AN ASSESSMENT OF THE RELATION BETWEEN COLD-HARDINESS AND BIOCHEMICAL CONTENTS OF WINTER BUDS OF GRAPEVINE CV. 'KARAERIK' IN ACCLIMATION-HARDENING-DEACCLIMATION PHASES

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This study was conducted to evaluate the correlations between the low-temperature tolerance level of buds and biochemical parameters such as malondialdehyde (MDA) as a lipid peroxidation marker, total soluble carbohydrate content, total soluble protein content, peroxidase (POD), superoxide radical ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2) in buds of *Vitis vinifera cv. 'Karaerik'*. For the experiments, the dormant grape buds in the cold acclimation (CA), hardening (HA) and deacclimation (DA) stages were used. The tolerance levels of buds were determined by measuring low-temperature exotherms (LTEs) obtained from thermal analysis (TA). It is the first report, that mLTE is related to mHTE (mean temperature at which high-temperature exotherms occurred). The mLTE is the temperature at which intracellular ice formation occurred. Intracellular ice formation is lethal. The mHTE is the temperature at which extracellular ice formation occurred. Extracellular ice formation is non-lethal. mHTE was statistically correlated ($r = 0.676$) with mLTE. Also, the relationships between the mLTE and total soluble protein ($r = -0.433$), total soluble carbohydrate ($r = -0.486$), $O_2^{\bullet-}$ ($r = -0.515$), H_2O_2 ($r = -0.360$), peroxidases (POD) ($r = 0.586$) and MDA ($r = -0.490$) were statistically significant. In conclusion, we suggest that $O_2^{\bullet-}$, H_2O_2, and MDA can be used as indicator for low-temperature stress of grapevine, but although there was a correlation between POD activity and the mLTE value of dormant bud, it has been hypothesised that POD did not affect cold-tolerance of 'Karaerik' grapevines. Furthermore, it can be accepted as the first report with respect to the fact that decreasing extracellular freezing temperatures decrease the temperature of intracellular ice formation.

Keywords: Erzincan, grapevine, 'Karaerik', mHTE, mLTE

Beurteilung des Zusammenhangs zwischen Winterhärte und den Gehalten biochemischer Verbindungen in Winterknospen der Rebsorte 'Karaerik' in Phasen der Akklimatisation, Abhärtung und Deakklimatisation. Diese Studie wurde durchgeführt, um den Zusammenhang zwischen dem Toleranzniveau von Knospen (*Vitis vinifera cv. 'Karaerik'*) gegenüber tiefen Temperaturen und biochemischen Parametern, wie Malondialdehyd (MDA) als Lipidperoxidationsmarker, Gesamtgehalten an löslichen Kohlehydraten und löslichem Protein, Peroxidase (POD), Superoxid-Radikal-Anion ($O_2^{\bullet-}$) und Wasserstoffperoxid (H_2O_2) zu untersuchen. Für die Experimente wurden ruhende Knospen in den Stadien der Kaltakklimatisation (CA), der Abhärtung (HA) und der Deakklimatisation (DA) verwendet. Die Toleranzniveaus der Knospen wurden durch Messung von Tieftemperatur-Exothermen (LTEs) bestimmt, die durch thermische Analyse (TA) erhalten wurden. Dies ist der erste Bericht darüber, dass mLTE mit mHTE (mittlere Temperatur, bei welcher Hochtemperatur-Exotherme auftreten) in Beziehung steht. mLTE ist die Temperatur, bei der die intrazelluläre Eisbildung erfolgt, die zum Zelltod führt. mHTE ist die Temperatur, bei der extrazelluläre Eisbildung auftritt, die nicht zum Zelltod führt. mHTE korrelierte statistisch (r = 0,676) mit mLTE. Auch die Zusammenhänge zwischen mLTE und dem Gesamtgehalt an löslichem Protein (r = -0,433), dem Gesamtgehalt an löslichen Kohlenhydraten (r = -0,486), $O_2^{\bullet-}$ (r = -0,515), H_2O_2 (r = -0,360), Peroxidasen (POD) (r = 0,586) und MDA (r = -0,490) waren statistisch signifikant. Zusammenfassend schlagen wir vor, dass $O_2^{\bullet-}$, H_2O_2 und MDA als Indikatoren für Niedertemperaturstress bei Weinrebe verwendet werden können, aber obwohl eine Korrelation zwischen der POD-Aktivität und dem mLTE-Wert der ruhenden Knospe besteht, wird angenommen, dass POD keinen Einfluss auf die Kältetoleranz der Karaerik-Reben hatte. Darüber hinaus kann diese Arbeit als erster Bericht in Bezug auf die Tatsache angesehen werden, dass abnehmende extrazelluläre Gefriertemperaturen die Temperatur der intrazellulären Eisbildung verringern.
Schlagwörter: Erzincan, Weinrebe, Karaerik, mHTE, mLTE

Tolerance to cold damage is a basic feature that determines the suitability of grapes for cultivation in Northern climates where the climate is characterised by low temperatures (Fallahi et al., 2001; Lisek, 2012). In these regions severe low temperatures can significantly influence crop productivity, quality and even survival through tissue and organ destruction caused by freezing injury (Ershadi et al., 2016). However, the grapevines have developed a series of mechanisms for resisting low temperatures (Burke et al., 1976). These mechanisms occur in winter buds during seasonal events of growth and dormancy. Towards the end of the autumn season, grapevines cease growing and undertake a dormant and cold-hardiness status protecting buds against inconvenient hard winter temperatures (Badulescu and Ernst, 2006). In grapevines, like many other woody plants, cold-tolerance traces a general series of cold-acclimation (CA), hardening (HA) and deacclimation (DA) phases (Ferguson et al., 2014). CA leads to an increase in cold-tolerance which enables grape buds to survive low-temperature stress (Grant and Dami, 2015), and full CA needs a short photoperiod together with low temperatures (Fennell and Hoover, 1991). Therewith, during HA, growth is suppressed by endogenous substances within the dormant buds and cold-demand absolutely needs to be satisfied before growth is regained (Perez and Lira, 2005). The DA occurs in response to seasonal increases in temperature (Fennell, 2004), but bud break is suppressed by unfavorable environmental conditions, and usually DA occurs during spring and late winter. Grapevines have different tolerance levels to low temperatures at CA, HA and DA phases. Thus, understanding the factors related to cold-hardiness in these phases is required. At the same time, cold-hardiness is the result of a complicated process including a number of biochemical and physiological events (Wisniewski et al., 2003), such as changes in lipid-membrane compounds (Chinnusamy et al., 2007), backlog of specific proteins (Thomashow, 1999), increased amounts of sugars and actuation of antioxidative mechanisms (Thomashow, 1999).

