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ORIGINAL ARTICLE

Bud Death and Its Relationship with Lateral Shoot, Water Content and Soluble Carbohydrates in Four Grapevine Cultivars Following Winter Cold

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Abstract
The objectives of this study was to compare bud death of four different grapevine cultivars ('Bronx Seedless', 'Cardinal', 'Autumn Royal', 'Superior Seedless') after the winter cold of 2018–2019, and was to investigate the relationship between bud death and water content, the presence of lateral shoot and soluble carbohydrates of dormant buds. Bud death rates were determined after exposure to winter temperatures by using binocular microscope after opening buds with a razor blade. Significant differences ($p \leq 0.0$) were found among the low temperature hardiness of dormant buds at nodes with or without lateral shoots of four grape cultivar following winter cold. This investigation illustrates that buds in nodes with lateral shoots were bud death at higher temperatures than buds in nodes without lateral shoots. It is revealed that buds in nodes with lateral shoots had less tolerance to winter temperatures. Cultivars were classified as hardy ('Bronx Seedless' and 'Cardinal'), moderately hardy ('Superior Seedless') and least hardy ('Autumn Royal') based on the bud mortality rates. It was observed that water and soluble carbohydrate contents were much higher in the buds in nodes without lateral shoots against to buds in nodes with lateral shoots in cold hardy cultivars namely 'Bronx Seedless', 'Cardinal' compared to the other cultivars. The pattern of water content and soluble carbohydrates were highly consistent with the bud death rate. The correlation analysis of cold hardiness indices with bud mortality indicated that the bud death rate is positively correlated with water content and is negatively correlated with soluble carbohydrate content. Because of the presence of lateral shoots reduces the resistance of buds to low temperatures, it is highly suggested to those who grow with these varieties could remove the lateral shoots in time period of summer pruning in cold regions.

Keywords Grapevine · Cold hardiness · Presence of lateral shoot · Water content

Knospensterben in Relation zu Seitentrieben, Wassergehalt und löslichen Kohlenhydraten in vier Weinsorten nach Winterfrost

Schlüsselwörter Wein · Frosthärte · Vorkommen von Seitentrieben · Wassergehalt

Introduction

Grape, which has big importance among fruit species, is one of the most produced fruits in the world (Fennell 2004). Table grape and raisin, which can be found easily in every season, are full of nutrition values. The grapes containing A, B1, C, D vitamins, calcium, iron, magnesium, phosphorus, potassium and pyridoxine (Keller 2015) could be used as a natural drug for diseases (Dohadwala and Vita 2009). Because of these important properties, grapes are cultivated in many regions of the world (Grant 2012). Although this may seem a significant advantage of growing grapes all around the world, this could often increase the damages of environmental stress during grape production. In particular, low temperatures are one of the environmental stresses could occur in autumn, winter and spring (Quamme 1995). As it happens all around the world, the low temperature

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stress during winter affects productivity of the grapevine in Turkey. Indeed, winter temperatures could dramatically reduce grape yield or even be a limiting factor to grow grapevines in the northern part of Turkey, where seriously freezing injury could occur (Kaya and Köse 2017; Buztepe et al. 2017; Kalkan et al. 2017; Kaya and Köse 2019). This situation is a more serious problem especially for the grape varieties cultivated in the regions dominated by the continental climate (Köse and Kaya 2017; Rende et al. 2018). In regions that are important for grape production, while growers are trying to minimize the cold damage with cultural practices, they are also looking for new cultivars that could adapt to low temperatures (Fennell 2004; Kaya and Köse 2018). Although all cultivars of grapes are able to survive at a certain extent low temperatures, the level of cold hardiness varies among them (Grant 2012). They are generally divided into groups based on the maximum cold hardiness of their buds on the vines achieved in mid-winter: very hardy, hardy, moderately hardy, very tender, tender and moderately tender (Zabadal et al. 2007). The cultivars that are currently production of premium wines and dominate the market in acreage are derived from the species of *Vitis vinifera* and therefore they are desired by wine and grape producers. However, these grape cultivars are sensitive to winter temperatures below –20 °C (Kaya and Köse 2018). Currently more than 90% of grape expansion is cultivated with cultivars of *Vitis vinifera* in Turkey and little knowledge exists about the freezing response of these grape cultivars during winter conditions. Therefore, it could be useful to determine the cold hardiness of grape cultivars in Turkey to define their potential for breeding studies.

