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Effect of Late-Season Defoliation on Vine Cold Tolerance and Chilling Requirement: An Introduction to Greenhouse Grape Production

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ABSTRACT

The late-season source leaves play a decisive role in the grapevine's cold tolerance and chilling requirements. The objective was to evaluate the effect of manual late-season defoliation on the cold tolerance and chilling requirements of 'Bidaneh-Sefid' grapes. Treatments were applied on October 23 via manual leaf removal of the canes at four levels: 1) control (no leaf removal), 2) apical-node defoliation, 3) Basal-node defoliation, and 4) All-node defoliation of the canes. Based on the results, the lowest cold tolerance (highest electrolyte leakage, or EL) as assessed via the EL method was observed in the 'all-node defoliation' treatment, whereas the highest cold tolerance (lowest EL) was found in the control vines. The highest levels of glucose, fructose, sucrose, and raffinose were recorded in the control treatment, while the lowest levels of these soluble sugars were observed in the 'all-node defoliation' treatment. Similarly, the highest levels of putrescine, spermidine, and spermine were found in the control treatment. Abscisic acid content in the vines subjected to 'all-node defoliation' treatment was 28.5% lower than that of the control vines. The highest levels of gibberellin and auxin were observed in the control plants. In the defoliation treatments group, budbreak (time to 50% budburst) occurred 11 days earlier than in the control group. 'All-node defoliation' delayed the rate of budbreak. Overall, it was determined that late-season defoliation can reduce cold tolerance in vineyard vines, however, this horticultural practice can be employed to accelerate budbreak following a standard 400-hour chilling treatment for greenhouse grape production.

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1. Introduction

The grapevine (*Vitis vinifera* L.) is a perennial plant belonging to the Vitaceae family. This family comprises 17 genera and approximately 1,000 species, which mostly grow as shrubs or climbing vines. It is one of the most important horticultural crops globally and in Iran, cultivated in over 90 countries as a significant fruit crop with diverse applications (Keller, 2015; Zaeri-Behrooz et al., 2026). Low temperature stress is one of the environmental factors affecting plant growth and development, as well as causing morphological, physiological, and biochemical changes in plants (Cragin *et al*., 2017; Ghasemi et al., 2026). As the temperature decreases, the rate of metabolic processes first decreases, and if the cold persists, it may stop completely (Eom *et al*., 2022). If the cold is sudden, the plant experiences cold shock and is unable to produce an appropriate physiological response (Beheshti Rooy *et al*., 2017). However, if the temperature decrease is gradual, the plant adapts to cold conditions as much as possible by inducing various responses, which help stabilize the membranes by changing the composition of membrane lipids, accumulating simple sugars, and hydrophilic proteins (Beheshti Rooy *et al*., 2017; Cragin *et al*., 2017). In addition to these changes, increased antioxidant mechanisms and increased levels of polyamines and the hormone abscisic acid in the intercellular space can play an important role in reducing cell damage caused by cold stress (Karimi, 2017; Totonchi et al., 2026).

One practice employed in many vineyards especially during late season is the sudden removal of the entire vine's foliage through livestock grazing—a method based on the misconception that the consumption of leaves by animals eliminates pathogens and insect eggs. Naturally, this improper practice disrupts the vine's acclimation process and reduces the buds' capacity to withstand freezing winter temperatures (Sarikhani *et al*., 2020); it may also influence the timing of spring budburst and increase susceptibility to spring frost damage. The removal of photosynthetically active leaves at various stages of vine development is a technique utilized for a range of purposes (Sivilotti *et al*., 2016). The timing of basal-node leaf removal is particularly critical. If performed too early (e.g., prior to flowering), it results in a loss of leaf area and a reduction in the photosynthesis required to produce carbohydrates for berry development (Cragin *et al*., 2017). Basal-node leaf removal is often employed to increase the exposure of grape clusters to sunlight and heat. Removing leaves before bloom can lead to looser clusters with improved light and air penetration, ultimately enhancing fruit composition; leaf removal regulates the vine's total leaf area and influences the relationship between photosynthetically active leaf area and yield (Smith and Holzapfel, 2009; Sarikhani *et al*., 2020).

