'Perlette' under the I-shape and T-shape cordon system, and 'Khalili' cultivar under the creeping system (K × C; Table 3). In 2021 (Feb. stage), no statistical difference was observed between the H_2O_2 content measured in the grape buds of 'Perlette' and 'Khalili' cultivars under the creeping system (Table 3).

Table 2. Effect of four training systems (one non-trellis or creeping (C) and three bilateral cordon trellis (I-shape, T-shape, Y-shape) systems) on bud malondialdehyde (μmol g^{-1} FW) of 'Perlette' (P) and 'Khalili' (K) grape cultivars from Dec. to Feb. of 2020 and 2021.

Cultivar × Training systems	14 Dec.		18 Jan.		20 Feb.	
	2020	2021	2020	2021	2020	2021
P × C	8.8 ± 0.16^{a}	9.0 ± 0.42^{a}	11.8 ± 0.8^{a}	12.6 ± 0.37^{a}	7.7 ± 0.36^{a}	8.0 ± 0.88^{a}
P × I	6.8 ± 0.04^{b}	7.7 ± 0.04^{b}	9.7 ± 0.87^{b}	10.4 ± 0.1^{b}	6.2 ± 0.38^{b}	7.4 ± 0.46^{b}
P × T	6.3 ± 0.96^{bc}	6.5 ± 0.73^{c}	9.4 ± 0.72^{c}	8.4 ± 0.72^{d}	5.3 ± 0.81^{c}	6.4 ± 0.47^{c}
P × Y	5.7 ± 0.19^{c}	6.1 ± 0.19^{c}	8.8 ± 0.34^{c}	9.1 ± 0.32^{c}	5.1 ± 0.18^{c}	6.5 ± 0.3^{c}
K × C	5.9 ± 0.30^{c}	7.3 ± 0.24^{b}	8.3 ± 0.72^{c}	9.4 ± 0.01^{bc}	5.8 ± 0.66^{bc}	6.8 ± 0.23^{bc}
K × I	4.8 ± 0.39^{d}	5.75 ± 0.16^{d}	6.7 ± 0.22^{d}	8.2 ± 0.0^{d}	4.5 ± 0.39^{cd}	5.3 ± 0.4^{cd}
K × T	4.4 ± 0.72^{de}	5.40 ± 0.22^{d}	5.9 ± 0.17^{de}	7.2 ± 0.74^{e}	3.1 ± 0.72^{d}	4.9 ± 0.26^{d}
K × Y	4.3 ± 0.21^{e}	4.51 ± 0.43^{e}	4.2 ± 0.33^{e}	6.5 ± 0.21^{e}	3.81 ± 0.21^{d}	4.4 ± 0.07^{d}

ǂ Means with the same letters are not significantly different according to Duncan test at 5% level of probability.

Table 3. Effect of the four training systems (one non-trellis or creeping (C) and three bilateral cordon trellis (I-shape, T-shape, Y-shape) systems) on bud hydrogen peroxide (μmol g^{-1} FW) of 'Perlette' (P) and 'Khalili' (K) grape cultivars from Dec. to Feb. of 2020 and 2021.

Cultivar × Training systems	14 Dec.		18 Jan.		20 Feb.	
	2020	2021	2020	2021	2020	2021
P × C	7.5 ± 0.01^{a}	7.9 ± 0.01^{a}	5.3 ± 0.05^{a}	5.9 ± 0.05^{a}	5.1 ± 0.12^{a}	5.6 ± 0.12^{a}
P × I	6.3 ± 0.01^{b}	6.7 ± 0.21^{b}	4.7 ± 0.18^{bc}	5.3 ± 0.8^{bc}	4.7 ± 0.03^{ab}	5.2 ± 0.24^{b}
P × T	5.1 ± 0.2 ^{c}	6.7 ± 0.08^{b}	5.1 ± 0.12^{ab}	5.2 ± 0.26^{bc}	4.7 ± 0.19^{ab}	5.3 ± 0.19^{b}
P × Y	5.4 ± 0.06^{c}	6.5 ± 0.15^{b}	4.6 ± 0.02^{bc}	5.1 ± 0.024^{bc}	4.1 ± 0.12^{c}	4.7 ± 0.12^{c}
K × C	6.6 ± 0.01^{b}	6.6 ± 0.1^{b}	4.7 ± 0.08^{bc}	5.5 ± 0.14^{ab}	4.9 ± 0.1^{a}	5.5 ± 0.1^{ab}
K × I	6.3 ± 0.01^{b}	6.5 ± 0.12^{b}	4.5 ± 0.03^{cd}	5.4 ± 0.16^{ab}	4.6 ± 0.04^{bc}	4.9 ± 0.04^{bc}
K × T	5.3 ± 0.01^{c}	5.7 ± 0.2^{c}	4.08 ± 0.12^{d}	4.6 ± 0.12^{c}	4.3 ± 0.02^{c}	5.2 ± 0.02^{b}
K × Y	4.6 ± 0.25^{d}	4.5 ± 0.26^{d}	3.3 ± 0.13^{e}	3.9 ± 0.13^{d}	3.2 ± 0.18^{d}	3.7 ± 0.2^{d}

ǂ Means with the same letters are not significantly different according to Duncan test at 5% level of probability.