In both dormant buds and woody tissues of grapevine, it has been indicated that water content, soluble carbohydrates and the presence of lateral shoot play an important role in the maintenance of cold hardiness (Wolpert and Howell 1986; Sakai and Larcher 1987; Wample and Bary 1992; Hamman et al. 1996; Grant and Dami 2015; Kaya and Köse 2017; Rende et al. 2018). During the low winter temperatures, the increase of soluble carbohydrates concentration in dormant bud tissues is often associated with a rise of cold hardiness (Grant and Dami 2015; Kaya and Köse 2017; Rende et al. 2018; Jiang et al. 2014). Proposed roles associated with the accumulation of soluble carbohydrates include the decrease in the crystallization of water in the cell, which reduces freezing-induced dehydration, provision of metabolic energy, cryoprotection of cellular constituents, freezing point depression and glass formation, which could stop most physical activity and all biochemical (Santarius 1973; Franks 1985; Burke 1986; Lineberger and Steponkus 1980; Crowe et al. 1988; Koster and Leopold 1988; Caffrey et al. 1988; Jeffrey and Huang 1990). Thus, it is important to understand the factors that regulate soluble carbohydrates metabolism in the grape cultivars have different cold hardiness. Besides soluble carbohydrates, the decrease in bud water content is effectively related with increasement in cold hardiness and is thought to contribute to an increased ability to supercooling, a mechanism that allows water to remain in the liquid phase below sub-freezing temperatures (Salzman et al. 1996; Bourne and Moore 1991; Valle 2002; Kaya and Köse 2017). Therefore, it is feasible to use alterations of soluble carbohydrates and water content in dormant buds exposed to cold stress as indicators to assess cold hardiness in different grapevine cultivars. On the other hand, published works have illustrated the close correlation between cold hardiness and lateral shoot present in grapevines (Howell and Shaulis 1980; Köse and Güleryuüz 2011; Kaya and Köse 2017). However, the mechanism of present lateral shoot on cold hardiness of dormant buds of different grapevine cultivars has not been fully investigated. The oxidative browning method followed by accurate viability assays provide useful means for evaluation of new varieties, genotypes and rootstocks and help in identifying main cultural and ecological influences on established vines (Stergios and Howell 1973; Barka and Audran 1997; Jones et al. 1999; Linden 2002). The oxidative browning of tissues as a visual indication of bud death rate is the most common method that could be used reliably in both laboratory and field to evaluate the cold hardiness of grape cultivars (Wolf and Cook 1992; Rekika et al. 2004; Kalkan et al. 2017; Köse and Kaya 2017). Therefore, the aims of the current study were: (1) to determine cold hardiness of different grape varieties following winter cold by using the oxidative browning method (2) to define the relationship between bud death and the presence of lateral shoot, water content, soluble carbohydrates of dormant buds.

Materials and Methods

Plant Material

In this experiment, the average lowest temperature of Erzincan is –19.8 °C during winter season (Fig. 1). Though this province has generally hot and long summers, the winter temperatures could plunge down to –22 °C or even lower, causing significant injury to grape cultivars. Daily minimum, maximum and mean air temperatures were recorded from 1 December 2018 to 25 January 2019 at a local weather station that is 700 m from the study region (Fig. 1). In this study, four different cultivars from 6 years old own-rooted vines were evaluated following winter 2018–19. Vines have been planted with designing of randomized complete block of three blocks, with three-vine plots in each block. Four different cultivar vines planted at a spacing of 2.5 m by 2.5 m (vine by row) apart, and trained to a bilateral low cordon training system, and spur-pruned to