As is known, the main target of frost damage are the cell

membranes (Levitt, 1980). Stress in cell membranes caused by frost damage can increase the rate of reactive oxygen species (ROS) and then results in intense oxidative damage which fosters malondialdehyde (MDA) formation, protein degradation and alteration of metabolic functions (Hashempour et al., 2014). Exposure to low temperatures induces widespread changes of other plant metabolites playing a key-role in cold-tolerance (Jiang et al., 2014). Antioxidants can mitigate the injury caused by ROS to the membrane under freezing temperatures and lead to general alterations in the antioxidant system (Alberdi and Corcuera, 1991). These changes are expressed as a resistance mechanism against the unfavorable impacts of ROS (Selman et al., 2000). In order to reduce oxidative damage and ROS, grapevine like many other plants has to alert its antioxidant protection system consisting of enzymes like peroxidases (POD).

The antioxidant system prevents or reduces oxidative injury by scavenging the ROS like $O_2^{\cdot}$-. and H_2O_2 (Kose et al., 2011). Therefore, grapevines should maintain the antioxidant enzyme activities for overcoming low-temperature damages. Nevertheless, antioxidant capacity of grapes may not be adequate to reduce the detrimental influences of oxidative injury under hard cold-stress circumstances. In these circumstances, augmentation of cold-tolerance in grapevines is vital and necessary. The total contents of carbohydrates and soluble proteins have a fundamental role in maintening the frost tolerance in plants. (Grant and Dami, 2015). Therefore, it is possible to use changes in these biochemicals in bud tissues exposed to cold-stress to evaluate low-temperature tolerance in grapevine varieties during the CA, HA and DA phases.

Tolerance to low temperatures of the grapevine is a well-researched phenomenon. Grapevines, like other woody plants, avoid freezing in their tissues or survive by deep supercooling (Grant and Dami, 2015). Deep supercooling is an avoidance mechanism that allows cellular water to remain in the liquid phase at low, subfreezing temperatures (Wisniewski and Arora, 2000). Supercooling has a significant role in determination of cold-tolerance distribution of grapes and has been studied by a lot of scientists (Mills et al., 2006; Ferguson et al., 2014; Grant and Dami, 2015). Thermal analysis (TA) is a method utilized to measure HTE and LTE exotherms that result from freezing of dormant tissues (Andrews et al., 1984). TA is based on detecting the heat released by the hidden heat of fusion, called high-temperature exotherms (HTE) and low-temperature exotherms (LTE), that is given off when ice is formed in the tissues as measured by using thermocouples or thermoelectric modules. The freezing of extracellular water, particularly in the bud primordia, produces an exotherm called the HTE. This extracellular freezing does not damage the bud tissue and is accepted as non-lethal. The mHTE is the mean of temperature at which the high-temperature exotherms (HTE) are observed.

On the other side, the freezing of intracellular water creates an exotherm named LTE. The initiation of the LTE coincides with the intracellular ice formation which generally is fatal for tissues, leading to cell death (Wisniewski, 1995; Mills et al., 2006). The lethal temperature is defined as mLTE (determined by calculating the heat that kills 50 % of the bud population). The mLTE is calculated as the mean of temperature at which low-temperature exotherms occur. The idea that mLTE is closely related to the temperature, at which the buds die, has been raised by numerous scientists studying cold-hardiness by TA, (Quamme, 1972; Nus et al., 1981; Andrews et al., 1984; Fennell, 2004; Mills et al., 2006; Ferguson et al., 2014).

Many investigations have shown the close correlation between cold-hardiness and carbohydrate contents in dormant buds of the grapevines, but the full nature of the changes of antioxidant systems in response to cold-temperature stress during cold acclimation (CA), hardening (HA) and deacclimation (DA) phases in dormant grapevine buds has not been fully understood (Wample and Bary, 1992; Zhang et al., 2012).

In our previous studies, the amount of water, total soluble protein contents, and antioxidant enzyme activities of dormant buds have been determined in order to explain the difference between the frost tolerance levels of the dormant buds at nodes with or without lateral shoots (Kaya and Kose, 2017). Furthermore the MDA contents of dormant buds at different positions have been measured in order to confirm the natural mortality rate based on tissue browning of dormant buds exposed to low temperature in vineyard condition (Kose and Kaya, 2017). However, the aims of the current study were to

(1) investigate the temperature of extracellular and of intracellular ice formation at different stages of cold-tolerance, (2) identify relationships between the temperature of extracellular ice formation and the temperature of intracellular ice formation, (3) determine the relation of biochemical parameters such as MDA, total soluble carbohydrate content, total soluble protein content, POD, $O_2^{\cdot -}$ and H_2O_2 and the temperature at which extracellular and intracellular ice formation occur. All studies included dormant buds during CA, HA and DA phases. Finally, the possible mechanisms for cold-tolerance in bud tissue and its relationships with these physiological changes are discussed.

MATERIALS AND METHODS

PLANT MATERIAL

The study was carried out with *V. vinifera* L., cv. 'Karaerik' buds found in the 1st, 2nd, 3rd, and 4th nodes of one-year-old canes obtained from a 20-years-old vineyard at Uzumlu distric of Erzincan, Turkey. Vines were spaced 2.5 m × 2.5 m (row × vine) apart, fertilized and watered regularly and the amounts of spur-pruned canes were 10 to 12 per vine. The height of head was 0.3 to 0.4 m above ground. Shoots were collected from the vineyard at 6 sampling dates (30th Nov. 2015; 20th Dec. 2015, for cold acclimation (CA) phase; 10th Jan. 2016; 30th Jan. 2016, for hardening (HA) phase; 20th Feb. 2016 and 10th Mar. 2016, for deacclimation (DA) phase) during the dormant season of 2015/2016. Approximately 150 to 160 canes with 4 to 5 dormant buds were cut in the vineyard, put into plastic bags, and transferred to the laboratory. In order to eliminate the effects of bud position on both cold-tolerance levels and biochemical parameters, a similar number of dormant buds obtained from each 1st, 2nd, 3rd, and 4th node was used for thermal test and the other analyzes. In other words, obtained values were a mean of 4 buds from different positions.

EVALUATION OF COLD-TOLERANCE OF DORMANT BUDS BY THERMAL ANALYSIS (TA)

Copper-constant thermocouples (36 gauge) were inserted in the unspoilt dormant primary buds and then wrapped with flexible band. Silicon grease was utilized to cover the thermocouple entry to catch maximum heat transfer in dormant buds. TA of dormant primary buds was realized on 36 unspoilt buds (9 replicates, and 4 buds per replicate) both in 2015 and in 2016 and repeated twice for each sampling date. Then, measurement of cold-tolerance of dormant buds by TA was carried out according to the methodology proposed by Kaya and Kose (2017).

EVALUATION OF PEROXIDASE ACTIVITY OF DORMANT BUDS

For the determination of peroxidase (POD) activity bud tissue (0.5 g) was homogenized in 5 ml of 10 mM phosphate buffer (pH 7.0) including 4 % (w/v) polyvinylpyrolidone and 1 mM ethylenediaminetetraacetic acid (EDTA). The homogenate was centrifuged at 15.000 × g for 15 min at 4 °C and the supernatant obtained was utilized as enzyme extract. The extraction was carried out at 4 °C. POD activity of dormant buds was determined according to the methodology used by (Yee et al., 2003). Guaicol was used as a hydrogen donor. The activity of POD was assayed from the measurement of absorbance at 470 nm.