Water content

In December 2020, the lowest bud water content (WC) was observed in the 'Khalili' cultivar under the T-shaped cordon system, while the highest bud WC was found in the 'Perlette' cultivar under the creeping system. However, the bud WC of the 'Perlette' cultivar was not significantly different from that of the 'Khalili' cultivar under the creeping system (Table 3). In December 2021, bud WC in all treatments was lower than in the previous year. Similarly, the lowest and highest bud WC were associated with the 'Perlette' cultivar under the creeping system and the 'Khalili' cultivar under the T-shaped training system, respectively.

Bud WC was reduced at the full cold acclimation stage in January compared to December in both years. During this stage, the 'Perlette' cultivar under the creeping system exhibited the highest bud WC, while the 'Khalili' cultivar under the Y-shaped system showed the lowest bud WC (Table 4). In February, compared to January, bud WC increased in all vines. At this stage, vines under the training systems had lower bud WC compared to those under the creeping system (Table 4).

Table 4. Effect of four training systems (one non-trellis or creeping (C) and three bilateral cordon trellis (I-shape, T-shape, Y-shape) systems) on bud water content (%) of 'Perlette' (P) and 'Khalili' (K) grape cultivars from Dec. to Feb. of 2020 and 2021.

Cultivar × Training systems	14 Dec.		18 Jan.		20 Feb.	
	2020	2021	2020	2021	2020	2021
P × C	65.5 ± 0.61^{a}	59.6 ± 3.3^{a}	57.9 ± 1.4^{a}	50.8 ± 3.4^{a}	63.3 ± 1.18^{a}	61.6 ± 3.5^{a}
P × I	56.9 ± 0.92^{b}	48.0 ± 1.6^{c}	51.0 ± 1.6^{c}	41.8 ± 0.51^{c}	56.3 ± 0.45^{b}	49.2 ± 1.64^{c}
P × T	54.3 ± 0.12^{bc}	45.1 ± 0.12^{d}	48.2 ± 0.1^{d}	36.9 ± 0.12^{d}	52.5 ± 0.71^{c}	46.3 ± 0.12^{d}
P × Y	54.1 ± 1.0^{bc}	47.9 ± 0.50^{cd}	49.5 ± 0.8^{cd}	39.4 ± 0.38^{cd}	51.9 ± 2.3^{c}	49.2 ± 0.50^{c}
K × C	63.9 ± 1.5^{a}	57.7 ± 1.4^{b}	55.0 ± 0.63^{b}	45.6 ± 1.9^{b}	52.7 ± 1.9^{b}	53.4 ± 1.7^{b}
K × I	52.7 ± 1.2^{cd}	46.5 ± 0.47^{cd}	45.5 ± 1.1^{e}	37.6 ± 0.81^{d}	50 ± 0.36^{c}	47.1 ± 1.3^{cd}
K × T	49.1 ± 0.1^{cd}	39.9 ± 0.12^{e}	44.5 ± 0.7^{e}	32.3 ± 0.18^{e}	46.9 ± 0.83^{d}	40.9 ± 0.55^{e}
K × Y	50.6 ± 0.6^{cd}	39.1 ± 2.02^{e}	42.9 ± 0.12^{f}	30.8 ± 1.52^{e}	43.8 ± 2.7^{e}	38.1 ± 2.36^{e}

ǂ Means with the same letters are not significantly different according to Duncan test at 5% level of probability.

Proline

Analysis of bud proline content across all sampling stages during both years revealed that proline accumulation was higher in vines grown under bilateral cordon training systems (Y, T, I) compared to those under the creeping system. Notably, the 'Khalili' cultivar exhibited greater proline accumulation than the 'Perlette' cultivar (Table 5). Among the bilateral cordon training systems, the highest proline content was observed in the buds of the 'Khalili' cultivar under the Y-shaped training system, although this was not statistically significantly different from the proline content in the buds of the same cultivar under the T-shaped bilateral cordon training system. In 2021, proline content in the buds of both cultivars was higher than in 2020. However, the trend of proline changes across grape cultivars and training systems remained consistent in both years (Table 5).

Table 5. Effect of four training systems (one non-trellis or creeping (C) and three bilateral cordon trellis (I-shape, T-shape, Y-shape) systems) on bud proline (μ mol g^{-1} FW) of 'Perlette' (P) and 'Khalili' (K) grape cultivars from Dec. to Feb. of 2020 and 2021.