Appropriate viticultural practices—such as proper structural pruning suited to the region, fruiting pruning tailored to the vine's vigor, balanced nutrition, avoidance of leaf removal (especially late in the season), and other measures that boost end-of-season carbohydrate reserves in the shoots, cordons, and trunk—significantly influence flower bud development, crop load regulation, and the winter hardiness of grapevine buds (Smith and Holzapfel, 2009; Zaeri-Behrooz et al., 2026). Leaves are the primary photosynthetic source organs and contribute substantially to carbohydrate production and storage; consequently, excessive leaf removal weakens the grapevine. Leaf removal is typically practiced only in cold regions, as the growing season there is short and the procedure is recommended solely to enhance sunlight exposure for the clusters; conversely, in hot regions, the shade provided by the foliage prevents the clusters and shoots from scorching, and thus, the leaves should not be removed (Kaya *et al*., 2020; Sarikhani *et al*., 2020).

Insufficient accumulation of winter chill negatively affects the vegetative and reproductive growth of fruit trees (Eshghi et al., 2024; Kazemie et al., 2025). As with other deciduous fruit species, the timing of flowering in grapevines is determined by the interplay between chilling and heat requirements, with each factor playing a specific role depending on the local climate (Dokoozlian, 1999; Totonchi et al., 2026). In other words, the extent of bud development is crucial for breaking dormancy and resuming growth, and is influenced by factors such as temperature, nutrition, and irrigation (Cragin *et al*., 2017; Jabbari et al., 2025). Abscisic acid and certain phenolic compounds are among the internal factors determining bud dormancy in grapes; these substances are translocated from the leaves to the buds at the end of the season, and their accumulation levels dictate the intensity of winter dormancy and the timing of dormancy release (Cragin *et al*., 2017; Karimi, 2017; Zaeri-Behrooz et al., 2026). Furthermore, the presence of this hormone is essential for enhancing freezing tolerance during the winter dormancy period (Cragin *et al*., 2017; Karimi, 2017).

Given the recent trend toward expanding greenhouse grape cultivation, the application of horticultural techniques to reduce chilling requirements is of particular importance. Indeed, reducing or eliminating the chilling requirement is essential for achieving early budbreak, flowering, fruit set, and subsequent rapid fruit ripening, thereby enabling the supply of out-of-season fruit. Beyond the conventional method of satisfying chilling requirements by exposing vines to temperatures of 7°C, a process that entails significant time and cost (Dokoozlian, 1999; Eshghi et al., 2024), we hypothesized that leaf removal could influence chilling requirements, the timing of budbreak, and physiological indices related to cold tolerance in grapevines by affecting abscisic acid accumulation. Therefore, the present study investigated the effects of late-season defoliation on the cold tolerance and chilling requirements of the ‘Bidaneh-Sefid’ grape cultivar.

2. Materials and Methods

2.1. Plant materials and experimental design

This study was conducted during the 2020–2021 growing season at Malayer University's Research Vineyard (#3) to evaluate the effect of defoliation on cold tolerance and chilling requirements of the 'Bidaneh-Sefid' grape cultivar, based on randomized complete block design (RCBD). Each experimental unit consisted of two vines, and each treatment was represented by three independent experimental units, giving a total of six vines per treatment. A commercial, fruit-bearing vineyard with normal growing conditions was selected, and manual defoliation was performed on October 23 (with ambient temperature of 18ºC) at four levels: 1) control (no defoliation), 2) removal of leaves from the upper half of the cane (apical-node defoliation), 3) removal of leaves from the lower half of the cane (basal-node defoliation), and 4) complete defoliation of cane (all-node defoliation) (Bennett *et al*., 2005). In terms of intensity of defoliation, almost all leaves on the upper or lower half of the cane were removed in 'apical-node' or 'basal-node' defoliation treatments, respectively. Three months after the treatments, physiological and biochemical indices in the vine buds were assessed in January when the vines were in deep winter dormancy.

2.2. Temperature treatments

For cold hardiness related physiological indices measurements, samples were collected from three randomly chosen vines for each treatment (each vine as an experimental unit), and at least two representative cuttings (20-30 cm in length) per each vine were collected in January (with ambient temperature of 10ºC). The cane sections were packed in polyethylene bags, and shipped on ice in Styrofoam boxes to the Laboratory.

Cane segments were rinsed under cold running demineralized water to remove surface contaminants. Samples were wrapped in moist paper towels to ensure ice nucleation, closed in polyethylene bags and were randomly assigned to predetermined test temperatures. Six replicates were assigned for each defoliation/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 -15ºC (selected based on the LT50 results determined for this cultivar in our previous study) freeze test temperature in January (Ershadi *et al*., 2016). The control samples were kept in polyethylene bags at 4 ºC. Freeze test temperatures were determined following preliminary tests on canes collected two days before each sampling date (Karimi, 2020). The freezing rate was 2 ºC h^{-1}, and samples were held at -15ºC test temperature for 90 min, before removing from freezing chamber (Ershadi *et al*., 2016) then, samples were taken from buds of the canes and used for other physiological and biochemical measurements as follows.