EVALUATION OF LIPID PEROXIDATION, SIGLENT OXYGEN AND HYDROGEN PEROXIDE CONTENTS OF DORMANT BUDS

MDA content in dormant buds was assayed according to the thiobarbituric acid method used by Heath and Packer (1968). Absorbance was recorded at 600 and 532 nm. MDA content in dormant buds was calculated using the following equation: MDA (nmol/ml) = $[(A_{532-}A_{600})/155000] \times 10^6$.

The superoxide radical ($O_2^{\bullet -}$) content in dormant buds was measured according to the methodology described by Elstner and Heupel (1976) with a slight modification. The absorbance wavelength was 530 nm, and sodium nitrite ($NaNO_2$) was used as a standard solution to calculate the formation rate of $O_2^{\bullet -}$.
The hydrogen peroxide level of dormant buds was determined by monitoring the absorbance at 410 nm wavelength in titanium reagent (He et al., 2005).

EVALUATION OF SOLUBLE PROTEIN AND TOTAL SOLUBLE CARBOHYDRATE CONTENTS

Soluble protein content of dormant grapevine buds was measured according to the methodology based on monitoring the absorbance in bicinchoninic acid at 562 nm, described by Smith et al., (1985). Total carbohydrate content from dormant grapevine buds was determined with a spectrophotometer (at 630 nm wavelength), using Anthron reagent (Dische et al., 1962).

STATISTICAL ANALYSIS

The test was determined using a completely randomized design with three replications. Data were analyzed using the statistics software SPSS 16.0, with correlation analyses and principal component analysis being used to determine the relationship between temperatures of exotherms and the variables. One-way analysis of variance (ANOVA) was performed on the data set. Mean separations were performed by Duncan's multiple range tests. Differences at $p \leq 0.05$ or 0.01 were considered as significant.

RESULTS

FREEZING-HARDINESS AS ESTIMATED BY TA MEASUREMENTS

In the current study, the mHTE and mLTE values, indicators for freezing of apoplastic and symplastic water, respectively, were clearly idendified in the bud primordia. Both mHTE and mLTE values of dormant buds changed according to acclimatization phases (Table 1). The mean temperature of freezing of extracellular water (mHTE) ranged from -5.79 °C (10 Mar.) to -7.67 °C (10 Jan.). At the DA phase (20 Feb. and 10 Mar.) mHTE values were significantly higher than during the other two phases (CA phase and HA phase). The mLTE values obtained from this experiment are shown in Table 1. According to our results mLTE's of dormant buds at CA, HA and DA phase were between -9.07 °C and -9.37 °C, -11.60 °C and -12.70 °C, -8.60 °C and -8.63 °C, respectively. Cold-tolerance in buds began to increase from 30 Nov. through 10 Jan., and then decreased through 10 Mar. At the DA phase mLTE values were significantly higher than during the previous HA phase. In this study, it was determined that the temperature of extracellular freezing might affect the temperature of intracellular freezing. Indeed, a statistically significant relationship between mHTE and mLTE was found ($p \leq 0.01$) ($r = 0.676^{**}$).

Table 1: Mean values of HTE and LTE of dormant buds at CA, HA and DA phases

Exotherm Temperatures	Sampling dates					
	CA phase		HA phase		DA phase	
	30 Nov. 2015	20 Dec. 2015	10 Jan. 2016	30 Jan. 2016	20 Feb 2016	10 Mar. 2016
mHTE	-7.06 ± 0.31ab	-7.17 ± 0.25ab	-7.67 ± 0.30a	-6.98 ± 0.23 ab	-6.25 ± 0.10 bc	-5.79 ± 0.44 c
mLTE	-9.07 ± 0.13 bc	-9.37 ± 004 bc	-12.70 ± 0.16 a	-11.60 ± 0.29 b	-8.63 ± 0.10 c	-8.60 ± 0.14 c

Data are means (± SE) of at least four determinations with 9 replicates.
Different letters in the same column indicate statistically significant differences ($p \leq 0.01$).

CONTENTS OF H_2O_2, $O_2^{\bullet-}$ AND MDA OF DORMANT BUDS AT ACCLIMATION, HARDENING AND DEACCLIMATION PHASES

The ROS level was evaluated by measuring the H_2O_2 and $O_2^{\bullet-}$ content in dormant buds at CA, HA and DA phases. H_2O_2 content of dormant bud was the highest at 30 Nov. (1.118 µmol/g FW), whereas after this date, a continuous decrease in the content of H_2O_2 to the minimum level at 10 Mar. (0.523 µmol/g FW) was recorded (Table 3). In addition it was determined that decreasing temperatures of extracellular and intracellular freezing were associated with increasing H_2O_2 contents of the buds. The relationship between H_2O_2 content and mLTE value of buds was not statistically significant (r = -0.360ns), while the H_2O_2 content significantly correlated (r = -0.623**) with the mHTE value of the buds.

In terms of the O_2•- content of dormant buds, 20 Dec. and 10 Jan. were statistically different from the other sampling dates. The content of $O_2^{\bullet-}$ was higher in dormant buds taken at 20 Dec. and 10 Jan. than in buds taken at other sampling dates (Table 2). It was also determined that after November 30th, superoxide content of dormant buds reached its maximum level at December 20th, afterwards a rapid decline in the superoxide content was observed until March 10th. The amount of $O_2^{\bullet-}$ in dormant buds was negatively correlated with both mHTE (r = -0.550**) and mLTE (r = -0.515**) values (Table 3).

The oxidative stress was assayed by measuring the amount of MDA in the dormant buds. The MDA content of dormant buds, except for the DA phase, was significantly increased at the CA and HA phases. The MDA content of dormant buds increased till 20 Dec. sampling date, and decreased gradually afterwards. Significant differences in MDA contents of dormant buds between the DA phase and the other phases were recorded whereas buds collected in the CA phase and the HA phase were not statistically discernible (Table 2). The MDA content of buds was negatively correlated with mHTE (r = -0.678**) and mLTE (r = 0.490*) values (Table 3).

Table 2: Changes in POD activity (EU/mg protein), $O2^{\bullet-}$ (µmol/g FW), MDA (nmol/g FW), soluble protein (mg/g FW), total soluble carbohydrate (mg/g FW) contents of dormant buds at CA, HA and DA phases

Biochemical parameters	Sampling dates					
	CA phase		HA phase		DA phase	
	30 Nov. 2015	20 Dec. 2015	10 Jan. 2016	30 Jan. 2016	20 Feb 2016	10 Mar. 2016
POD activity	19.39 ± 1.07 b	20.31 ± 4.50b	11.90 ± 2.41 b	18.02 ± 0.+90b	29.55 ± 1.97 a	20.84 ± 1.59 b
H_2O_2 content	1.12 ± 0.014a	0.98 ± 0.001b	0.97 ± 0.034b	0.65 ± 0.014c	0.56 ± 0.014d	0.52 ± 0.044d
$O_2^{\bullet-}$ content	5.36 ± 0.40b	7.07 ± 0.25a	6.93 ± 0.38a	4.76 ± 0.21b	4.73 ± 0.18b	4.69 ± 0.07b
MDA content	148.6 ± 3.28a	162.7 ± 2.49a	157.9 ± 1.96a	137.3 ± 10.48ab	119.6 ± 4.63bc	103.2 ± 5.43c
Soluble protein content	192.42 ± 20.27a	182.59 ± 17.96a	183.80 ± 8.02a	184.13 ± 6.63 a	115.71 ± 1.48 b	144.57 ± 8.81ab
Total soluble carbohydrate content	3.09 ± 0.64c	9.75 ± 1.40a	8.14 ± 0.87ab	7.69 ± 0.88bc	5.17 ± 0.17bc	5.04 ± 0.28 bc

Data are means (± SE) of at least four determinations with 3 replicates.
Different letters in the same column indicate statistically significant differences ($p \leq 0.01$ and $p \leq 0.05$).

