

# Evaluation of Cold Tolerance of Dormant Buds According to Position Using Thermal Analysis in Karaerik (*V. vinifera* L.) Grape

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## ABSTRACT

The study was conducted on Karaerik grape cultivar the aim was to determine the low temperature tolerances of winter buds taken from different positions during the dormant season. In this study, mean temperature values which were mean high temperature exotherms (mHTE) and mean low temperature exotherms (mLTE) known as indicator of low temperature tolerance in primary buds of winter, buds found in the 1st, 2nd, 3rd, 4th, 5th and 6th nodes of one-year-old shoot were determined. In the examination, mHTE and mLTE values of primary buds of winter bud during the dormant season for first year were found to be  $-5.54^{\circ}\text{C}$  and  $-9.01^{\circ}\text{C}$  respectively, mHTE and mLTE values of those of second years were established as  $-6.86^{\circ}\text{C}$  and  $-9.06^{\circ}\text{C}$ , respectively. In Karaerik grape cultivar, tolerance levels of primary buds of winter bud to low temperatures were determined to be in maximum levels in the end of November, December and January. Also, winter buds on 4th, 1st and 2nd nodes were found to be more tolerant than other buds, and cold hardiness of primary buds in Karaerik grape cultivar is affected differently by temperature at different time during the dormancy season in buds taken from different positions. But, studying the cold hardiness of winter buds at dormant phases and their correlation with physiological and biochemical changes during the dormancy cycle of winter buds taken from different positions is worthy to be considered in future researches.

**Keywords:** Grapevine, Erzincan, frost tolerance, low temperatures exotherms, Karaerik

## INTRODUCTION

Grapevines are often exposed to low temperature stresses caused by changes in environmental conditions. Especially low temperatures are one of the most important environmental stresses in grapevine growing regions. <sup>(1)</sup> In general, low temperatures occur in spring, fall, or winter, causing the significant losses in yield and quality in many grape growing regions in the world. <sup>(2)</sup> For this, understanding of the mechanisms involved in freezing tolerance, acclimation, hardening, and deacclimation periods in grapevines is extremely important. <sup>(3)</sup> Also, cold hardiness can occur over a range of temperatures, even in samples taken from

the same planting and from shoots of position and similar diameter in the canopy. One of the most widely reported differences in grapevine cold hardiness is that of bud or shoot tissues in basal and apical regions of shoots. <sup>(4)</sup> Thus the apical buds in grapes, are more susceptible to freeze injury than are the basal buds during acclimation, hardening, and deacclimation stages. <sup>(5-6)</sup>

The freezing of water in supercooled tissue releases heat of fusion that can be defined on the thermal profile during cooling as low temperature exotherms. When the supercooled buds does freeze, intracellular ice forms, resulting in bud death. <sup>(7)</sup> Thermal analysis (TA), is mostly

used to measure cold tolerance of tissues and organs that avoid freezing by supercooling like buds of grapevine. <sup>(8-10)</sup> In practice, TA, uses sensitive thermocouples to detect the latent heat of fusion released when supercooled bud tissues freeze, <sup>(8)</sup> and the heat can be given off as two exotherms as is seen in grape buds that do supercool. <sup>(11)</sup> These exotherms are called high temperature exotherm (HTE) and low temperature exotherm (LTE). In TA experiments, dormant buds exhibit a non-lethal HTE, representing extracellular ice formation. <sup>(12)</sup> The HTE is followed by one LTE associated with intracellular freezing of supercooled water in the dormant bud primordia. <sup>(13)</sup> Thus, TA has been widely used to determine the exotherms of supercooled buds and organs. As well as frost tolerance differences between tissues and organs can be determined by this method. Therefore, the study was carried out to determine the tolerance level of winter buds (1st, 2nd, 3rd, 4th 5th and 6th buds) of Karaerik cv. grape cultivar which have a significant share in Erzincan viticulture during winter colds that occurred in 8 different sampling dates in 2010-2011 and 2011-2012. Also, in addition to a better understanding of tolerance processes and mechanisms of winter buds altered under Baran training system, the information from this research will help to improve pruning management strategies for this important variety, after years in which low temperature damage occurs.