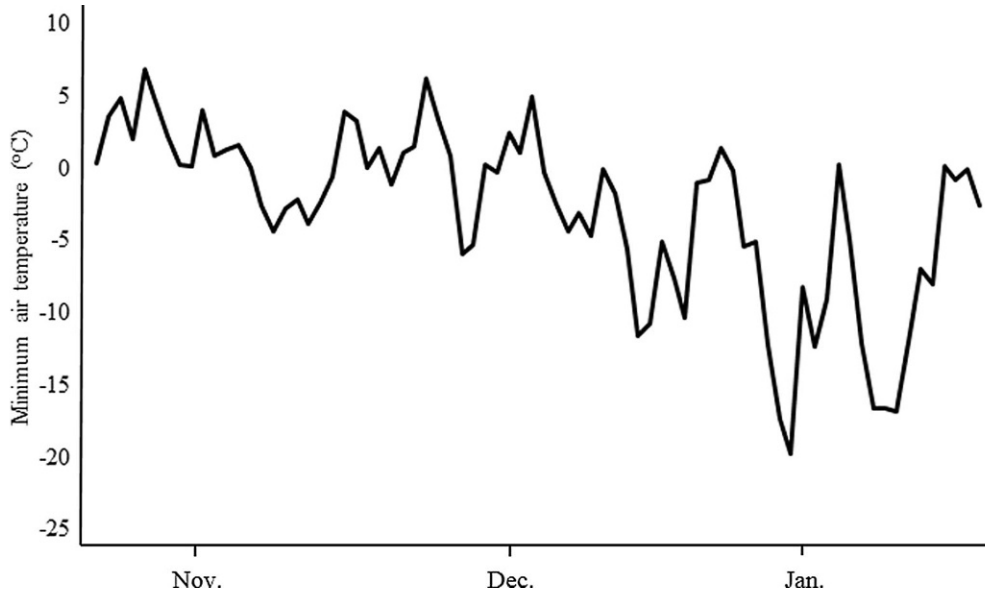

Fig. 1 Minimum daily temperatures recorded in Erzincan Horticultural Research Institute Weather Station (Nov. 2018–Jan. 2019)

28 buds per vine. The number of shoots was ranged from 10 to 12 (shoots with 2 or 3 buds) per vine. One-year-old, cane sections were collected from vineyard at one date (12 Jan. 2019) after low winter temperature. The single bud one-year old cuttings of *Vitis vinifera* L. cv. 'Bronx Seedless', 'Cardinal', 'Autumn Royal', and 'Superior Seedless' were placed in nylon sacks, and transferred to laboratory. In this study, the dormant buds at nodes without lateral shoots (nLNB) and with lateral shoots (LNB) were used from first 4 nodes of the cane section. The LNB and nLNB were also taken at the same number for each nodes at the same position of cane sections. Then, both nLNB and LNB were separated into two group lots to assess the freezing injury, water content and soluble carbohydrates.

Bud Browning Assay

After freezing damage, both nLNB and LNB buds were held at room temperature for 24 h. Following the 24 h anticipation, a longitudinal and/or cross sectioning were made on both nLNB and LNB buds with a single-edged razor blade to confirm freeze damage manifested by tissue discoloration or browning. Dormant buds showing brown tissue judged to be dead, whereas buds showing vibrant green tissue judged to be viable dead. These buds were determined under a stereomicroscope (Model BX50F4; Olympus, Japan), and just the primary buds were considered for discoloration (Stergios and Howell 1977). The proportion of injured buds for each cultivars were calculated based on the number of deaths of buds (Odneal 1984).

Soluble Carbohydrates

Soluble carbohydrates of buds both in LNB and nLNB were measured by performed the anthrone method (Yemm and Willis 1954). Dormant buds taken from the first 4 nodes of an old canes were oven-dried at 80 °C for 72 h. Then samples were ground in a grinder and were stored in lightless condition until analysis. Soluble carbohydrates of buds both in LNB and nLNB were extracted four times from 0.2 g of milled dry tissue with 5 mL of 80% ethanol and centrifuged for 20 min at 4000 gn. Two mL of 0.2% anthrone reagent (0.2 g anthrone in 100 mL of 72% sulfuric acid) was added to 50 µL of the ethanolic extract. The mixture in glass tubes were incubated in a water bath at 90 °C for 15 min and then glass tubes were rapidly cooled in ice water. Absorbance of the extract was read at 620 nm using a Thermo Fisher Multiskan Sky (model-51119700DP) Microplate Spectrophotometer (Olympus, Japan). The concentration of soluble carbohydrates of buds both in LNB and nLNB were eventually calculated by using a standard glucose curve and expressed as mg g^{-1} dry weight (DW).

Water Content

Buds both in LNB and nLNB were collected at the day after the cold injury. Dormant buds taken from the first 4 nodes of an old canes were excised and were weighed immediately with precision scales and after placing in an oven for 2 days at 85 °C. Bud both in LNB and nLNB water content were determined as percent of fresh tissue weight by using the following formula:

$$\text{Bud water content} = [(\text{fresh weight} - \text{dry weight})/\text{fresh weight}] \times 100$$

Statistical Analysis

Statistical analyses were carried out using JMP statistical software (version. 7.0, SAS Institute Inc., Cary, NC). Student's t test was used to determine that mean values of bud death rate, water content and soluble carbohydrate parameters and results were significantly different between nLNB and LNB within each grape cultivars with a level of significance $p \leq 0.05$ or 0.01. Relationship between bud death and water content, the presence of lateral shoot, soluble carbohydrates were also tested using correlation analysis. Data for cultivar comparison and the measurement parameters were tested using one-way analysis of variance. Duncan's new multiple range test was analyzed for post-hoc comparison of mean values.