2.3. Electrolyte leakage (EL) and water content (WC)

As declared above, before EL determination the bud tissues were exposed to controlled freezing temperatures and EL measurements were started immediately after thawing canes by cutting five compound buds with approximately 2 mm of intact cane tissue surrounding and underlying the bud. The bud segments were placed separately into 70-mL test tubes containing 40 mL of demineralized water. The tubes were capped with aluminum foil and incubated at room temperature under constant shaking at 120 rpm. After 24 h of incubation, the electrical conductivity (EC_1) of the bathing solutions was measured using an electrical conductivity meter (Otago, Japan). The secondary electrical conductivity (EC_2) of the samples was determined after they were placed in an autoclave (temperature 121°C; 15 minutes) and the relative ion leakage (REL) was calculated using the following equation (Campos *et al*., 2003):

$$REL = (EC_1 / EC_2) \times 100$$

For bud water content (WC) measurements, samples were collected at early January and excised and weighed before and after placing in an oven for 3 days at 70 ºC. Bud water content was expressed as percent of fresh weight using the following equation (Ershadi *et al*., 2016):

Bud water content = [(fresh weight - dry weight) / fresh weight] x 100.

2.4. Proline

To assay proline, 0.5 g of buds was ground in the presence of liquid nitrogen, mixed with 3% sulfosalicylic acid (10 ml) and centrifuged. Two ml of the resulting solution was combined with two ml of acetic acid and two ml of ninhydrin reagent (1.25 g ninhydrin + 30 ml glacial acetic acid + 20 ml 6M phosphoric acid) and incubated for 45 minutes in a water bath (90°C). After rapid cooling of the samples, four milliliters of toluene were added and vortexed vigorously to form two phases. The upper phase was used for proline at 520 nm (Bates *et al*., 1973).

2.5. Soluble sugars

The frozen bud tissues were grounded and of powdered tissues (0.5 g) used for soluble sugars extraction using 80% ethanol (10 ml) and the solution centrifuged (8000g) for 15 min. After that, the upper phase of solution filtered using 0.2 µM filters and was used for soluble sugars measurement (Shin *et al*., 2002) with HPLC. The HPLC pump equipped with a SPD UV –Vis detector and a Spherisorb ODS-2 Column (0.3 µm, 150 mm × 4.6 mm i.d.). The used materials for mobile phase were acetonitrile (1:99, v/v) and sodium citrate (pH 5.5) at 0.1 ml min^{-1} flow rate and injection volume of 10 µl. Different concentration of glucose, fructose, sucrose and raffinose were used as standard (Comis *et al*., 2001).

2.6. Endogenous hormones

For analysis of endogenous hormone including of abscisic acid (ABA), gibberellins (GA) and auxin (Indole acetic acid; IAA), 1 g buds tissues were grounded in liquid nitrogen and subjected to extraction in 80% methanol (10 ml), and homogenized 0.01 g vitamin C and 0.01% polyvinylpyrrolidone. Samples were placed in a refrigerator- containing shaker at rate of 120 rpm for 12 h and centrifuged (4000 g_n) for 15 min under low temperature condition (4°C). After collecting of supernatant, its pH was adjusted using 10% phosphate buffer to 8, then filtered through Whatman filters (0.45 µ) and desiccated under stream of air. Next, 0.2 N hydrogen chloride was added to extract and its pH adjusted around 2.5 and then mixed with ethyl acetate (10 m). Ethyl acetate was evaporated from homogenate and obtained extracts were dissolved in acetic acid (0.1 M) and 3% methanol (1 ml) and filtered again. Samples quantitative analysis began by 20 µL injection of filtered extract to a reversed phase high-performance liquid chromatography (Crystal 200 series HPLC; ATI Unicam, Cambridge, UK). The HPLC was 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). The column was eluted by different concentration of methanol (20–80%) in 0.4% (w/v) aqueous acetic acid. The used materials for mobile phase were 20–75% methanol in 1% acetic acid (v/v) at 0.1 ml min^{-1} flow rate for GA and IAA and with at a flow rate of 1.2 mL min^{-1} for ABA (Li *et al*., 2010).