Cultivar × Training systems	14 Dec.		18 Jan.		20 Feb.	
	2020	2021	2020	2021	2020	2021
P × C	3.7 ± 0.41^{d}	4.5 ± 0.25^{b}	6.0 ± 0.34^{d}	5.6 ± 0.08^{e}	4.03 ± 0.35^{d}	3.4 ± 0.07^{c}
P × I	4.4 ± 0.18^{c}	5.5 ± 0.23^{b}	7.5 ± 0.17^{c}	6.9 ± 0.05^{d}	5.0 ± 0.05^{c}	4.9 ± 0.26^{b}
P × T	4.6 ± 0.01^{bc}	5.0 ± 0.31^{b}	7.7 ± 0.09^{c}	6.7 ± 0.34^{d}	5.8 ± 0.15^{c}	5.1 ± 0.5^{b}
P × Y	4.6 ± 0.17^{bc}	4.6 ± 0.15^{b}	8.5 ± 0.48^{ab}	7.6 ± 0.15^{c}	5.8 ± 0.15^{c}	4.3 ± 0.15^{c}
K × C	5.2 ± 0.08^{b}	4.1 ± 0.20^{b}	7.4 ± 0.40^{c}	7.7 ± 0.14^{c}	5.1 ± 0.12^{c}	4.8 ± 0.08^{b}
K × I	4.9 ± 0.26^{bc}	5.5 ± 0.26^{b}	8.1 ± 0.19^{b}	8.0 ± 0.16^{b}	5.6 ± 0.24^{b}	4.6 ± 0.07^{b}
K × T	5.8 ± 0.22^{ab}	7.3 ± 0.13^{a}	9.3 ± 0.10^{a}	8.9 ± 0.11^{ab}	6.2 ± 0.08^{a}	5.6 ± 0.01^{a}
K × Y	6.03 ± 0.06^{a}	7.5 ± 0.19^{a}	9.5 ± 0.33^{a}	9.9 ± 0.26^{a}	6.7 ± 0.32^{a}	5.7 ± 0.26^{a}

ǂMeans with the same letters are not significantly different according to Duncan test at 5% level of probability.

Abscisic acids

In the December sampling phase of both 2020 and 2021, bud abscisic acid (ABA) content was influenced by cultivar and training system. At this stage, the highest ABA content was observed in the vines of the 'Khalili' cultivar under the Y-shaped cordon training system, while the lowest ABA content was recorded in the 'Perlette' cultivar under the creeping system (Table 6). In the 'Perlette' grapes, the bud ABA content among vines under different bilateral cordon training systems did not show any statistically significant differences. Similarly, the ABA content of the 'Khalili' cultivar under the I-shaped and T-shaped systems was not statistically different (Table 6).

In January of both years, bud ABA content significantly increased compared to the December stage, with a more pronounced increase observed in the 'Khalili' cultivar compared to the 'Perlette' cultivar. Additionally, the ABA contents of both 'Perlette' and 'Khalili' grape cultivars under the Y-shaped system were higher than those in other training systems (Table 6).

During the third sampling stage in February, bud ABA content in all vines decreased compared to January. At this stage, vines grown under the bilateral cordon training systems exhibited higher ABA content than those under the creeping system. Furthermore, the bud ABA content was greater in the 'Khalili' cultivar than in the 'Perlette' cultivar. Specifically, the ABA content of grape buds from the 'Khalili' cultivar under the Y-shaped system was higher by 38.8% and 20.9% compared to that of the 'Perlette' cultivar under the same training system in 2020 and 2021, respectively (Table 6).

Table 6. Effect of four training systems (one non-trellis or creeping (C) and three bilateral cordon trellis (I-shape, T-shape, Y-shape) systems) on bud abscisic acid (nmol g^{-1} FW) of 'Perlette' (P) and 'Khalili' (K) grape cultivars from Dec. to Feb. of 2020 and 2021.