## MATERIALS AND METHODS

### Plant material

This study has been taken into account on a homogeneous plant material consists of a single vine variety of *V. vinifera* L. cv. Karaerik. In the study, dormant bud cold hardiness of cultivar from 25 years old own-rooted vines grown in Erzincan, was evaluated in 8 different times starting with November, with periods of 10-15 days (including acclimation, hardening and deacclimation) using TA in 2010-2011 (15 December 2010, 25 December 2010 -

acclimation phase, 9 January 2011, 2 February 2011, 18 February 2011 - hardening phase, 5 March 2011, 19 March 2011, 1 April 2011 - deacclimation phase) and 2011-2012 (20 November 2011, 2 December 2011, 17 December 2011 - acclimation phase, 31 December 2011, 28 January 2012, 12 February 2012 - hardening phase, 26 February 2012, 11 March 2012 - deacclimation phase) sampling dates. The vines were own-rooted, planted in sandy loam soil at a spacing of 2.5 x 2.5 m (vine x row) in east-west rows and trained to Baran system. (Baran system-trained is the system in which it is use of tiny soil hills that are 50 cm above the ground as support system (Fig. 1). The trunk is short, typically 40 to 50 cm (grape trunks which is buried underground) and the cane is unsupported by wires. Because of the hard cold winter in Erzincan, vines must be protected in order to survive in the season, otherwise they will die. Generally speaking, the easiest and effective way is to bury the grape trunks underground). Vines pruned to 2- to 3-node spurs each dormant season before the research but were not pruned before the collection of buds for this research. Cultural practices such as pruning, fertilization, irrigation, and pest control were uniform across the vineyards. One-year-old canes were randomly collected from all the vines in cultivar and the sample size ranged from 40 to 50 canes for each sampling date. Winter buds 1 to 6 from the basal of one-year-old canes were used for cold hardiness evaluation. All sections were cut outside, placed in plastic bags, and transferred to laboratory in less than 4h. With one-node segment of 5-7cm in length and 6-8 mm in diameter of dormant buds were removed from first 6 nodes of the cane sections and TA was performed. Then, of dormant buds for each node position was determined tolerance level for low temperatures using TA method.

### Determination of cold hardiness of dormant buds

Temperature exotherms of dormant buds, including segment first 6 of nodal

tissue, 5-7cm in length were determined by observing temperature recording for sudden temperature deflections from primary buds. (14-15) Copper-constant thermocouples were inserted in the intact dormant primary buds and then, fixed with elastic band. Silicon grease was used to cover the thermocouple junction to provide maximum heat transfer. Then the one-node canes were wrapped with aluminium foil and placed in Dewar Flaks container which was pre-chilled to 4°C. Dewar Flaks were placed a programmable freezer, the Tenney Junior Environmental Test Chamber (model TU-JR, Thermal Product Solutions, Williamsport, PA), equipped with a temperature controller, (Partlow MIC 1462, The Partlow West Company, New Hartford, NY) to achieve a constant cooling rate that was 4°C/h<sup>-1</sup>. (16) The freezer was programmed to hold at 4°C for 1 hr, drop to -40°C in 11 hr (a cooling rate of 4°C/hr) and ended at -40°C. Low temperature exotherms were determined from temperature data recorded at 3-sec intervals using multi-channel data acquisition system, Ahlborn Data Acquisition System (Model MA 5990-0, Ahlborn Mess-und Regelungstechnik GmbH, Holzkirchen) in computer. (10) Lethal temperatures for dormant primary buds were reported as LTE<sub>50</sub> (the temperature of the median LTE's), the temperatures at which according to literature data 50% of the buds were killed. (8-10) The experiment was conducted using a completely randomized design with six replications and eighteen dormant buds per node (1st, 2nd, 3rd, 4th 5th and 6th nodes) for each position and sampling date.

### Statistical analysis

To evaluate buds per node (1st, 2nd, 3rd, 4th 5th and 6th buds) effect on cold hardiness, a one-way analysis of variance (ANOVA) was conducted. The comparison of mean HTE-LTE values between buds and years was assessed using the Fisher's least-significant difference test (LSD). The statistical analyses were conducted at the 1 - 5 % probability level using SAS version 9.4 (SAS Institute, Cary, NC 2013).