Results and Discussion

Vineyard daily low temperatures from Nov. 2018 through Jan. 2019 are shown in Fig. 1. The first sub-zero temperature recorded on Nov. 15, and the lowest temperature recorded at the vineyard was –19.8 °C on Jan. 11. The frost injury in dormant buds of grapevine cultivars were investigated with the bud browning assay following the lowest air temperature recorded on Jan. 11. Researchers working on frost injury of fruit and dormant buds have noted that oxidative browning of bud structure after a frost event is a good indication of the death tissues, as a result, there will not be a successful fruit set (Odneal 1984; Ershadi et al. 2016; Kalkan et al. 2017; Köse and Kaya 2017). To assess cold injury following lowest air temperature, one-year-old canes from a vineyard need to be held for a minimum of 24 h at 23 °C (room temperature) before cutting to maximize tissue browning (Odneal 1984; Kalkan et al. 2017; Köse and Kaya 2017). In this study, buds both in LNB and nLNB were cut one day after the lowest air temperature recorded and kept at room temperature (23 °C) for one day. Tissue browning was occurred within all cultivars after a day and significant difference ($p \leq 0.01$) was found both among cultivar and between LNB and nLNB (Tables 1 and 2). Bud death rates for all grape cultivars were influenced by the presence of the lateral shoot and were lower in the nLNB compared to LNB (Table 1). These findings are similar to previous

Table 1 Contents of soluble carbohydrates (mg. g^{-1} DW), water (%), and bud death rate (%) of LNB or nLNB of 'Bronx Seedless', 'Cardinal', 'Autumn Royal', 'Superior Seedless' grape cultivars

Measured parameters	Lateral presence	Bronx Seedless	Cardinal	Autumn Royal	Superior seedless
Bud death rate	nLNB	11.38 ± 2.51	22.00 ± 3.03	65.33 ± 2.51	47.97 ± 2.98
	LNB	42.00 ± 2.01	52.00 ± 3.11	79.34 ± 1.53	79.94 ± 2.65
	t testi	*	*	*	*
Soluble carbohydrates	nLNB	16.13 ± 0.26	13.05 ± 0.06	12.71 ± 0.31	12.71 ± 0.31
	LNB	13.55 ± 0.12	11.75 ± 0.66	10.29 ± 1.66	11.47 ± 1.66
	t testi	*	**	*	**
Water	nLNB	23.35 ± 1.34	24.47 ± 0.43	25.14 ± 1.74	25.34 ± 0.48
	LNB	27.52 ± 0.88	27.33 ± 0.73	28.15 ± 1.46	31.77 ± 0.82
	t testi	*	*	*	*

*Significant at $p \leq 0.01$, **significant at $p \leq 0.05$

Table 2 Comparison of LNB or nLNB of 'Bronx Seedless', 'Cardinal', 'Autumn Royal', 'Superior Seedless' grape cultivars according to water (%), soluble carbohydrate contents (mg.g^{-1} DW) and bud death rate (%)

Grape cultivars	nLNB			LNB		
	Bud death rate	Soluble carbohydrates	Water	Bud death rate	Soluble carbohydrates	Water
Bronx Seedless	11.38 ± 2.51^{a*}	16.13 ± 0.26^{a*}	23.35 ± 1.34^{a*}	42.00 ± 2.01^{a*}	13.55 ± 0.12^{a*}	27.52 ± 0.88^{a*}
Cardinal	22.00 ± 3.03^{b}	13.05 ± 0.06^{b}	24.47 ± 0.43^{ab}	52.00 ± 3.11^{b}	11.75 ± 0.66^{ab}	27.33 ± 0.73^{a}
Autumn Royal	65.33 ± 2.51^{d}	12.71 ± 0.31^{b}	25.14 ± 1.74^{b}	79.34 ± 1.53^{c}	10.29 ± 1.66^{b}	28.15 ± 1.46^{b}
Superior seedless	47.97 ± 2.98^{c}	12.71 ± 0.31^{b}	25.34 ± 0.48^{b}	79.94 ± 2.65^{c}	11.47 ± 1.66^{b}	31.77 ± 0.82^{c}

*Means within columns followed by the lowercase letters do differ significantly among grape cultivars at 0.01 level based on adjusted *p*-values using Duncan's new multiple range test