Cultivar × Training systems	14 December		18 January		20 February	
	2020	2021	2020	2021	2020	2021
P × C	24.2 ± 0.38^{d}	34.2 ± 0.38^{d}	33.8 ± 1.9^{e}	36.7 ± 0.38^{d}	16.0 ± 1.60^{d}	28.1 ± 0.38^{d}
P × I	33.3 ± 0.93^{c}	41.0 ± 1.60^{bc}	40.6 ± 2.2^{c}	45.5 ± 1.6^{c}	23.8 ± 1.64^{c}	34.9 ± 1.6^{bc}
P × T	33.1 ± 0.38^{c}	43.1 ± 0.38^{bc}	42.7 ± 0.3^{c}	45.6 ± 0.38^{b}	28.2 ± 0.38^{c}	37.0 ± 0.37^{bc}
P × Y	34.3 ± 1.59^{c}	44.3 ± 1.5^{b}	43.8 ± 3.1^{c}	46.8 ± 1.59^{b}	29.4 ± 1.9^{c}	38.2 ± 1.50^{b}
K × C	33.0 ± 2.26^{c}	36.3 ± 0.38^{cd}	37.9 ± 2.2^{d}	44.2 ± 1.6^{c}	28.1 ± 2.26^{c}	30.2 ± 0.38^{dc}
K × I	43.4 ± 2.17^{b}	43.1 ± 2.2^{b}	46.0 ± 1.60^{b}	49.6 ± 2.26^{b}	37.5 ± 1.40^{b}	37.0 ± 2.26^{bc}
K × T	45.9 ± 1.7^{b}	45.2 ± 0.38^{b}	48.1 ± 0.38^{b}	52.7 ± 0.38^{b}	39.7 ± 0.53^{b}	39.1 ± 0.40^{b}
K × Y	55.7 ± 1.6^{a}	60.0 ± 0.61^{a}	56.9 ± 0.38^{a}	61.5 ± 0.38^{a}	48.1 ± 0.38^{a}	48.3 ± 1.10^{a}

ǂ Means with the same letters are not significantly different according to Duncan test at 5% level of probability.

Sucrose, glucose and fructose

According to the results presented in Table 7, significant differences were observed between various breeding systems and cultivars regarding bud soluble sugar content (glucose, fructose, and sucrose) during both years. The highest sucrose content was found in the vines of the 'Khalili' cultivar under the Y-shaped bilateral cordon training system. Conversely, the lowest sucrose values in the first year were recorded for both cultivars under the non-trellised training system. In the second year, the 'Perlette' cultivar under the non-trellised training system exhibited the lowest sucrose content.

Regarding glucose content, the highest levels were also found in the 'Khalili' cultivar under the Y-shaped bilateral cordon training system, while the lowest glucose content was recorded in the 'Perlette' cultivar under the non-trellised training system. Notably, during the January and February sampling phases of the first year, the 'Perlette' cultivar under the I-shaped bilateral cordon training system also displayed the lowest glucose content.

Fructose content was highest in the 'Khalili' cultivar under the Y-shaped bilateral cordon training systems, whereas the lowest fructose levels were found in the 'Perlette' cultivar under the non-trellised training system (Table 7).

Putrescine, spermidine and spermine

The results presented in Table 8 indicate a significant difference ($p < 0.01$) between various training systems and cultivars regarding spermidine and spermine polyamine content during both years. The highest spermidine content was found in the vines of both cultivars under the T-shaped and Y-shaped bilateral cordon training systems, while the lowest levels of this polyamine were recorded in the 'Perlette' cultivar under the non-trellised training system.

For spermine content, the highest amounts were also observed in the vines under the T-shaped and Y-shaped bilateral cordon training systems. In contrast, the lowest spermine levels were found in the vines of both cultivars under the non-trellised training system. Putrescine content was significantly different only during the third sampling stage (February), with the highest levels in the 'Khalili' cultivar under cordon training systems and the lowest levels in the 'Perlette' cultivar under the non-trellised training system (Table 8).

Discussion

Training systems significantly influence tissue maturation and carbohydrate accumulation in grape canes and buds by affecting the distribution of incoming sunlight within the canopy (Yu et al., 2022). Specifically, the cold acclimation process and frost tolerance of grapevines are strongly influenced by vine training methods. In this study, vines grown on bilateral cordon trellis systems (I-shaped, T-shaped, Y-shaped) exhibited greater cold tolerance compared to those under the creeping training system across all three measurement stages over two years.

Table 7. Effect of four training systems (one non-trellis or creeping (C) and three bilateral cordon trellis (I-shape, T-shape, Y-shape) systems) on bud soluble sugars (μ mol g^{-1} FW) of 'Perlette' (P) and 'Khalili' (K) grape cultivars from Dec. to Feb. of 2020 and 2021.