## RESULTS AND DISCUSSION

High and low temperature exotherm values (mHTE and mLTE) of winter buds taken from different positions were identified using thermal analysis. The mHTE and mLTE values of buds were clearly identified according to positions in both years. Each sampling date and the mLTE temperatures of the buds (lethal temperatures for buds as bud LTE<sub>50</sub>, the temperatures at which 50%, of the buds were killed) were calculated (Table 2). The HTE of buds is associated with initial freezing or nonlethal formation of extracellular ice. (8) For buds according to positions in both years of the study and each sampling date that were evaluated the mHTEs were observed between -8.75°C and -2.37°C, (Table 1). The mHTE values of dormant buds between -5°C and -10°C were reported to be associated with non-lethal freezing of extracellular water. (17) Also, the results showed that mHTE of all studied nodes increased significantly during acclimation and hardening stages, in compared to deacclimation stage for each years. At acclimation stage of first year, the lowest mHTE values were found with 4th node (mHTE -7.93°C), 1st node (mHTE -7.57°C), 5th node (mHTE -7.53°C) and 2nd node (mHTE -7.03°C) nodes, respectively, whereas 3rd node (mHTE -6.20°C) and 6th node (mHTE -6.03°C) were found to be the most sensitive nodes, respectively. At hardening stage of first year, the lowest mHTE values were found with 6th node (mHTE -6.87°C), 5th node (mHTE -6.80°C) and 2nd node (mHTE -6.80°C), respectively, whereas 3rd node (mHTE -5.50°C) were found to be the most sensitive nodes. At deacclimation stage of first year, the lowest mHTE values were found with 1st node (mHTE -6.43°C), 6th node (mHTE -5.67°C) and 4th node (mHTE -5.37°C) nodes, respectively, whereas 3rd node (mHTE -3.80°C) were found to be the most sensitive nodes. On the other hand, during the all stages of second years, 5th node (mHTE -8.75°C), 6th node (mHTE -

8.45°C), 3rd node (mHTE -8.40°C), and 2nd node (mHTE -8.30°C), were generally the most extracellular freezing resistant, respectively, whereas 1st node (mHTE -3.65°C) and 4th node (mHTE -6.55°C), were the most sensitive nodes, respectively (Table 1). Cold hardiness has been previously determined in buds of *V. vinifera* L. cv. Karaerik in the same location and with the same methodology, finding similar results. (6-18-19) On the other hand, our findings show that the death of buds were generally between the mHTE and mLTE values, indicating that damage begins at temperatures just below the mHTE and that tissue injury is showing at temperatures above the mLTE. Also, LTE values for each position were correlated with years and sampling date and is consistent with the previous finding by (20) and (21). Thus, for buds according to positions in both years of the study and each sampling date that were evaluated the mLTEs were observed between -13.63°C and -4.63°C (Table 2). In first year, the buds of 1st node (mLTE -13.63°C), 4th node (mLTE -13.55°C) and 2nd node (mLTE -13.30°C) had the highest supercooling points, respectively (Table 2). Besides, the buds of 6th node (mLTE -4.80°C), 3rd node (mLTE -5.50°C) and 5th node (mLTE -6.10°C) had the lowest supercooling points, respectively. Similarly, in the second year, the lowest mLTE values were found with 1st node (mLTE -12.55°C), 2nd node (mLTE -12.20°C), and 4th node (mLTE -11.50°C), nodes, respectively, whereas 3rd node (mLTE -4.65°C) and 6th node (mLTE -5.07°C) were found to be the most sensitive nodes, respectively (Table 2). Differences in the tolerances of the winter buds to low temperatures according to their positions are approved by researchers, which is consistent with previous findings. (6-18) For example, while the winter buds at the 1st and 4th nodes according to positions were found to be the most tolerant buds, whereas, the winter buds found on the 2nd and 3rd nodes according to positions were found to be the most sensitive buds. (6) But, the mHTE and mLTE temperatures and

supercooling points of the buds could not be detected in the research conducted by (6,22) reported that the winter buds on first four nodes on the shoot of 15 different grape varieties were evaluated for tolerance to low temperatures and 1st winter buds were determined to have the highest tolerance. In a similar way, Concord grape variety were found that basal buds, were able to withstand freezing stress at lower temperatures than middle buds, then apical buds. (4) Also, (11) reported that basal buds were generally more freezing tolerant compared to the other node positions.