2.7. Endogenous polyamines (PAs)

Buds were picked and kept at -70 °C until upcoming extraction and measurement of polyamines (PAs) based on Walter and Geuns, (1987) method. For extraction, a volume of 2 ml $HClO_4$ (4%) in comprising 1, 7 diaminoheptane-2HCl were added to the grounded tissues of frozen buds or leaves (250 mg) and placed for 60 min at 4 °C. The homogenates were filtered through Whatman filter (0.45 µ). One ml of dansyl chloride solution (10 mg ml^{-1} acetone) and 1 ml of carbonate buffer (pH 9) were added to 0.2 ml obtained homogenates and then heated for 60 min at 60 °C. For extraction of dansylated polyamines, toluene (3 ml) was added to homogenate. The extract was loaded on a column (0.5 g silica gel) and washed with toluol- triethylamine (5 ml, 10/0.3, v/v) and toluol (5 ml). A volume of 3 ml ethyl acetate was used to elute dansylated polyamines and under N_2 volume reduction occurred. Isocratic HPLC-analysis with acetonitrile/H_2O (72/28, v/v) on a 10-cm long 3 mm octadecyl silica column took 8 min and solvent flow of 2 ml min^{-1}. Dansylated putrescine (Put), spermidine (Spm) and spermine (Spd) were used as standard.

2.8. Budbreak test assay

For budbreak test assay after chilling, five 10-15 cm cuttings (containing three buds) were assigned to cooling chamber (5°C for 400 h) to ensure the completion of bud dormancy. Chilling requirement was determined under a controlled forcing experiment based on chilling accumulation. After applying the cold treatments, the cuttings of each treatment were planted in plastic pots containing a mixture of perlite and peat moss (3/1 v/v) and placed in the research greenhouse of the horticulture group with temperature conditions of 20 and 15°C (day/night). Bud break was assessed and the number of buds reaching the balloon or green-tip stage was recorded daily; the index used to determine chilling requirement fulfillment was the number of days until 50% bud break (Dokoozlian, 1999).

For field chilling requirement assay the bud break timing was recorded and compared as an indicator of chilling requirement fulfillment in defoliation-treated and control grapevines. The criterion for bud break was the attainment of the "green tip" stage. The time required for 50% of the buds to open was measured and designated as the date of bud break (Saberi *et al*., 2019).

2.9. Data analysis

Analysis of variation among all measured physiological and biochemical indices were done using GLM procedure of SAS (SAS Institute, Cary, NC). Moreover, the means separation of data was done by Duncan's multiple range tests at $p \leq 0.05$.

3. Results

3.1. Soluble sugars

3.1.1. Glucose

Analysis of variance revealed that the treatment effect (defoliation) on glucose content in the grapevine buds was significant at the 1% probability level (Table 1). Mean comparison results showed that the highest glucose level (48.6 mg/100 gr) was observed in the control treatment, while the lowest (40.4 mg/100 gr) was recorded in the complete leaf removal treatment (Table 2). Complete defoliation reduced glucose content by approximately 17% relative to the control vines (Table 2).

Table1. Analysis of variance of the effect of defoliation on bud soluble sugars content of 'Bidaneh-Sefid' grapevine.

S.O.V	df	Mean of Squares			
		Glucose	Fructose	Sucrose	Raffinose
Treatment	3	36.8**	26.5**	18.83**	3.15**
Block	2	553.4**	410.4**	301.7**	30.3**
Error	6	0.52	0.29	0.84	0.085
C.V	-	1.62	1.33	3.57	3.63

**, * and ns indicate significance at the 1% and 5% levels, respectively, and non-significant ($p > 0.05$) according to Duncan's multiple range test

Table 2. The effect of defoliation on bud soluble sugars content of 'Bidaneh-Sefid' grapevine.

Treatments	Glucose (mg/100 gr)	Fructose (mg/100 gr)	Sucrose (mg/100 gr)	Raffinose (mg/100 gr)
Control	48.6a	43.5a	28.0a	9.0a
Apical-node defoliation	45.2b	42.8b	25.6ab	8.1b
Basal-node defoliation	46.7b	42.0c	26.8b	8.4b
All-node defoliation	40.4c	36.6d	22.2c	6.6c

The same letters indicate no significant difference at the probability level of $P<0.05$.