Cultivar × Training systems	Sucrose			Glucose			Fructose		
2020	**14 Dec.**	**18 Jan.**	**20 Feb.**	**14 Dec.**	**18 Jan.**	**20 Feb.**	**14 Dec.**	**18 Jan.**	**20 Feb.**
P × C	41.4 ± 1.22[cd]	41.1 ± 1.69[d]	42 ± 2.08[d]	51.9 ± 1.92[d]	64.3 ± 1.4[c]	51.9 ± 1.92[c]	48.4 ± 1.92[d]	60.7 ± 1.4[d]	48.4 ± 1.9[e]
P × I	45.6 ± 2.5[cd]	47.24 ± 2.8[cd]	48.03 ± 2.6[cd]	59.7 ± 2.4[c]	66.8 ± 3.6[c]	59.1 ± 2.6[c]	56.2 ± 2.4[c]	62.9 ± 3.5[cd]	57.6 ± 1.9[cd]
P × T	47.7 ± 1.02[cd]	51.4 ± 1.6[cd]	52.3 ± 1.8[bc]	69.3 ± 1.64[bc]	76.7 ± 0.65[b]	72.5 ± 0.55[b]	65.7 ± 1.64[b]	73.1 ± 0.65[b]	67.7 ± 1.15[d]
P × Y	50.2 ± 1.9[bcd]	57 ± 1.33[cd]	56.2 ± 3.32[bc]	68.8 ± 1.41[b]	76.1 ± 1.48[b]	70.50 ± 2.2[b]	65.3 ± 1.41[b]	72.5 ± 1.48[b]	65.0 ± 2.5[bc]
K × C	39.5 ± 0.32[d]	40.23 ± 1.6[d]	40.8 ± 1.46[d]	59.3 ± 0.20[c]	71.2 ± 0.88[bc]	67.8 ± 0.98[bc]	55.8 ± 6.20[c]	65.6 ± 0.8[bc]	54.3 ± 0.98[ad]
K × I	53.5 ± 2.0[bc]	60.5 ± 1.9[b]	61.7 ± 2.11[b]	67.01 ± 1.6[b]	74.8 ± 1.69[b]	70.1 ± 1.28[b]	65.8 ± 1.66[b]	69.5 ± 1.9[bc]	66.5 ± 1.28[b]
K × T	60.6 ± 2.4[b]	58.9 ± 2.4[b]	61.8 ± 1.8[b]	77.8 ± 0.73[b]	0.79 ± 0.0[ab]	72.5 ± 1.7[b]	68.01 ± 2[b]	72.2 ± 0.73[b]	69 ± 1.67[b]
K × Y	78.1 ± 3.39[a]	78.1 ± 2.17[a]	77.05 ± 1.5[a]	91.5 ± 0.57[a]	0.82 ± 0.01[a]	90.8 ± 1.21[a]	81.9 ± 0.74[a]	89. ± 1.9[a]	87.3 ± 1.21[a]
2021									
P × C	23.4 ± 1.91[d]	30.4 ± 2.7[c]	34 ± 1.8[c]	22.1 ± 0.02[d]	48 ± 0.63[d]	43.8 ± 1.2[f]	26.4 ± 1.9[d]	54.4 ± 2.7[c]	49 ± 1.85[c]
P × I	39.2 ± 3.3[bc]	39.2 ± 2.81[bc]	40 ± 2.67[bc]	39.9 ± 2.6[c]	60.8 ± 2.3[bc]	52.2 ± 3.4[def]	42.2 ± 3.3[bc]	63.3 ± 2.8[cb]	55.1 ± 2.6[bc]
P × T	43.7 ± 1.67[bc]	43.4 ± 1.68[b]	44.3 ± 1.89[b]	44.4 ± 0.96[c]	72.3 ± 1.74[b]	67.5 ± 2.6[bc]	46.7 ± 1.63[bc]	67.4 ± 1.6[b]	59.3 ± 1.89[b]
P × Y	45.5 ± 1.90[b]	45.7 ± 1.92[b]	44.6 ± 1.91[b]	42.2 ± 0.76[c]	61.9 ± 0.19[bc]	56.5 ± 0.01[cde]	48.5 ± 1.9[b]	69.7 ± 1.92[b]	59.6 ± 1.91[b]
K × C	34.2 ± 1.8[bc]	38.9 ± 2.6[bc]	39.6 ± 2.5[bc]	24.8 ± 2.3[d]	50.9 ± 1.23[cd]	47.9 ± 2.5[ef]	37.1 1.82[c]	62.9 ± 2.6[bc]	54.5 ± 2.5[bc]
K × I	40.8 ± 1.6[bc]	45.8 ± 1.9[b]	47 ± 1.8[b]	53.1 ± 1.7[b]	62.8 ± 1.7[bc]	59.5 ± 0.59[cd]	43.8 ± 1.66[bc]	69.8 ± 1.95[b]	62.5 ± 1.86[b]
K × T	46.3 ± 1.8[b]	47.6 ± 0.83[b]	47.1 ± 1.4[b]	47.7 ± 2.2[bc]	72.5 ± 4.6[b]	71.3 ± 3.5[b]	45.3 ± 1.63[bc]	62.3 ± 1.06[bc]	61.8 ± 1.85[b]
K × Y	58.1 ± 0.57[a]	69.1 ± 0.33[a]	70.3 ± 0.25[a]	71.1 ± 0.19[a]	90.7 ± 1.98[a]	85.8 ± 1[a]	65.5 ± 2.05[a]	78.1 ± 1.9[a]	82.3 ± 1.0[a]

ǂ Means with the same letters are not significantly different according to Duncan test at 5% level of probability.