In our study, the capacity for supercooling of buds increased in all nodes with the overall trend of declining temperatures from acclimation through hardening stages. Buds then lost their ability to supercool as they moved from hardening stage to deacclimation stage (Table 2). This phenomenon has been associated with modification in membrane activity, which plays a significant role in inhibition intracellular ice formation. (13-23) Also, the increase in grapevine hardiness is greater with the approach of autumn and the tolerance of winter buds to low temperatures is directly associated with cane maturation. (4) In grape maturation and dormancy in the over wintering buds proceed from the base of the cane to the apex and the apical buds, are more susceptible to freeze injury than are the basal buds during phase both of development and dormancy. (24) In our results the reason to be more tolerant of winter buds at the 1st and 2nd nodes according to positions may be related to earlier maturation and decreased water content. (25) reported that changes in bud water content are directly correlated with the increases in freezing tolerance. Our results are in agreement with this finding but, changes in freezing tolerance of the bud are correlated with not only with decreasing water content but also starch and water soluble carbohydrate accumulation in the bud. (26) Thus, vines trained with baran training system occurs a bending in the 4th node on the shoot during the vegetation



period and in these buds 4th node may have caused the accumulation of carbohydrate substance. Due to these reasons may have increased the tolerance of the winter buds at

4th node. Also, this view supports to be more sensitive to low temperatures of the winter buds at 3rd and 5th node on the shoot in both years.

**Table 1. The mHTE values of winter buds taken from different positions in 8 different times in 2010-2011 and 2011-2012.**

Sampling date	Node positions	mHTEs (2010/2011 years)	Sampling date	Node positions	mHTE values (2011/2012 years)
15 December 2010	1st	-7.57 ± 1.15a	20 November 2011	1st	-7.00 ± 0.14b
	2nd	-7.03 ± 0.90ab		2nd	-8.30 ± 0.70ab
	3rd	-6.20 ± 1.49 b		3rd	-7.90 ± 0.07ab
	4th	-7.93 ± 0.49a		4th	-7.40 ± 0.56ab
	5th	-7.53 ± 0.40a		5th	-8.75 ± 0.35a
	6th	-7.17 ± 0.51ab		6th	-8.45 ± 0.63 ab
	<i>F-value</i>	10.196 **		<i>F-value</i>	6.739 **
25 December 2010	1st	-7.33 ± 0.36a	2 December 2011	1st	-7.80 ± 0.14
	2nd	-6.37 ± 1.02b		2nd	-7.90 ± 0.28
	3rd	-6.67 ± 0.32ab		3rd	-7.50 ± 1.27
	4th	-6.50 ± 0.36b		4th	-7.50 ± 0.56
	5th	-6.07 ± 0.95b		5th	-7.95 ± 0.77
	6th	-6.03 ± 1.05b		6th	-7.50 ± 0.28
	<i>F-value</i>	13.021 **		<i>F-value</i>	1.485 ns
9 January 2011	1st	-5.93 ± 0.56bc	17 December 2011	1st	-8.00 ± 0.70a
	2nd	-5.80 ± 0.43bc		2nd	-7.80 ± 1.97ab
	3rd	-5.50 ± 1.34c		3rd	-6.65 ± 0.49bc
	4th	-6.43 ± 0.41 ab		4th	-6.55 ± 0.63c
	5th	-6.80 ± 0.52a		5th	-7.30 ± 0.14abc
	6th	-6.87 ± 0.05a		6th	-7.50 ± 0.14abc
	<i>F-value</i>	18.314 **		<i>F-value</i>	8.416 **
2 February 2011	1st	-6.53 ± 1.05	31 December 2011	1st	-7.00 ± 0.28ab
	2nd	-6.27 ± 1.10		2nd	-5.85 ± 0.35c
	3rd	-6.70 ± 0.60		3rd	-6.95 ± 0.36ab
	4th	-6.27 ± 0.56		4th	-6.65 ± 0.49abc
	5th	-6.57 ± 1.49		5th	-7.70 ± 0.14a
	6th	-6.37 ± 0.92		6th	-6.20 ± 1.41bc
	<i>F-value</i>	0.875 ns		<i>F-value</i>	13.964 **
18 February 2011	1st	-6.33 ± 0.65ab	28 January 2012	1st	-7.40 ± 0.07a
	2nd	-6.80 ± 0.72a		2nd	-7.30 ± 0.56a
	3rd	-6.33 ± 1.10ab		3rd	-5.85 ± 1.06b
	4th	-5.93 ± 0.58b		4th	-7.20 ± 0.84a
	5th	-6.47 ± 0.75ab		5th	-7.00 ± 0.42ab
	6th	-6.03 ± 0.96b		6th	-7.00 ± 0.28ab
	<i>F-value</i>	4.884 *		<i>F-value</i>	4.757 *
5 March 2011	1st	-5.43 ± 1.19a	12 February 2012	1st	-6.45 ± 0.49b
	2nd	-4.63 ± 1.71ab		2nd	-6.40 ± 0.42b
	3rd	-4.03 ± 1.02b		3rd	-8.40 ± 0.84a
	4th	-5.37 ± 0.05a		4th	-7.45 ± 0.07ab
	5th	-5.13 ± 1.20ab		5th	-7.75 ± 0.49a
	6th	-5.67 ± 1.74a		6th	-8.05 ± 0.07a
	<i>F-value</i>	8.610 **		<i>F-value</i>	17.734 **
19 March 2011	1st	-6.43 ± 0.65a	26 February 2012	1st	-5.10 ± 1.97b
	2nd	-4.60 ± 0.35bc		2nd	-6.60 ± 1.13ab
	3rd	-3.80 ± 1.21c		3rd	-6.90 ± 0.70ab
	4th	-5.23 ± 1.91 ab		4th	-6.60 ± 0.14ab
	5th	-5.03 ± 1.30abc		5th	-6.40 ± 0.98ab
	6th	-5.30 ± 1.55 ab		6th	-7.25 ± 0.91a
	<i>F-value</i>	14.299 **		<i>F-value</i>	5.502 **
1 April 2011	1st	-4.27 ± 1.19a	11 March 2012	1st	-3.65 ± 0.63b
	2nd	-2.67 ± 0.86b		2nd	-4.95 ± 0.49a
	3rd	-2.80 ± 1.21b		3rd	-4.10 ± 1.27ab
	4th	-3.03 ± 0.55ab		4th	-4.90 ± 0.07a
	5th	-2.37 ± 0.20b		5th	-4.05 ± 0.21ab
	6th	-2.50 ± 0.62b		6th	-4.85 ± 1.06a
	<i>F-value</i>	11.552 **		<i>F-value</i>	13.100 **