3.1.2. Fructose

Based on the analysis of variance, the effect of defoliation treatments on the fructose content of the grapevine buds was significant at the 1% probability level (Table 1). Mean comparison results indicated that the treatment effect on fructose content was significant at the 5% level according to Duncan's test; the highest fructose content (43.5 mg/100 gr) was observed in the control treatment, while the lowest its content (36.6 mg/100 gr) was found in 'all-node defoliation' treatment (Table 2). For instance, the buds fructose content in vines subjected to 'all-node defoliation' treatment decreased by 15.9% compared to the control vines.

3.1.3. Sucrose

Analysis of variance indicated that the defoliation treatments effect on bud sucrose content in the grapevine was significant at the 1% probability level (Table 1). Mean comparison results showed that the highest bud sucrose content (28.0 mg/100 gr) was observed in the control treatment, while the lowest level (22.2 mg/100 gr) was found in 'all-node defoliation' treatment (Table 2). Complete defoliation reduced sucrose content by approximately 21% relative to the control vines (Table 2).

3.1.4. Raffinose

Analysis of variance indicated that the defoliation treatment effect on bud raffinose content in the grapevine was significant at the 1% probability level (Table 1). Mean comparison results showed that the highest raffinose content (9.0 mg/100 gr) was observed in the control treatment, while the lowest level (6.6 mg/100 gr) was found in 'all-node defoliation' treatment (Table 2). Complete defoliation reduced raffinose content by approximately 27% relative to the control vines (Table 2).

3.2. Free polyamines

3.2.1. Putrescine

Bud putrescine content was affected significantly (1% probability) by defoliation treatments in grapevine plants (Table 3). Based on results, the highest bud putrescine content (12.0 µmol/gr) was observed in the control treatment, while the lowest content of this polyamine (10.0 µmol/gr) was found in 'all-node defoliation' treatment (Table 4). Complete defoliation reduced putrescine content by approximately 17% relative to the control vines (Table 4).

Table 3. Analysis of variance of the effect of defoliation on bud polyamines content of 'Bidaneh-Sefid' grapevine.

S.O.V	df	Mean of Squares		
		Putrescine	Spermidine	Spermine
Treatment	3	2.34**	68.8**	117.3**
Block	2	20.18**	658.5**	903.9**
Error	6	1.88	92	1.42
C.V	-	1.44	1.53	1.75

**, * and ns indicate significance at the 1% and 5% levels, respectively, and non-significant ($p > 0.05$) according to Duncan's multiple range test

3.2.2. Spermidine

Bud spermidine content was affected significantly (1% probability) by defoliation treatments in grapevine plants (Table 3). Mean comparison results showed that the highest bud spermidine content (67.6 µmol/gr) was observed in the control treatment, while the lowest content of this polyamine (56.3 µmol/gr) was found in 'all-node defoliation' treatment (Table 4). Bud spermidine content in vines subjected to 'all-node defoliation' treatment decreased approximately by 17% compared to the control vines.

3.2.3. Spermine

Analysis of variance results indicated that the treatment effect on spermine levels in the grapevine was significant at the 1% probability level (Table 3). Mean comparison results (Duncan's test) showed that the treatment effect on spermine levels was significant at the 5% level; the highest spermine level (85.9 µmol/gr) was observed in the control plants, while the lowest (71.2 µmol/gr) was recorded in 'all-node defoliation' treatment (Table 4). Bud spermine content in vines subjected to 'all-node defoliation' treatment decreased approximately by 16.9% relative to the control vines.

Table 4. The effect of defoliation on bud polyamines content of 'Bidaneh-Sefid' grapevine.

Treatments	Putrescine (µmol/gr)	Spermidine (µmol/gr)	Spermine (µmol/gr)
Control	12.0a	67.6a	85.9a
Apical-node defoliation	11.0b	62.5c	79.7c
Basal-node defoliation	11.6ab	64.7b	82.5b
All-node defoliation	10.0c	56.3d	71.2d

The same letters indicate no significant difference at the probability level of $P<0.05$.

3.3. Endogenous hormones

3.3.1. Abscisic acid

Bud abscisic acid content was affected significantly (1% probability) by defoliation treatments in grapevine plants (Table 5). The highest abscisic acid content (233.3) was observed in the control group, while the lowest (166.7) was recorded in 'all-node defoliation' treatment (Table 6). Bud abscisic acid content in vines subjected to 'all-node defoliation' treatment decreased approximately by 29% compared to the control vines.