Table 8. Effect of four training systems (one non-trellis or creeping (C) and three bilateral cordon trellis (I-shape, T-shape, Y-shape) systems) on bud polyamines (n mol g^{-1} FW) of 'Perlette' (P) and 'Khalili' (K) grape cultivars from Dec. to Feb. of 2020 and 2021.

Cultivar × Training systems	Putrescine			Spermine			Spermidine		
2020	14 Dec.	18 Jan.	20 Feb.	14 Dec.	18 Jan.	20 Feb.	14 Dec.	18 Jan.	20 Feb.
P × C	8.0 ± 0.1^{g}	12.4 ± 1.20^{c}	7.3 ± 1.2^{c}	9.8 ± 0.65^{d}	12.6 ± 0.53^{d}	7.5 ± 0.38^{d}	9.9 ± 0.13^{d}	13.9 ± 0.39^{d}	9.4 ± 0.4^{e}
P × I	10.1 ± 0.69^{f}	12.8 ± 1.4^{c}	10.1 ± 1.4^{c}	16.3 ± 0.49^{bc}	20 ± 0.43^{c}	14.6 ± 0.33^{bc}	19.7 ± 0.03^{b}	23.7 ± 0.09^{b}	16.1 ± 0.17^{dc}
P × T	11.9 ± 0.20^{de}	15.1 ± 0.8^{bc}	11.4 ± 0.8^{b}	16.5 ± 0.15^{bc}	18.6 ± 0.13^{c}	12.6 ± 0.13^{c}	26.9 ± 0.57^{a}	30.9 ± 1.73^{a}	19.3 ± 1^{abc}
P × Y	13.3 ± 0.45^{cd}	16.5 ± 2^{ab}	12.2 ± 2^{ab}	17.4 ± 0.2^{ab}	23.2 ± 0.33^{b}	17.2 ± 0.33^{b}	26.9 ± 0.11^{a}	30.5± 0.32^{a}	17.4 ± 0.5^{bcd}
K × C	11.3 ± 0.46^{ef}	12.9 ± 1.1^{c}	10 ± 1.1^{c}	14.7 ± 0.9^{c}	14.9 ± 0.62^{d}	9.2 ± 0.5^{d}	15.4 ± 0.17^{c}	19.4 ± 0.53^{c}	14.4 ± 0.8^{d}
K × I	13.6 ± 0.47^{bc}	12.5 ± 0.33^{c}	12.1 ± 0.33^{ab}	16.3 ± 0.1^{bc}	22.8 ± 0.38^{b}	16.5 ± 0.19^{b}	19.2 ± 0.02^{b}	23.2 ± 0.08^{b}	18.6 ± 0.5^{abc}
K × T	15 ± 0.3^{ab}	19 ± 2.7^{a}	12.6 ± 2.7^{ab}	18.2 ± 0.3^{a}	23.3 ± 0.62^{b}	16.6 ± 0.57^{b}	25.4 ± 0.06^{a}	29.4 ± 0.18^{a}	20.3 ± 0.37^{ab}
K × Y	15.4 ± 0.79^{a}	19.1 ± 1.9^{a}	12.7 ± 1.9^{a}	17.9 ± 0.1^{a}	29.2 ± 0.97^{a}	23.5 ± 0.96^{a}	29.1 ± 0.02^{a}	33.1 ± 0.08^{a}	21.3 ± 0.37^{a}
2021									
P × C	9.4 ± 1.20^{d}	12.4 ± 1.20^{e}	7.3 ± 1.2^{c}	9.5 ± 0.28^{e}	10.9 ± 0.38^{d}	8.9 ± 0.21^{e}	8.5 ± 0.49^{c}	15.9 ± 0.26^{d}	11.8 ± 0.27^{d}
P × I	11 ± 1.4^{cd}	12.8 ± 1.4^{de}	10.2 ± 1.4^{b}	13.9 ± 0.31^{d}	16.08 ± 046^{c}	12.7 ± 0.09^{d}	17.3 ± 0.28^{b}	18 ± 0.08^{cd}	14 ± 0.42^{bcd}
P × T	12.4 ± 0.80^{bc}	4.5 ± 0.80^{dc}	11.6 ± 0.8^{ab}	14 ± 0.20^{bcd}	19.2 ± 0.87^{ab}	14.5 ± 0.53^{bc}	22.7 ± 0.10^{a}	26.5 ± 0.34^{ab}	17.7 ± 0.79^{ab}
P × Y	11.5 ± 2^{bc}	15.2 ± 2^{bc}	10.9 ± 2^{ab}	16.3 ± 0.20^{ab}	19.7 ± 0.59^{ab}	16.7 ± 0.51^{a}	22.1 ± 0.65^{a}	24.5 ± 1.8^{ab}	16.1 ± 0.5^{8abc}
K × C	13.5 ± 1.1^{b}	13.9 ± 1.1^{dc}	10.6 ± 1.1^{b}	14 ± 0.55^{d}	14.9 ± 0.28^{c}	10.6 ± 0.34^{e}	14.6 ± 0.54^{b}	22.7 ± 0.25^{bc}	3.7 ± 1.23^{dc}
K × I	17.5 ± 0.33^{a}	13.6 ± 0.33^{ce}	13.1 ± 0.33^{a}	14.3 ± 0.11^{cd}	17.6 ± 0.46^{b}	13.6 ± 0.33^{cd}	17.1 ± 0.08^{b}	24.2 ± 0.36^{ab}	16 ± 0.48^{abc}
K × T	15.5 ± 2.7^{a}	16.3 ± 2.7^{ab}	12.6 ± 2.7^{a}	16 ± 0.36^{abc}	20.9 ± 0.56^{a}	15.6 ± 0.41^{ab}	22.6 ± 1.54^{a}	25 ± 1.5^{ab}	17.4 ± 0.28^{ab}
K × Y	16.6 ± 1.9^{a}	17.7 ± 1.9^{a}	13.1 ± 1.9^{a}	16.9 ± 0.42^{a}	20.2 ± 0.70^{a}	15.7 ± 0.13^{a}	23.9 ± 0.91^{a}	27.1 ± 0.06^{a}	18.5 ± 0.68^{a}