Data are means (± SD) of at least 18 determinations with 6 replicates

Different letters in the same column indicate statistically significant differences (p≤0.01 and p≤0.05)

**Table 2. The mLTE values of winter buds taken from different positions in 8 different times in 2010-2011 and 2011-2012.**

Sampling date	Node positions	mLTEs (2010/2011 years)	Sampling date	Node positions	mLTE values (2011/2012 years)
15 December 2010	1st	-11.65 ± 0.41 bc	20 November 2011	1st	-11.80 ± 1.97a
	2nd	-12.20 ± 0.64ab		2nd	-9.00 ± 1.69b
	3rd	-11.90 ± 1.15b		3rd	-10.37 ± 1.48ab
	4th	-13.55 ± 1.87a		4th	-10.17 ± 0.63ab
	5th	-10.15 ± 1.15c		5th	-8.75 ± 1.06b
	6th	-11.50 ± 0.26bc		6th	-10.37 ± 0.49ab
	<i>F-value</i>	18.698 **		<i>F-value</i>	16.387 **
25 December 2010	1st	-13.63 ± 1.01a	2 December 2011	1st	-8.05 ± 0.07c
	2nd	-9.60 ± 1.21bc		2nd	-12.20 ± 1.55a
	3rd	-8.93 ± 0.25c		3rd	-9.80 ± 0.42b
	4th	-11.20 ± 1.03b		4th	-11.47 ± 0.77a
	5th	-10.83 ± 0.75bc		5th	-11.20 ± 0.70a
	6th	-9.00 ± 1.22bc		6th	-11.20 ± 0.14a
	<i>F-value</i>	23.791 **		<i>F-value</i>	63.169 **
9 January 2011	1st	-11.20 ± 0.79b	17 December 2011	1st	-10.87 ± 0.77a
	2nd	-13.30 ± 0.96a		2nd	-9.27 ± 0.21b
	3rd	-10.17 ± 1.06bc		3rd	-10.90 ± 0.98a
	4th	-10.40 ± 1.70bc		4th	-7.07 ± 0.07c
	5th	-10.40 ± 1.35bc		5th	-9.90 ± 0.14ab
	6th	-9.45 ± 0.49c		6th	-10.73 ± 1.90a
	<i>F-value</i>	31.413 **		<i>F-value</i>	50.478 **
2 February 2011	1st	-11.20 ± 1.09a	31 December 2011	1st	-12.55 ± 0.07a
	2nd	-10.20 ± 1.56ab		2nd	-7.67 ± 0.21d
	3rd	-8.70 ± 1.01bc		3rd	-9.13 ± 1.06c
	4th	-10.70 ± 0.72a		4th	-11.37 ± 1.48ab
	5th	-8.30 ± 1.20c		5th	-10.05 ± 0.63bc
	6th	-7.47 ± 1.84c		6th	-7.27 ± 0.21d
	<i>F-value</i>	24.552 **		<i>F-value</i>	78.727 **
18 February 2011	1st	-9.35 ± 1.77b	28 January 2012	1st	-10.37 ± 0.07ab
	2nd	-7.93 ± 0.95c		2nd	-9.60 ± 0.42bc
	3rd	-11.77 ± 1.25a		3rd	-7.83 ± 1.55d
	4th	-7.45 ± 1.30cd		4th	-11.50 ± 0.98a
	5th	-9.60 ± 1.59b		5th	-9.70 ± 1.11bc
	6th	-6.50 ± 1.78d		6th	-8.20 ± 0.84cd
	<i>F-value</i>	78.064 **		<i>F-value</i>	30.130 **
5 March 2011	1st	-6.75 ± 0.51ab	12 February 2012	1st	-10.17 ± 0.63a
	2nd	-6.93 ± 1.45a		2nd	-8.50 ± 0.56b
	3rd	-5.50 ± 0.75b		3rd	-9.80 ± 0.14ab
	4th	-6.60 ± 0.70ab		4th	-10.87 ± 0.49a
	5th	-6.10 ± 1.57ab		5th	-8.35 ± 1.34b
	6th	-6.45 ± 1.76ab		6th	-8.40 ± 0.56b
	<i>F-value</i>	3.096 *		<i>F-value</i>	18.312 **
19 March 2011	1st	-8.67 ± 0.75b	26 February 2012	1st	-6.97 ± 1.32b