3.3.2. Gibberellin

Bud gibberellin content was affected significantly (1% probability) by defoliation treatments in grapevine plants (Table 5). The highest gibberellin content (41.7 nmol/gr) was found in the control grapevine plants and the lowest its content (24.0 nmol/gr) was observed in 'all-node defoliation' treatment (Table 6).

3.3.3. Auxin

Bud auxin content was affected significantly (1% probability) by defoliation treatments in grapevine plants (Table 5). Based on results the highest auxin content (17.7 nmol/gr) was observed in the control plants, while the lowest content of this endogenous hormone (11.4 nmol/gr) was found in 'all-node defoliation' treatment (Table 6).

Table 5. Analysis of variance of the effect of defoliation on bud endogenous hormones content of 'Bidaneh-Sefid' grapevine.

S.O.V	df	Mean of Squares		
		Abscisic acid	Gibberellin	Auxin
Treatment	3	2452.1**	166.4**	21.47**
Block	2	702.1**	6.78**	156.3**
Error	6	2.08	0.088	0.11
C.V	-	1.5	2.01	1.04

**, * and ns indicate significance at the 1% and 5% levels, respectively, and non-significant (p > 0.05) according to Duncan's multiple range test

Table 6. The effect of defoliation on bud endogenous hormones content of 'Bidaneh-Sefid' grapevine.

Treatments	Abscisic acid (nmol/gr)	Gibberellin (nmol/gr)	Auxin (nmol/gr)
Control	233.3a	41.7a	17.7a
Apical-node defoliation	196.7b	36.0b	15.8b
Basal-node defoliation	181.7c	31.7c	14.0b
All-node defoliation	166.7d	24.0d	11.4c

The same letters indicate no significant difference at the probability level of P<0.05.

3.4. Proline

Bud proline content was affected significantly (1% probability) by defoliation treatments in grapevine plants (Table 7). Mean comparison results showed that the highest proline content (4.29 µmol/gr) was observed in the control group, while the lowest content of this amino acid (28.6 µmol/gr) was recorded in 'all-node defoliation' treatment (Table 8).

Table 7. Analysis of variance of the effect of defoliation on bud electrolyte leakage, proline water content and chilling requirement (day to budbreak, 5º C, 400 h), of 'Bidaneh-Sefid' grapevine.

S.O.V	df	Mean of Squares			
		Proline	Water content	Electrolyte leakage	Day to budbreak
Treatment	3	113.6**	56.00**	93.86**	56.24**
Block	2	60.02**	16.0**	29.1**	15.9**
Error	6	0.55	1.08	1.86	1.47
C.V	-	2.13	1.05	4.95	2.44

**, * and ns indicate significance at the 1% and 5% levels, respectively, and non-significant (p > 0.05) according to Duncan's multiple range test

3.5. Bud water content

Analysis of variance revealed that the treatment effect on bud water content in the grapevine was significant at the 1% probability level (Table 7). The highest bud water content (60.0%) was obtained in the control group, whereas the lowest (50.0%) was observed in 'all-node defoliation' treatment (Table 8).

Table 8. The effect of defoliation on bud electrolyte leakage, proline water content and chilling requirement (day to budbreak, 5º C, 400 h) of 'Bidaneh-Sefid' grapevine.

Treatments	Proline (µmol/gr)	Water content (%)	Electrolyte leakage (%)	Day to budbreak (day)
Control	4.29a	68.2a	41.7d	25.5a
Apical-node defoliation	3.56b	61.5b	45.7c	19.0b
Basal-node defoliation	3.18b	58.7c	48.0b	22.5c
All-node defoliation	2.86c	53.9d	55.0a	14.5c

The same letters indicate no significant difference at the probability level of P<0.05.

3.6. Electrolyte Leakage

Bud electrolyte leakage was affected significantly (1% probability) by defoliation treatments in grapevine plants (Table 7). According to Duncan's test, the treatment effect on electrolyte leakage was also significant at the 5% level. The highest percentage of electrolyte leakage (55.0%) was observed in the complete leaf removal treatment, while the lowest percentage (41.7%) was recorded in the control group (Table 8).

3.7. Day to budbreak

Chilling requirement was determined by day to budbreak based on day to budbreak under 5º C for 400 h. Based on the result of Table 7, defoliation significantly ($p \leq 0.01$) affected days to budbreak in all treatments. The lowest day to budbreak (lowest chilling requirements) was related to those vines that 'all-node' of their canes defoliated in cold acclimation stage which didn't significantly differed with vine under 'Basal nodes defoliation' treatment. There was no significant difference in the timing of budbreak between 'all-node defoliation' treatment and 'basal nodes defoliation' treatment; however, 'all-node defoliation' accelerated the time to budbreak (based on 50% budburst) by 11 day relative to control vines (Table 8).