ǂ Means with the same letters are not significantly different according to Duncan test at 5% level of probability.

Cordon training systems (T and Y shapes) enhance light penetration in the canopy, facilitating cane maturation (i.e., periderm development) and subsequently increasing vine tolerance to low temperatures. Conversely, I-shaped cordon systems, characterized by a dense and undivided canopy, create a high degree of shading. These vines often continue to grow until late autumn, resulting in inadequate hardening of the canes and buds, making them more susceptible to low temperatures compared to other cordon systems. The horizontal distribution of canes in bilateral Y and T shapes reduces cell sap movement and vegetative growth. In contrast, canes in the I-shaped or creeping systems are positioned to shade one another, leading to less efficient growth.

The positioning of canes on the Y and T-shaped systems promotes better spatial distribution than the I-shaped cordon system, resulting in larger diameters and enhanced periderm development in canes, alongside increased carbohydrate reserves in permanent tissues (Mehrpour et al., 2022). Furthermore, the horizontal orientation of cordons in T or Y-shaped training systems raises the carbon-to-nitrogen (C/N) ratio by reducing apical dominance and vascular sap flow, thereby controlling the vegetative growth of the vine (Mamun et al., 2012; Du et al., 2023).

The 'Khalili' cultivar trained on the Y-shaped bilateral cordon training system exhibited lower MDA, H_2O_2, and WC across all three sampling stages compared to other training/cultivar treatments. The stability of the cell membrane (evidenced by lower MDA and H_2O_2) and the cold tolerance of 'Khalili' grapes can be attributed to several factors: 1) the early ripening of this cultivar, 2) larger cane diameter and bud size, 3) greater accumulation of suberin and lignin on bud scales, and 4) a higher ratio of xylem to pith in canes compared to the 'Perlette' cultivar. Notably, 'Khalili' is one of the cultivars with high tolerance to winter cold (-23 ºC) and has a late bloom time in spring but ripens early in summer. This results in a flowering-to-fruit ripening interval of less than two months, extending the period between fruit harvest and leaf fall, which can enhance the cold acclimation process (Karimi, 2014).

The 'Khalili' cultivar, grown under the Y-shaped bilateral cordon training system, exhibited higher proline and polyamine content across all three sampling stages compared to other treatments. In contrast, the content of these nitrogenous compounds was lower in the vines of the 'Perlette' cultivar under the non-trellised (creeping) training system (P × C) throughout all sampling phases in both years (2020-2021). Proline, a non-structural amino acid, is synthesized in response to biotic and abiotic stresses, helping to prevent cell acidification and stress. Its antioxidant properties allow proline to neutralize free radicals, thereby protecting membrane proteins from oxidative damage (Ershadi et al., 2016). Previous studies have reported a positive correlation between proline content and increased cold tolerance in grapes (Karimi, 2020) and pomegranates (Soloklui et al., 2012).