	2nd	-5.10 ± 0.41d		2nd	-7.25 ± 0.35b
	3rd	-6.33 ± 0.89c		3rd	-8.07 ± 0.21ab
	4th	-10.30 ± 0.51a		4th	-8.42 ± 0.35ab
	5th	-6.40 ± 1.25c		5th	-7.30 ± 0.28b
	6th	-7.25 ± 1.34c		6th	-9.03 ± 1.34a
	<i>F-value</i>	116.475 **		<i>F-value</i>	8.508 **
1 April 2011	1st	-5.37 ± 1.91cd	11 March 2012	1st	-5.10 ± 1.55c
	2nd	-9.27 ± 1.26a		2nd	-7.45 ± 1.20a
	3rd	-6.70 ± 1.81bc		3rd	-4.65 ± 1.34c
	4th	-8.10 ± 1.05ab		4th	-6.33 ± 1.06b
	5th	-6.90 ± 1.05bc		5th	-6.07 ± 0.77b
	6th	-4.80 ± 1.81d		6th	-5.07 ± 0.74c
	<i>F-value</i>	34.899 **		<i>F-value</i>	50.731 **

Data are means (± SD) of at least 18 determinations with 6 replicates

Different letters in the same column indicate statistically significant differences ( $p \leq 0.01$  and  $p \leq 0.05$ )



**Fig. 1. A general view of Baran system-trained (photo by O. Kaya)**

## CONCLUSION

In research, we provide evidences to determine the low temperature tolerances of winter buds in Karaerik (*V. vinifera* L.) grapevine cultivar taken from different positions during the dormant season. In this study, results from the thermal analysis studies, evaluation of bud injury under controlled freezing showed that the winter buds at the 1st, 2nd and 4th node on the shoot occurred most tolerant to low temperatures. Also, cold damage of Karaerik variety is influenced by three factors: the time of entering dormancy, and the hardening stage, and the time of deacclimation stage. We suggested that vines prune to 4-5 bud spurs rather than the standard 2-3 bud spurs at winter pruning in order to less the yield losses following the years which low temperature damage occurs. Also, studying the cold hardiness of winter buds at acclimation-hardening-deacclimation phases and their relation to physiological and biochemical changes during the dormancy cycle of winter buds taken from different positions is worthy to be considered in future researches.

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### Author contribution statement

AB was responsible for collection of the plant material, field and laboratory experiments. CK supervised the experiment, reviewed the manuscript and in editing the manuscript. OK was responsible for collection of the plant material, TA test and laboratory experiments. All the authors read and approved the final manuscript.

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