Table 3: Correlations between mHTE and mLTE with biochemical parameters of dormant buds during dormancy phases

	mHTE	$O_2^{\bullet-}$	H_2O_2	MDA	POD	Soluble protein	Total soluble carbohydrate
mHTE	1,00**	-0,550**	-0,623**	-0,678**	0,570**	-0,483*	-0,414*
mLTE	0,676**	-0,515**	$-0{,}360^{ns}$	-0,490*	0,586**	-0,433*	-0,486*

ns: not significant, *: significant at $p \leq 0{,}05$, **: significant at $p \leq 0{,}01$

POD ACTIVITY OF DORMANT BUDS AT ACCLIMATION, HARDENING AND DEACCLIMATION PHASES

As seen from Table 2, POD activity of dormant buds showed a fluctuating change at CA, HA and DA phases, and there was no statistically significant difference in POD activity between the HA and CA phase. In general, the lowest POD activity was observed at the HA phase, while the highest POD activity was determined at the DA phase. Significant differences between sampling dates were only recorded for the buds collected on 20 Feb., at this date POD activity was statistically higher than at all the other sampling times. At the same time statistically significant positive correlations between POD activity of dormant buds and both mHTE (r = 0.570**) and mLTE values (r = 0.586**) were observed (Table 3).

SOLUBLE PROTEIN CONTENT AND TOTAL SOLUBLE CARBOHYDRATES OF DORMANT BUDS AT ACCLIMATION, HARDENING AND DEACCLIMATION PHASES

According to Table 2, soluble protein content of dormant buds was higher at the CA and HA phases compared to the DA phase, whereas soluble protein contents in samples taken at the CA and HA phases were statistically similar to each other. During CA, HA and DA phases, the maximum and minimum soluble protein content was observed in samples taken on 30 Nov. (192.41 mg/g FW) and in samples taken on 20 Feb. (115.71 mg/g FW), respectively (Table 2).

As shown in Table 2, total soluble carbohydrate contents of dormant buds showed significant differences ($p \leq 0.01$) at CA, HA and DA phases. Total soluble carbohydrate content was the lowest (3.09 mg/g FW) in samples taken on 30 Nov., and then it reached the highest level (9.75 mg/g FW) at the following sampling date on 20 December and decreased gradually towards the last sampling date on 10 March. Statistical analysis proved a significant ($p \leq 0.05$) correlation between soluble protein contents of dormant buds and their mHTE (r = -0.483*) and mLTE (r = -0.433*) values (Table 3).

DISCUSSION

The results on cold-hardiness of dormant buds at cold-acclimation, hardening and deacclimation phases, investigated in this research, were in accordance with the results of previous studies (Jones et al., 1999; Kaya and Kose, 2017). At CA phase (30 Nov. to 20 Dec.), 'Karaerik' grape variety increased in cold-tolerance as shown by a reduction in mLTE. Minimum mLTE value of buds in midwinter (at HA phase) is the most common published index to estimate cold-tolerance of dormant buds of grapevine varieties (Andrews et al., 1984; Mills et al., 2006; Shellie et al., 2014). It is well known that during the first phase (CA phase), in late summer, cold-acclimation begins; the second phase (HA phase) at which cold-hardiness of dormant buds is at its maximum, continues from the time of first fall frost to late January; and at the third phase (DA phase), late February, deacclimation begins and cold-hardiness of dormant buds decreases (Mills et al., 2006; Grant and Dami, 2015). This knowledge and our results prove that dates of CA, HA and DA phase analyzed in this study were selected correctly. On the other hand, Mills et al. (2006) determined that dormant grapevine buds' LTE range was wide (3 °C to 7 °C) during the acclimation and deacclimation phase in fall and spring but, it was narrow (2 °C to 3 °C) when buds were fully acclimated in winter. In our study, at HA phase mLTE range was 1,1 °C, which is consistent with previous findings (Mills et al., 2006). However, mLTE range at HA phase was wider than mLTE range at the other phases (0.3 °C at CA phase, and 0.03 °C at DA phase). The reason why the ranges of mLTE were narrower at CA and DA compared to HA can be the fact, that our sampling dates were late in fall, early in spring, and/or acclimation and deacclimation phases are nested with the hardening phase. Indeed, it is clearly known that to gain cold-tolerance, plants follow different phases and it is impossible to separate the phases with exact time limits (Ferguson et al., 2014).

The correlation between the mHTE and mLTE of the dormant buds observed in our study indicated that a decrease of the temperature of extracellular ice formation also reduced the temperature of intracellular, lethal freezing. In other words, we think that extracellular free-

zing temperatures may change the intracellular freezing temperatures. To our knowledge this relation between mHTE and mLTE values of dormant grapevine buds is described for the first time.

Up to now there are no studies explaining the relationship between extracellular and intracellular freezing temperatures. FENNELL (2004) stated that water content of tissue is strongly related to cold-tolerance and decreased water content is thought to contribute to an increased capability to supercooling. To tolerate low temperatures, dormant buds supercool, and thus water in the bud tissue remains un-frozen at subzero temperatures. It was also reported that plants generally survive low temperatures by extracellular freezing (RAJASHEKAR and LAFTA, 1996). Characteristically during freezing some of the intracellular water within the primordium migrates to the growing extracellular ice crystals, due to water potential differences (ASHWORTH, 1992). Because of the fact that the extracellular ice crystals grow, the intracellular solution becomes more concentrated. As a result, concentrating the intracellular solution could lower the temperature of ice nucleation (JONES, 1997).

Cold-stress could initiate the accumulation of reactive oxygen species (ROS) causing oxidative damage. ROS such as singlet oxygen ($^1O_2$), superoxide radical ($O_2^{\bullet-}$), the hydroxly radical (OH^-) and hydrogen peroxide (H_2O_2) are indicators for cold-stress or damage in plants. ROS have also been implicated as important regulatory and signalling elements in a variety of cellular processes. However the exact mechanisms, e. g. how the balance between ROS production and elimination is regulated and how signals are transmitted to the cell machinery to trigger senescence processes in plant organs, are still being unveiled (BAJGUZ and HAYAT, 2009).