Polyamine synthesis is influenced by various factors, including light, environmental stresses, injuries, growth regulators, and nutrition. These factors trigger the regulation of polyamine concentration by affecting enzymes such as arginine decarboxylase, ornithine decarboxylase, and spermidine synthase (Groppa and Benavides, 2008). Polyamines possess a positive charge and can bind to anionic molecules like proteins, phosphate groups, nucleic acids, membrane phospholipids, and pectin polysaccharides. This interaction contributes to membrane stability and prevents deterioration under low-temperature conditions (Groppa and Benavides, 2008).

The content of soluble sugars (glucose, fructose, and sucrose) in the buds was significantly influenced by the type of training system and cultivar. Generally, at different stages of cold acclimation, the soluble sugar content in the 'Khalili' cultivar trained on Y or T-shaped cordon systems was higher than in other treatments. The quantity of stored carbohydrates can vary due to seasonal changes (Karimi and Naserpour, 2024), the yield of the previous year's crop (Bavougian et al., 2012), and the spatial arrangement of canes and leaves (Wimmer et al., 2018), among other factors. The lower sugar content observed in the creeping training system compared to the trellised cordon systems may be attributed to reduced light interception, leading to diminished photosynthetic capacity of the leaves. This ultimately results in delayed leaf fall in these training systems at the onset of the cold season, disrupting the aging process of the leaves and the storage of materials in permanent tissues.

Open canopies enhance photosynthesis, carbohydrate storage, and the maturation of canes and buds (Poni and Intrieri, 2001; Zoecklein et al., 2008). In comparing cordon training systems, the vertical training system (I-shape) exhibits greater leaf and cane density in the lower canopy layers, which results in less efficient sugar production and storage compared to the T and Y-shaped bilateral cordon systems. The accumulation and increase of sugars and proline during the cold season have been documented in various cold-climate trees

(Ershadi et al., 2016; Karimi and Naserpour, 2024). The observed differences in sugar levels between the T and Y-shaped bilateral cordon training systems are likely attributed to the orientation and type of trellised support for the canes. The T-shape training system typically has more geotropic canes than the Y-shape system, leading to increased auxin hormone levels in the apex and proximal sections of the canes. This results in smaller diameters and reduced xylem development, ultimately decreasing hydraulic and stomatal conductivity in the plant (Wimmer et al., 2018).

Previous studies have noted that the orientation of geotropic branches can diminish vegetative growth and leaf density, corroborating our findings (Heuvel et al., 2004; Bavougian et al., 2012). One critical function of trellised training systems is to maintain the phototropic orientation of the branches. Several reports indicate a reduction in shoot growth, leaf area, and net photosynthesis, as well as lower protein levels, decreased activity of ribulose 1,5-bisphosphate (RuBP), and diminished glucose and sucrose levels in geotropic branches compared to phototropic branches (Pisciotta et al., 2004; Schubert et al., 1995; Lovisolo and Schubert, 2000).

Cold injury predominantly affects the proximal part of the cane, which typically has a higher water content than other sections and is thus more susceptible to freezing (Fennell, 2004). The bud ABA content was significantly influenced by both the training system and cultivar. Overall, during various stages of cold acclimation, the ABA content in the 'Khalili' cultivar was higher under the Y or T-shaped training systems than in other treatments. The elevated ABA content in the Y-shaped bilateral cordon system, particularly for the 'Khalili' cultivar compared to non-trellised systems, underscores the role of this phytohormone in grapevine cold acclimation. The capacity of cultivars to accumulate ABA is a genetically determined trait that significantly impacts their cold tolerance.

Moreover, the role of viticulture practices during the growing season and their effect on winter cold tolerance should not be overlooked, especially for vines with a trellised training system that promotes optimal sunlight distribution within the canopy. Vines with favorable conditions regarding carbohydrate reserves and ABA content enter the cold adaptation phase more effectively, developing cold tolerance more rapidly under cordon trellis systems than in creeping systems. Increased levels of ABA reduce the oxidation of membrane lipids, stabilizing cell membranes and preserving intracellular proline (Karimi et al., 2016). A relationship between ABA accumulation and cold tolerance has been reported across various grape cultivars (Karimi and Ershadi, 2014; Karimi, 2017; Mehrpour et al., 2022).

Conclusions

The vines under the Y-shape training system demonstrated superior cold acclimation, as indicated by the physiological indices measured. This advantage can be attributed to the effective control of vegetative growth, regulation of shading, and improved light penetration within the canopy, as well as the vertical development of canes, which reduces the risk of freezing injury during winter. While the T-shape cordon system produced similar results in many indices, its inability to fully support the canes and the downward orientation of the canes limited further enhancement of cold acclimation. Among the various cordon training systems, the I-shape cordon system exhibited the lowest cold tolerance. Based on the findings of this research, the Y-shape cordon training system, combined with the 'Khalili' cultivar, is identified as the most suitable choice for establishing vineyards in cold regions such as Malayer.

Conflict of Interest

The authors indicate no conflict of interest in this work.

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