4. Discussion

The effect of defoliation on plant yield and quality depends on factors such as the timing of leaf removal, the intensity of the treatment, and the position of the leaf on the cane. Defoliation is recognized as a technique that can yield vastly different results depending on the implementation method (Sarikhani *et al*., 2020). According to the findings of this study, the highest levels of glucose, fructose, sucrose, and raffinose were observed in the control treatment, while the lowest levels were found in the 'all-node defoliation' treatment. Soluble sugar levels decreased following defoliation; this reduction may be attributed to the creation of additional metabolic sinks resulting from the removal process (Kaya, 2020). Another hypothesis suggests that defoliation eliminates major photosynthetic parts of the plant, thereby leading to a reduction in stored carbohydrates. The decrease in soluble sugar levels in defoliated grapevines is attributed to the creation of metabolic sinks and the proliferation of flowering shoots (Sivilotti *et al*., 2016; Ghasemi et al., 2026). Defoliation increases the sink-to-source ratio for photosynthates, thereby alleviating any carbohydrate-induced feedback inhibition of photosynthetic capacity; these changes may stem from alterations in hormonal dynamics or the fact that the vine was not operating at peak activity prior to pruning (Greven *et al*., 2016). The direct relationship between carbon assimilation and specific leaf area following defoliation creates conditions that enhance the translocation of carbohydrates from the remaining leaves to other parts of the plant (Greven *et al*., 2016).

The highest content of putrescine, spermidine, and spermine were observed in the control treatment, while the lowest levels were found in 'all-node defoliation' treatment. Due to their polycationic nature, polyamines bind to cell wall pectins, protecting them from damage caused by reactive oxygen species (Gill and Tuteja, 2010). Polyamines may also function as signaling molecules; by stimulating the activity of antioxidant enzymes, they reduce hydrogen peroxide concentrations through the scavenging of reactive oxygen species (Karimi, 2017; Yilmaz *et al*., 2021).

The highest contents of gibberellin and auxin were observed in the control plants, while the lowest levels were found in 'all-node defoliation' treatment. A reduction in leaf number and surface area may lead to decreased production of plant growth hormones. In fact, the removal of leaves reduces the source of growth hormone production, leading to growth cessation and the induction of early dormancy in the treated vines (Khalil -Ur –Rehman *et al*., 2017; Karimi, 2019).

In current study, the highest proline content in the grapevine was recorded in the control group, and the lowest in 'all-node defoliation' treatment. Proline accumulation in the vegetative parts of the plant may serve as a significant indicator of stress tolerance. Proline is a nitrogen-rich compound, and plants utilize this endogenous nitrogen source under abiotic stress conditions. In plants, proline functions as an osmolyte, playing a role in osmoregulation. It contributes to subcellular structural stability, the scavenging of free radicals, and the modulation of cellular redox potential under stress conditions (Karimi and Ershadi, 2015; Jafari et al., 2026).

The highest bud water content in the grapevine was observed in the control group, while the lowest content was recorded in 'all-node defoliation' treatment. A decrease in bud water content is typically interpreted as a mechanism to concentrate cell sap—specifically by increasing soluble sugars and proline—as part of the cold acclimation process (Khalil -Ur –Rehman *et al*., 2017).

The highest rate of electrolyte leakage was found in 'all-node defoliation' treatment, whereas the lowest rate occurred in the control group. Complete defoliation was associated with increased electrolyte leakage following freezing, indicating greater membrane injury or reduced freezing tolerance (Ghasemi et al., 2026). Under these conditions, plant cells shrivel and the cell wall loses its stability and structural integrity; furthermore, in addition to the inactivation of membrane proteins and lipid peroxidation, cell membrane permeability increases, leading to electrolyte leakage (Ershadi *et al*., 2016; Yilmaz *et al*., 2021). Electrolyte leakage is considered an indicator of

the extent of oxidative damage and is among the most significant plant responses to abiotic stresses (Campos *et al*., 2003; Karimi, 2020).