One of the undesirable biochemical changes that the cold-stress causes in the plants is the accumulation of $O_2^{\bullet-}$ (BAJGUZ and HAYAT, 2009). Although $O_2^{\bullet-}$ has been frequently studied during the last decade, no studies have been found about changing $O_2^{\bullet-}$ contents during the CA, HA and DA phases in dormant buds of grapevines. However, there is evidence that in different plant species exposed to low-temperature stress, the amount of superoxide in tissues increases (HOLA et al., 2007; BAJGUZ and HAYAT, 2009; LIU et al., 2009; JIANG et al., 2014; AAZAMI et al., 2014; MIN et al., 2014). Based on these evidences, we think that low-temperature stress during December 20th and January10th was higher than at other sampling dates, so the content of superoxide anion at the end of December and at the beginning of January was higher than at other sampling dates. Also, these changes observed in $O_2^{\bullet-}$ content may be linked to the adjustment in metabolic activity to survive the constraints imposed by cold-stress. It is well known that plants have improved mechanisms to wipe out these ROS by enzymatic and non-enzymatic antioxidant systems, such as superoxide dismutase (SOD), peroxidase (POD) etc. Thus, plants protect themselves from or alleviate oxidative injury caused by low temperatures (WISE, 1995; LEE and LEE, 2000; BAJGUZ and HAYAT, 2009). On the other hand, according to our results $O_2^{\bullet-}$ contents of dormant buds negatively correlate both with temperature of extracellular ice formation and temperature of intracellular ice formation. To our knowledge up to now there are no studies confirming this correlation. Based on our results we think that buds with lower freezing point have been exposed to more severe and longer stresses. Therefore, the amount of superoxide may have increased in the buds that freeze at lower temperature.

Another ROS known to accumulate during cold-stress is H_2O_2, (JIANG et al., 2014). In the present study, however, the highest level of H_2O_2 in dormant buds was recorded on 30 Nov. (early CA) and then it gradually decreased during the following colder months. The minimum level of H_2O_2 at the DA phase can be explained by the absence of low-temperature stress in this period. Qsaib et al. (2014) detected that the amount of H_2O_2 of dormant buds of grapevine cv. 'Merlot' was the highest in July and then decreased until the end of October. The amount of H_2O_2 increased from the end of October to mid-November. It decreased again until mid-December. The findings of QSAIB et al. (2014) are similar to our results. However, the high amount of H_2O_2 in November (before cold-stress did occur), indicated that the amount of H_2O_2 may have been affected by the other factors in addition to as well as low temperature. VERSLUES et al. (2007) reported that daily decreases in photoperiod re-

duces the amount of H_2O_2. In addition, the fact that the amount of H_2O_2 was significantly negatively correlated (r = -0.623**) with only mHTE may suggest that slight cold-stress may have a bigger effect on the amount of H_2O_2. Indeed, the temperature of extracellular ice formation occurs at higher temperatures than the temperature of the intracellular ice formation.

In plants, cellular functions are affected by ROS damaging nucleic acids, oxidizing proteins, and causing lipid peroxidation (Gill and Tuteja, 2010; Xi et al., 2013). Therefore, low temperature leads to MDA production. It was determined that changes observed in MDA contents of dormant buds depending on sampling date were similar to those of ROS investigated in this research, especially superoxide radical. Our results are consistent with previous findings, indicating that the superoxide radical may trigger the creation of other reactive oxygen species like hydroxyl radical ($OH^{\cdot -}$) and more possible singlet oxygen ($^1O_2$), each of which may cause peroxidation of membrane lipids and cellular weakening (Bielski et al., 1983; Halliwell, 2006; Gill and Tuteja, 2010). In our research, MDA contents of dormant buds at DA phase were lower than those at CA and HA phases, which is consistent with previous findings (Zhang et al., 2012; Karimi et al., 2015). In a study conducted with six grape varieties, Karimi et al. (2015) found that the lowest MDA content was in the DA phase and the highest MDA content was in the HA phase with all varieties. It was reported by Zhang et al. (2012) that MDA content of dormant buds is linearly related to cold-stress. Observations of Zhang et al. (2012), Theocharis et al. (2012), Xi et al. (2013) and Karimi et al. (2015) support our findings that the MDA contents of dormant buds statistically significantly correlate with mHTE values and mLTE values. Indeed, they have reported that the correlation between MDA content of buds and low-temperature stress is positive while there is a negative correlation between MDA content and low-temperature tolerance of dormant buds.

In order to reduce or avoid cold-induced oxidative damage, plants have developed mechanisms to scavenge reactive oxygen species by antioxidant systems, such as ascorbate peroxidase (APX), POD, SOD and catalase (CAT) (Hasempour et al., 2014). POD is an important multifunctional enzyme and is involved in a large number of biochemical and physiological processes in plants. PODs regulate H_2O_2 levels and ROS production within cells and catalyse the reduction of H_2O_2 by employing various substrates (Barcelo et al., 2003; Kashefi et al., 2010). Also POD plays a key role in determining the final cell wall architecture and the turnover of phenolic metabolites in the plant cell vacuole (Barcelo et al., 2003). The results of several studies conducted on herbaceous and woody plants showed that the peroxidase activity in autumn and winter was higher than during the growth season (Ebermann and Stich, 1984; Zolfaghari et al., 2010; Chen et al., 2006; Thakur and Kapila, 2017). Zolfaghari et al. (2010) reported that the seasonal of POD activity in beech was related to the annual cycle of cambial activity, season, temperature and physiological stresses. These facts comfirm the course of the POD activity observed in our current study. We determined that POD activity did not show any significant difference between all sampling dates except for 20th February in DA phase. The reason for the rise of POD activity in the DA phase can not be fully explained due to the fact that studies on seasonal change of POD activity of dormant grapevine buds are limited. With respect to our sampling dates, the pattern of changes in the activity of POD obtained from this study was similar to seasonal changes observed in previous studies (Ebermann and Stich, 1984; Kuroda et al., 1990; Chen et al., 2006; Zolfaghari et al., 2010). Ebermann and Stich (1984) reported that in trunk tissue of oak, the POD activity which was stable during Sep. to Dec., increased during Jan. to Feb. and then decreased in Mar. Zolfaghari et al. (2010) found that in beech (*Fagus orientalis*) twigs, POD activity decreased simultaneously with the increase of air temperature and the onset of cambial activity at the end of March although peroxidase activity did not show any significant difference during all sampling months from Feb. to Nov. Similar results where obtained from apple flower buds, the POD activity increased from Sep. to Dec., then decreased in Jan. and again increased in Feb. as reported by Kuroda et al. (1990). In addition, the fact that the amount of H_2O_2 was lower in the DA phase than the amounts in the CA and HA phases could be indirectly explained by the high amount of POD pre-

sent at DA phase compared to CA and HA phases. Indeed, it is well known that POD converts H_2O_2 into water and oxygen (SUDHAKAR et al., 2001). The high POD activity observed during the DA phase in our study may have resulted from the increasing metabolic activity in this period. BARCELO et al. (2003) outlined that peroxidase levels are strongly modulated during plant cell development and in response to both biotic and abiotic environmental factors. Thus the rising temperatures in the DA phase may have initiated cell growth in the bud axis thus resulting in the observed high POD activity in the DA phase. In contrast to the general scientific knowledge, in the current study the correlations between POD activity and mHTE and mLTE values were found to be positive, and also the POD activity was higher at the DA phase compared to the CA and HA phases. Therefore, we suggest the hypothesis that POD may not play a role in protecting 'Karaerik' variety grapevines against freezing injury.