Table 3 Correlation of nLNB and LNB among 'Bronx Seedless', 'Cardinal', 'Autumn Royal', 'Superior Seedless' grape cultivars according to water content, soluble carbohydrates and bud death rate

Grape cultivars	*nLNB*	*Bud death rate*	*Soluble carbohydrates*	*Water*
Bronx Seedless	Bud death rate	1.000	–0.980**	0.994**
Cardinal		1.000	–0.960**	0.974**
Autumn Royal		1.000	–0.742**	0.980**
Superior seedless		1.000	–0.853**	0.999**
Grape cultivars	*LNB*	*Bud death rate*	*Soluble carbohydrates*	*Water*
Bronx Seedless	Bud death rate	1.000	–0.964**	0.891**
Cardinal		1.000	–0.845**	0.839**
Autumn Royal		1.000	–0.755**	0.912**
Superior seedless		1.000	–0.792**	0.963**

**Correlation is significant at the 0.05 level, values in the table represent the *r*

studies conducted by Kaya and Köse, (2017), Köse and Güleryüz (2009, 2011), Howell and Shaulis (1980) where they reported that winter cold caused more injury to LNB than those in nLNB. In the current study, the death rates of bud for nLNB were 11.38, 22.00, 65.33 and 47.97% for 'Bronx Seedless', 'Cardinal', 'Autumn Royal' and 'Superior Seedless', respectively, while the death rates of bud for LNB were 42.00, 52.00, 79.34 and 79.94% for 'Bronx Seedless', 'Cardinal', 'Autumn Royal' and 'Superior Seedless' (Table 1). Among the four grape cultivars, 'Bronx Seedless' had the lowest bud death rate in its nodes following lowest air temperature. 'Cardinal' was moderate tolerance, whereas 'Autumn Royal' and 'Superior Seedless' were most sensitive cultivars, respectively (Table 2). This result is consistent with previous investigations on bud death observed in same grape cultivars. The dormant buds of 'Superior Seedless' were exposed at –15 and –18 °C for 12, 18 and 24 h and sum of the percent of primary and secondary bud survival ranged between 72, 60 and 99% for 'Superior Seedless' (Aktan 2012). It also was reported in several investigations that grape cultivars have different degrees of cold hardiness (Fennell 2004; Zhang et al. 2012; Kaya and Köse 2018). The differences in the cold hardiness of the grape cultivars are possibly a combination of genetic factors, physiological, biochemical features, environmental conditions and day length and temperature (Fennell 2004; Zhang et al. 2012; Keller 2015; Kaya and Köse 2017; Rende et al. 2018). The cold hardiness is also influenced by various factors including cultural practices, rootstock, temperatures fluctuations, photoperiod, carbohydrate content, and bud water content (Fennell 2004; Ershadi et al. 2016; Rende et al. 2018).

The brown coloration in bud tissues exposed to low temperature which later resulted in oxidation of the phenolic compounds being released in the damaged tissues (Hamman et al. 1996; Jones et al. 1999; Ershadi et al. 2016; Kalkan et al. 2017). Severe injury results in more pronounced, deeper discoloration of injured or killed tissues. In this study, soluble carbohydrates for all grape cultivars were influenced by the presence of the lateral shoot and were higher in the nLNB compared to LNB (Table 1). The highest levels of soluble carbohydrates found in the cold hardy cultivars, such as 'Bronx seedless' and 'Cardinal' compared to the 'Autumn Royal' and 'Superior Seedless' (Table 2) which confirms the involvement of carbohydrates in frost protection and dormant conditions in grapevine. There also was negative correlation coefficient between soluble carbohydrates and bud death rate at both nLNB ($p \leq 0.05$) and LNB ($p \leq 0.05$) among all cultivars (Table 3).The negative relationship between the soluble carbohydrate content and the bud death in the buds in nodes with lateral shoots was determined for the 'Bronx seedless' ($r = -0.964$**), Cardinal ($r = -0.845$**), 'Autumn Royal' ($r = -0.755$**), and 'Superior Seedless' ($r = -0.792$**), respectively. The negative relationship between the soluble carbohydrate content and the bud death in the buds in nodes without lateral shoots was determined for the 'Bronx seedless' ($r = -0.980$**), 'Cardinal' ($r = -0.960$**), 'Autumn Royal' ($r = -0.742$**), and 'Superior Seedless' ($r = -0.853$**), respectively (Table 3). Indeed, some researchers expressed that changes in the contents of soluble carbohydrates could be used as an indicator to evaluate frost damage (Fennell 2004; Zabadal et al. 2007; Grant and Dami 2015; Ershadi et al. 2016; Kaya and Köse 2017). Interestingly, Wample and Bary (1992) identified that levels of carbohydrates in the grapevine have no any effects on frost tolerance illustrated which is contrary to the opinion that carbohydrates are playing role in cold hardiness. It has been well documented under cold conditions that, polysaccharides are hydrolyzed to soluble sugars, which leads to increase the osmotic potential of the cytoplasm and lower the temperatures of cell death (Zhang et al. 2012; Grant and Dami 2015; Ershadi et al. 2016; Kaya and Kose 2017; Rende et al. 2018). Additionally, the accumulation of soluble carbohydrates content in both nLNB and LNB cold tolerant cultivars might have provided a protection to the buds from the injury caused by lethal temperatures, because soluble carbohydrates are charged as protectants of proteins and cell plasma membranes from the effects of dehydration and frost injury (Gusta et al. 2002; Wang 1987; Fennell 2004; Jiang et al. 2014; Kaya and Köse 2017).