Abscisic acid plays a significant role in regulating the cold acclimation process in grapevines. Furthermore, it has been established that the levels of this endogenous hormone rise as the vines enter dormancy and decline thereafter, indicating that abscisic acid acts as an inhibitor of bud break in dormant grapevine buds (Cragin *et al*., 2017; Karimi, 2017). The lowest content of abscisic acid in the grapevine were observed following complete defoliation. Defoliation constitutes a form of environmental stress for the plant, triggering the activation of metabolic and physiological pathways associated with abiotic stress responses (Sarikhani *et al*., 2020).

Premature leaf removal reduces cold tolerance. This reduction in cold tolerance is associated with lower accumulation of abscisic acid, proline, soluble sugars, and proteins in the buds during the cold acclimation phase (Yilmaz *et al*., 2021). Alterations in growth physiology, stomatal closure, changes in growth regulator patterns, and metabolite accumulation are among the mechanisms of adaptation to stress conditions (Sun *et al*., 2017; Yilmaz *et al*., 2021). Plant hormones are key regulators of stress signaling pathways as well as plant growth and development; among these hormones, abscisic acid plays a crucial role in abiotic stress signaling pathways (Karimi, 2019).

In this study, it appears that in 'all-node defoliation'—or 'basal-node defoliation'—led to a reduction in abscisic acid content. Abscisic acid is a hormone in grapevines that inhibits growth and induces dormancy; it is synthesized in the root apical meristem and translocated to the leaves (Karimi and Ershadi, 2015; Cragin *et al*., 2017). This process is intensified when leaves are exposed to low temperatures; a portion of the translocated abscisic acid is stored in the buds in a conjugated form, where it not only confers cold tolerance but also prevents bud break until suitable temperatures are reached (Ghasemi et al., 2026). Indeed, early leaf removal diminishes abscisic acid reserves in the buds, thereby reducing cold tolerance and decreasing the grapevine's chilling requirement (defined as 400 hours at 5°C). Goodarzi (2011) demonstrated that increasing the duration of chilling exposure reduced the number of days required to reach 50% bud break in the studied cultivars ('Perlette', 'Laal', 'Yaghooti', 'Fakhri', 'Bidaneh-Sefid', and 'Khalili'); specifically, the 'Bidaneh-Sefid' cultivar reached 50% bud break after 65 days under the 300-hour chilling treatment, whereas extending the chilling duration to 1000 hours reduced this period to 28 days. Grapevine buds require cold exposure for development and budbreak. This requirement can vary depending on the bud's dormancy status, age, and position on the vine cane. Factors such as pruning, nutrition, irrigation, leaf drop or removal, and water stress can influence chilling requirements and budbreak (Khalil -Ur –Rehman *et al*., 2017; Karimi, 2019). Indeed, extending the duration of cold exposure increases both the number and uniformity of bursting buds. It has been reported that the total budburst percentage of the 'Bidaneh-Sefid' grape cultivar increased with the extension of the chilling period at 5°C; specifically, the rate of bud break rose from 13% in the control treatment (no chilling) to 100% after 1,000 hours of chilling (Goodarzi, 2011). Insufficient chilling leads to the death of floral primordia (Yin, 2001) by inhibiting carbohydrate influx into the bud and disrupting intercellular communication; it also causes delayed leaf and flower opening, leaf stunting, and poor shoot growth. The resulting blossoms are smaller than normal, deformed, and often produce poor-quality pollen; anthers become thickened, and buds may contain fewer cells and fail to develop properly, ultimately leading to flower bud drop (Keller, 2015; Jabbari et al., 2025). Earlier budbreak may also result from changes in dormancy status, hormonal balance, carbohydrate availability, tissue hydration, or heat responsiveness (Khalil -Ur –Rehman *et al*., 2017) which although need to be consider in future researches.

Conclusion

This study revealed that artificial defoliation (performed between harvest and shortly before natural senescence) affects grapevine growth and developmental physiology in two ways: 1. It reduces cold tolerance by disrupting hormonal balances and depleting carbohydrate, and impairing bud water status—a finding particularly relevant for vineyards where post-harvest livestock grazing occurs; and 2. It reduces chilling requirements and accelerate budbreak following a standardized 400-h chilling treatment (5ºC) by decreasing the accumulation of the growth-inhibiting hormone abscisic acid. The results of this study can serve as a warning regarding the impact of late-season defoliation (particularly due to livestock grazing) on cold acclimation in grapevines; conversely, the practice could be employed as a horticultural technique to reduce chilling requirements for greenhouse grape production.

Conflict of interest

The authors declare that there are no conflicts of interest.

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