Our finding that the amount of soluble protein of buds was lower at the DA phase as compared to the CA and HA phases corresponds to results of many studies (WANG, 1987; SALZMAN et al., 1996; WAKE and FENNELL, 2000; ZHANG et al., 2012). Also FENNELL (2004) noticed that soluble proteins and carbohydrates were related to rises in low-temperature tolerance during the transition from acclimation to hardening in grapevine buds. Also SALZMAN et al. (1996) and WAKE and FENNELL (2000) determined that soluble proteins increased under short photoperiod during acclimation, which is consistent with our findings in the present study. The statistically significant correlation between the amount of protein of dormant buds and mHTE, mLTE values was found to be negative. In other words, increasing contents of soluble protein were positively related to cold-hardiness of buds. GUSTA et al. (2009) stated that positive relations between protein content and cold-hardiness of buds exist in many species. Also SALZMAN et al. (1996) stated that the maximum amount of soluble protein was associated with the highest cold-tolerance of the grapevine bud cells. In our study, however, we found statistically comparable levels of protein in the CA and in the HA phase, while the mLTE value of the buds in HA phase was higher than in CA phase. Therefore, we consider that in addition to soluble proteins other factors contribute to the enhanced cold-tolerance in the HA phase. This presumption is in accordance with studies by FENNELL (2004) who established that rises in proteins may contribute to increases of cold-tolerance, but a direct effect has not been verified in grapevines.

The accumulation of soluble carbohydrates in dormant grapevine buds may protect the bud from the injury caused by cold-damage in many ways because soluble carbohydrates function as protectants of proteins and cell plasma membranes from the effects of cold-stress (JIANG et al., 2014). STUSHNOFF et al. (1993) determined that the total amount of carbohydrate started to increase in autumn as a reaction to low temperatures, reached the maximum level in mid-winter, and decreased again towards spring. GRANT (2012) also reported that carbohydrates have a special role in the acclimation period. In a study with the varieties 'Riesling' and 'Chardonnay' it was determined that total carbohydrates are associated with frost tolerance, and also started to increase in buds from Aug., and then reached the maximum level in Dec. and Jan. when cold-hardiness of dormant buds was at the highest level (HAMMAN et al., 1996). So, our results are consistent with results of previous studies. Our finding that the content of total soluble carbohydrates was lower at the DA phase than at the HA phase is also confirmed by a previous study (ERSHADI et al., 2016). In agreement with our study, Levitt (1980) as well as SAKAI and LARCHER (1987) demonstrated that the total soluble carbohydrates have been implicated in the cold-hardiness process of plants. The total soluble carbohydrates can function as protective materials and their amount is correlated with cold-tolerance (ZHANG et al., 2012). The amount of total soluble carbohydrates of dormant buds exhibited a similar pattern as soluble protein content of buds in this work. Statistically ($p \leq 0.05$) significant negative correlations were established between total soluble carbohydrates and both mHTE and mLTE values of dormant buds, which is in agrement with findings by HAMMAN and DAMI (2000). FENNELL (2004) also reported that total carbohydrates were rela-

ted to low-temperature tolerance of dormant buds, and it was stated that the relationship was stronger at CA phase than at HA phase (Wample and Bary, 1992; Jones et al., 1999). However, the correlation between soluble carbohydrates and cold-tolerance of dormant buds was the strongest during the HA phase as compared to the other phases presumably because starch converts into sugar at the HA phase (Hamman et al., 1996; Jones et al., 1999; Hamman and Dami, 2000).

CONCLUSIONS

Knowledge on the changes of the amounts of reactive oxygen species, antioxidant enzyme activities, contents of MDA, carbohydrates and soluble proteins, and on their relations to cold-tolerance is compulsory in understanding the mechanisms responsible for tolerance of grapevines to low temperatures. Results of this study have shown that cold-stress increases the amount of ROS and lipid peroxidation as generally known, but it can be hypothesized that the amount of H_2O_2 may be more affected by slight cold-stress at which the temperature is a few degrees below freezing point. Furthermore, in our current as well as our previous studies we have not detected an effect of PODs on cold-tolerance of dormant grapevine buds. This is the first report that decreasing of extracellular freezing temperatures decreases the temperature of intracellular ice formation. Further research on elongation of cell walls and on changes of negative pressures in the cells with different cold-resistant or cold-susceptible grapevine varieties are needed to elucidate the mechanism by which extracellular ice nucleation may contribute to cold-tolerance of dormant grapevine buds.

AUTHOR CONTRIBUTION STATEMENT

MR and CK designed this research and performed the experiments. MR was responsible for collection of the plant material and field and laboratory experiments. OK did TA test and laboratory experiments. CK supervised the experiment, wrote and reviewed the manuscript. All the authors read and approved the final manuscript.

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REFERENCES

Aazami, M.a., Mahna, N. and Hasani, R.n. 2014: Salicylic acid affects antioxidant system of some grape cultivar under cold stress conditions. Journal of Biodiversity and Environmental Sciences 5(5): 280-290.

Alberdi, M. and Corcuera, L.j. 1991: Cold acclimation in plants. Phytochemistry, 30: 3177-3184.

Andrews, P.k., Sandidge, C.R. and Toyoma, T.k. 1984: Deep supercooling of dormant and deacclimating *Vitis* buds. Amer. J. Enol. Viticult. 35: 175-177.

Ashworth, E.n. 1992. Formation and spread of ice in plant tissues. Hort. Rev 13: 215-255.

Badulescu, R. and Ernst, M. 2006: Changes of temperature exotherms and soluble sugars in grapevine (*Vitis vinifera* L.) buds during winter. J. Appl. Bot. Food Qual. 80: 165-170.

Bajguz, A. and Hayat, S. 2009: Effects of brassinosteroids on the plant responses to environmental stresses. Plant Physiology and Biochemistry 47(1): 1-8.

Barcelo, A.r., Pomar, F., Lopez-Serrano, M. and Pedreno, M.a. 2003: Peroxidase: a multifunctional enzyme in grapevines. Functional Plant Biology, 30(6): 577-591.

Bielski, B.h.j., Cohen, G. and Greenwald, R.a 1983:

Oxyradicals and Their Scavenger Systems. In International Conference on Superoxide and Superoxide Dismutase. Elsevier Biomedical, Ellenville, NY., 1983

Burke, M.j., Gusta, L.v., Quamme, H.A., Weiser, C.j. and Li, P.h. 1976: Freezing and injury in plants. Annu. Rev. Plant Physiol. 27: 507-528.