There were significant differences in water content between nLNB ($p \leq 0.01$) and LNB ($p \leq 0.01$) among cultivars following winter cold. The water content in the buds in nodes with lateral shoots were higher than in the buds in nodes without lateral shoots (Table 1). The lowest bud water content for nLNB found in the cold hardiness cultivars 'Bronx seedless' (23.35%), whereas the highest bud water content observed in the cold-sensitive cultivars 'Cardinal' (24.47%), 'Autumn Royal' (25.14%) and 'Superior Seedless' (25.34%). The lowest bud water content for nLNB found in the cold hardiness cultivars 'Bronx seedless' (27.52%) and 'Cardinal' (27.33%), whereas the highest bud water content observed in the cold-sensitive varieties 'Autumn Royal' (28.15%) and 'Superior Seedless' (31.77%) (Table 2). In addition, a significant positive correlation was found between the bud water content and bud death at both of nLNB and LNB (Table 3), and reducing of water content was associated with increased bud tolerance confirming previous observations in grape cultivars (Valle 2002; Salzman et al. 1996; Keinanen et al. 2006; Kalberer et al. 2006; Ershadi et al. 2016; Kaya and Köse 2017). The fact that the positive correlation between water content and bud death of all grape cultivars shows that cultivars with high bud water content can be affected by low temperatures more than varieties with low bud water content. Indeed, many researchers determined that the changes in water content of bud tissues are both contribute to a raised capability to supercooling and increases in cold hardiness (Pierquet et al. 1977; Quamme 1995; Wolpert and Howell 1985; Kovacs et al. 2002; Kaya and Köse 2017). In contrast to these findings, Hamman et al. (1990) reported that alterations in tissue water content was not strongly correlated with changes in dormant bud cold hardiness in grape cultivars. However, a close relationship between flower bud cold tolerance and water content has been observed in peach (*Prunus persica* L.) (Quamme 1983), sweet cherry (*Prunus avium* L.) (Kadir and Proebsting 1994), rhododendron (*Rhododendron* spp.) (Ishikawa and Sakai 1981) apple (*Malus domestica* Borkh.) (Kang et al. 1998), and blueberry (*Vaccinium australe* Small) (Bittenbender and Howell 1975). Quamme and Gusta, (1987) also reported that low water potential is involved in resistance to ice nucleation in flower tissues of peach.

Conclusions

The bud browning assay described herein is relatively not time-consuming and expensive and so could constitute a rapid method for assessment of grapevine cultivars winter injury before pruning. The number of cane and buds to be left in pruning time could be adjusted according to determination of the damage ratio in buds by using the browning method after winter frost. In current study, the bud death rate was evaluated according to the browning method, and the pattern of cold hardiness changes in four different grape cultivars was consistent with bud tissue soluble carbohydrates and water content. 'Bronx seedless' and 'Cardinal' cultivars were found to have the highest cold hardiness among the four studied grape cultivars. These cultivars could be considered in future breeding programs and are excellent to be used in regions under high risk of winter cold. In addition, the presence of lateral shoot increased the susceptibility of bud to low temperatures. Therefore, it could be concluded that while doing summer pruning removing the lateral shoots of vines will help preventing low temperature injury in the regions experiencing the cold winters. To sum up, further studies with other grape cultivars resistant or susceptible to low winter temperatures would be useful to evaluate if the hypothesis raised by the current study can be extended.

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Conflict of interest O. Kaya declares that he has no competing interests.

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