Chen, Y., Zhang, M., Chen, T., Zhang, Y. and An, L. 2006: The relationship between seasonal changes in anti-oxidative system and freezing tolerance in the leaves of evergreen woody plants of Sabina. South African Journal of Botany 72(2): 272-279.

Chinnusamy, V., Zhu, J. and Zhu, J.k. 2007: Cold stress regulation of gene expression in plants. Trends in Plant Science 12(10): 444-451.

Dische, Z., Pallavicini, C., Kavasaki, H., Smirnow, N., Cizek, L.J. and Chien, S. 1962: Influence of the nature of the secretory stimulus on the composition of the carbohydrate moiety of glycoproteins of the submaxillary saliva. Archives of Biochemistry and Biophysics 97(3): 459-469.

Ebermann, R. and Stich, K. 2007: Distribution and seasonal variation of wood peroxidase activity in oak (Quercus robur). Wood and Fiber Science 17(3): 391-396.

Elstner, E.f. and Heupel, A. 1976: Inhibition of nitrite formation from hydroxylammonium chloride: A simple assay for superoxide dismutase. Analytical Biochemistry 70(2): 616-620.

Ershadi, A., Karimi, R. and Mahdei, K.n. 2016: Freezing tolerance and its relationship with soluble carbohydrates, proline and water content in 12 grapevine cultivars. Acta Physiologiae Plantarum 38(1): 2.

Fallahi, E., Fallahi, B. and Chun, I.j. 2001: Adaptation, maturity and fruit quality of table grapes in the intermountain West region of USA. Small Fruits Review 1(4): 29-42.

Fennell, A. 2004: Freezing tolerance and injury in grapevines. Journal of Crop Improvement 10(1-2): 201-235.

Fennell, A., and Hoover, E. 1991: Photoperiod influences growth, bud dormancy, and cold acclimation in *Vitis labruscana* and *V. riparia*. J. Amer. Soc. Hort. Sci. 116: 270-273.

Ferguson, J.c., Moyer, M.m., Mills, Ljj., Hoogenboom, G. and Keller, M. 2014: Modeling dormant bud cold hardiness and bud-break in twenty three *Vitis* genotype reveals variation by region of origin. Am., J., Enol. and *Vitic*. 65: 59-71.

Gill, S.s. and Tuteja, N. 2010: Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants, Plant Physiol., Biochem. 48: 909-930.

Grant, T.n.l. 2012: Characterization of cold and short day acclimation in grape genotypes of contrasting freezing tolerance. The Ohio State University. Doctoral Dissertation.

Grant, T.n. and Dami, I.e. 2015: Physiological and biochemical seasonal changes in Vitis genotypes with contrasting freezing tolerance. American Journal of Enology and Viticulture, ajev. 2014. 14101

Gusta, L. V., Benning, N.t., Wu, G., Luo, X., Liu, X., Gusta, M. L. and Mchughen, A. 2009: Superoxide dismutase: an all-purpose gene for agri-biotechnology. Molecular Breeding 24(2): 103-115.

Halliwell, B. 2006: Oxidative stress and neurodegeneration: where are we now?. Journal of Neurochemistry 97(6): 1634-1658.

Hamman, Ra, Dami, I.e., Walsh, T.m. and Stushnoff, C. 1996: Seasonal carbohydrate changes and cold hardiness of Chardonnay and Riesling grapevines. American Journal of Enology and Viticulture 47(1): 31-36.

Hamman, R. a. and Dami, I. E. 2000: Effects of irrigation on wine grape growth and fruit quality. HortTechnology 10(1): 162-168.

Hashempour, A., Ghasemnezhad, M., Ghazvini, R.f. and Sohani, M.m. 2014: Olive (*Olea europaea L.*) freezing tolerance related to antioxidant enzymes activity during cold acclimation and non acclimation. Acta Physiologiae Plantarum 36(12): 3231-3241.

He, J.m., Xu, H., She, X.p., Song, X.g. and Zhao, W.m. 2005: The role and the interrelationship of hydrogen peroxide and nitric oxide in the UV-B-induced stomatal closure in broad bean. Functional Plant Biology, 32(3): 237-247.

Heath, R.l. and Packer, L. 1968: Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Arch Biochem Biophys 125: 189-198.

Hola, D., Kocova, M., Rothova, O., Wilhelmova, N. and Benesova M. 2007: Recovery of maize (*Zea mays* L.) inbreds and hybrids from chilling stress of various duration: Photosynthesis and antioxidant enzymes. Journal of Plant Physiology 164: 868-877.

Jiang, H.y., Li, W., He, B.j., Gao, Y.h. and Lu, J.x. 2014: Sucrose metabolism in grape (*Vitis vinifera* L.) branches under low temperature during overwintering covered with soil. Plant Growth Regulation 72(3): 229-238.

Jones, K.s. 1997: Relationship of metabolite levels and an ice nucleation barrier with depth of supercooling in overwintering grape buds. The University of Guelph, Mater Thesis.

Jones, K.s., Paroschy, J., Mckersie, B.d. and Bowley, S.r. 1999: Carbohydrate composition and freezing tolerance of canes and buds in *Vitis vinifera.* J. Plant Physiol., 155: 101-106.

Karimi, R., Ershadi, A., Esna, A.m., and Mashhadi, A.B.M. 2015: Seasonal changes in soluble proteins, total phenol and malondialdehyde content and their relationship with cold hardiness of some grapevine cultivars. Journal Of Crops Improvement (Journal Of Agriculture) 16 (4): 999-1013.

Kashefi, B., Tabaei-Aghdaei, S.R., Matinizadeh, M., Mousavi, A., Jafari, A. and Mirjani, L. 2010: Seasonal changes in leaf and twig antioxidant systems in Damask rose (*Rosa damascena* Mill.). Journal of Medicinal Plants Research 4(23): 2574-2583.

Kaya, O. and Kose, C. 2017: Determination of resistance to low temperatures of winter buds on lateral shoot present in Karaerik (*Vitis vinifera* L.) grape cultivar. Acta Physiologiae Plantarum 39(9): 209.

Kose, C., Erdal, S., Kaya, O. and Atici, O. 2011: Comparative evaluation of oxidative enzyme activities during adventitious rooting in the cuttings of grapevine rootstocks. J. Sci. Food. Agric. 91: 738-741.

Kose, C. and Kaya, O. 2017: Determination Of Resistance To Low Temperatures Of Winter Buds According To Position In Karaerik (*V. vinifera* L.) Grape Cultivar. International Journal of Scientific and Research Publications 7(4): 64-68.

Kuroda, H., Sagisaka, S. and Chiba, K. 1990: Frost induces cold acclimation and peroxide-scavenging systems coupled with the pentose phosphate cycle in apple twigs under natural conditions. Journal of The Japanese Society for Horticultural Science 59(2): 409-416.

Lee, D.h. and Lee, C.b. 2000: Chilling stress-induced changes of antioxidant enzymes in the leaves of cucumber: in gel enzyme activity assays. Plant Sci. 159: 75-85.

Levitt, J. 1980: Responses of Plants to Environmental Stress, Volume 1: Chilling, Freezing, and High Temperature Stresses. - NewYork: Academic Press, 1980

Lisek, J. 2012: Winter frost injury of buds on one-year-old grapevine shoots of *Vitis vinifera* cultivars and interspecific hybrids in Poland. Folia Horticulturae 24(1): 97-103.

Liu, Y., Zhao, Z., SI, J., DI, C., Han, J., and An, L. 2009: Brassinosteroids alleviate chilling-induced oxidative damage by enhancing antioxidant defense system in suspension cultured cells of Chorispora bungeana. Plant Growth Regul. 59 (3): 207–214.

Mills, L.J. Ferguson, J.C. and Keller, M. 2006: Cold-hardiness evaluation of grapevine buds and cane tissues. Am., J., Enol., Vitic. 57: 194-200.

Min, K., Chen, K. and Arora, R., 2014: Effect of short-term versus prolonged freezing on freeze–thaw injury and post-thaw recovery in spinach: Importance in laboratory freeze–thaw protocols. Environmental and Experimental Botany 106: 124-131.

Nus, J.l., Weigle, J.l. and Schradle, J.j. 1981: Su-

perimposed Amplified Exotherm Differential Thermal Analysis System (for detecting low-temperature exotherms of excised dormant flower buds, freezing). HortScience (USA).

Perez, F. j. and Lira, W. 2005: Possible role of catalase in post-dormancy bud break in grapevines. Journal of Plant Physiology 162(3): 301-308.

Qsaib, S., Faize, M. and Koussa, T. 2014: Changes in levels of hydrogen peroxide and phenolic compounds in grapevine latent buds during the annual cycle. The Febs Journal 281: 403.

Quamme, H.a. Stushnoff, C. and Weiser, C.j. 1972: The relationship of exotherms to cold injury in apple stem tissues. J. Amer. Soc. Hort. Sci 97: 608-13.

Rajashekar, C. B. and Lafta, A. 1996: Cell-wall changes and cell tension in response to cold acclimation and exogenous abscisic acid in leaves and cell cultures. Plant Physiology, 111(2): 605-612.

Sakai, A. and Larcher, W.l. 1987: Frost survival of plant: Responses and adaptation to freezing stress. Berlin: Spring- Verlag, 1987

Salzman, R.a., Bressan, R.a., Hasegawa, P.m., Ashworth, E.n. and Bordelon, B.p. 1996: Programmed accumulation of LEA-like protein during desiccation and cold acclimation of overwintering grape buds. Plant Cell Environ. 19: 713-720.

Selman, C., Mclaren, J.S. and Himanka, M.J. 2000: Effect of long-term cold exposure on antioxidant enzyme activities in a small mammal. Free Radic Biol Med 28 (8):1279-1285.

Shellie, K., Cragin, J. and Serpe, M. 2014: Performance of alternative European wine grape cultivars in southwestern Idaho: Cold hardiness, berry maturity, and yield. HortTechnology 24(1): 138-147.

Smith, P.k., Krohn, R.i., Hermanson, G.t., Mallia, A.k., Gartner, F.h., Provenzano, M. and Klenk, D.c. 1985: Measurement of protein using bicinchoninic acid. Analytical Biochemistry 150(1): 76-85.

Stushnoff, C.r.l.r., Remmele, R.l., essensee, V. and Mcneil, M. 1993: Low Temperature Induced Biochemical Mechanisms: Implications for Cold Acclimation and De-Acclimation. In: Jackson M.B., Black C.R. (eds) Interacting Stresses on Plants in a Changing Climate. NATO ASI Series (Series I: Global Environmental Change), vol 16. Springer, Berlin, Heidelberg, 1993

Sudhakar, C., Lakshmi, A. and Giridarakumar, S. 2001: Changes in the antioxidant enzyme efficacy in two high yielding genotypes of mulberry (*Morus alba* L.) under NaCl salinity. Plant Sci. 161: 613–619.

Thakur, S. and Kapila, S. 2017: Seasonal changes in antioxidant enzymes, polyphenol oxidase enzyme, flavonoids and phenolic content in three leafy liverworts. Lindbergia 40: 39–44.

Theocharis, A., Clèment, C. and Barka, E.A. 2012: Physiological and molecular changes in plants grown at low temperatures. Planta 235(6): 1091-1105.

Thomashow, M.f. 1999: Plant cold acclimation: freezing tolerance genes and regulatory mechanisms. Annual Review of Plant Biology 50(1): 571-599.

Verslues, P. e., Kim, Y. s. and Zhu, J. K. 2007: Altered ABA, proline and hydrogen peroxide in an Arabidopsis glutamate: glyoxylate aminotransferase mutant. Plant Molecular Biology 64(1-2): 205-217.

Wake, C. m. and Fennell, A. 2000: Morphological, physiological and dormancy responses of three Vitis genotypes to short photoperiod. Physiologia Plantarum 109(2): 203-210.

Wample, R.l. and Bary, A. 1992: Harvest date as a factor in carbohydrate storage and freezing tolerance of Cabernet Sauvignon grapevines. J. Am. Soc. Hort. Sci. 117: 32-36.

Wang, R.f. 1987: The kinds of plant hardiness criteria

and their application. Plant Physiology Communications (China) 03, 49-55. (In Chinese)

Wise, R.R. 1995: Chilling-enhanced photooxidation: the production, action and study of reactive oxygen species produced during chilling in the light. Photosynth Res 45: 79–97.

Wisniewski, M., Bassette, C. and Gusta, L.v 2003: An Overview of Cold Hardiness in Woody Plants: Seeing the Forest Through the Trees. Hortsci. 38(5): 952-959.

Wisniewski, M. and Arora, R. 2000: Structural and Biochemical Aspects of Cold Hardiness in Woody Plants. In: Jain S.M., Minocha S.C. (eds) Molecular Biology of Woody Plants. Forestry Sciences, vol 66. Springer, Dordrecht, 2000.

Wisniewski, M. and Davis, G. 1995: Immunogold localization of pectins and glycoproteins in tissues of peach with reference to deep supercooling. Trees 9: 253-260.

Xi, Z., Wang, Z., Fang, Y., Hu, Z., Hu, Y., Deng, M. and Zhang, Z. 2013: Effects of 24-epibrassinolide on antioxidation defense and osmoregulation systems of young grapevines (*V. vinifera* L.) under chilling stress. Plant Growth Regulation 71(1): 57-65.

Yee, Y., Tam, N.f.y, Wong, Y.s and Lr, C.y. 2003: Growth and physiological responses of two mangrove species (Bruguiragymnorrhiza and Kandeliacandel) to water logging. Environ. Exp. Bot.: 1-13.

Zhang, J. Wu, X. Niu, R. Liu, Y. Liu, N. Xu, W. Wang, Y. 2012: Cold-resistance evaluation in 25 wild grape species. Vitis 51(4): 153-160.

Zolfaghari, R., Hosseini, S.m. and Kororoi, S.A.A. 2010: Relationship between peroxidase and catalase with metabolism and environmental factors in Beech (*Fagus orientalis* Lipsky) in three different elevations. International Journal of Environmental Sciences 1(2): 243.

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