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Physiological impacts of early defoliation on the cold hardiness of grapevine (*Vitis vinifera* L.) 'Sultana'

H. Sarikhani, H. Delgarm and S. Mansouri

Department of Horticultural Sciences, Bu-Ali Sina University, Hamedan, Iran

Summary

Low winter temperatures are one of the limiting factors of grape production worldwide. This study was undertaken to inquire about the effects of postharvest early defoliation on the cold hardiness of grapevine. The grapevines samples, cv. 'Sultana', were defoliated at two stages (10 and 25 days after harvest), and then they were compared with natural leaf fall. Cane samples were collected in December 2017 and February 2018, and analyzed in terms of water content, soluble carbohydrate, and proline concentrations in both bud and cane tissues. The samples were then subjected to freezing treatments *i. e.* -8, -12, -15, -18, -21, and -24 °C for evaluating the levels of cold hardiness. Based on these results, early defoliation reduced proline and soluble carbohydrate concentrations but increased the water content compared to the control. Leaf removal also decreased abscisic acid concentration in the bud samples. Investigation of cold hardiness by electrolyte leakage and tetrazolium staining examinations showed that the defoliation decreased cold hardiness. Results demonstrated that leaf removal between the growing season and the beginning of the acclimation stage decreased the metabolite concentration in buds and canes and resulted to a reduction of cold hardiness.

K e y w o r d s : abscisic acid; cold hardiness; electrolyte leakage; tetrazolium staining; water content.

Introduction

Grape is one of the most important temperate-zone fruits, which is cultivated in a wide range of climatic conditions. One of the restricting factors diminishing the crop yield is winter temperature, which can cause significant economic losses to grape production (Creasy and Creasy 2009). Similar to other plants, the grapevine can withstand cold weather conditions to some extent against the damaging effects of frost (Goffinet 2000, Sarikhani *et al.* 2014). The acclimation process and cold hardiness in perennial plants can be reached in response to shortening day length and reducing temperature, resulting in molecular (Kim *et al.* 2017), physiological, biochemical, and physical changes in plant tissues (Ait Barka and Audran 1997, Ben Mohamed *et al.* 2010, Grant and Dami 2015, Khalil-ur-Rehman *et al.* 2019). Some of these changes might be named as accumulation of soluble carbohydrates, proteins, proline, and polyamines as well as reduction of tissues free water content, followed by changes in some growth regulators including increasing abscisic acid content (Ben Mohamed *et al.* 2010, Ferguson *et al.* 2014, Dami *et al.* 2015, Rubio *et al.* 2016, Beheshti-Rooy *et al.* 2017, Cragin *et al.* 2017, Khalil-ur-Rehman *et al.* 2019).

In general, cold hardiness is a quantitative feature affected by a complex set of environmental variables (Rubio *et al.* 2016). Maximum cold hardiness occurs during the coldest months of the winter, and then as the temperature rises, the deacclimation occurs (Ma *et al.* 2010, Jiang and Howell 2002). The cold hardiness degree in grape is a function of the plant genotype (Zhang *et al.* 2012, Ershadi *et al.* 2016, Londo and Kovaleski 2017). European *Vitis vinifera* grape cultivars are severely damaged below -25 °C, depending on the cultivar, phenological stage, location, nutritional conditions, and horticultural operations (Mills *et al.* 2006). During the cold acclimation process, the grape shifts from cold-tender to cold-hardy state (Zabadal *et al.* 2007).

In the northern hemisphere, the acclimation process in grapevines lasts from September to complete leaf fall and results in the increase of the cold hardiness of the plant (Grant and Dami 2015, Zabadal *et al.* 2007). The maximum cold hardiness in the tissues occurs at the end of the cold acclimation period, and persists at low temperatures until late February (Ma *et al.* 2010, Sarikhani *et al.* 2014). Similar to other plants, the cold acclimation process in grapevine is accompanied by a gradual decline, and eventually a complete stop in plant growth. At this stage, plant periderm develops and the leaf abscission occurs. The cold hardiness increases with decreasing cell water content (Jiang and Howell 2002).

Postharvest defoliation of grapevine is common in several growing regions of the world; because of early burying of canes for winter protection, grazing of the livestock, preventing pests and diseases, restricting chemical run off, and reducing chilling requirement (Pommer 2006, Mohamed 2008). The removal of grapevine leaves, immediately after harvest, and the reduction of photosynthetic products as well as of some acclimation metabolites (Greven *et al.* 2016) may affect the cold hardiness of the plant. Moreover, due to the role of abscisic acid on winter resistance of grape (Zhang and Dami 2012, Bowen 2016) and its production in leaves (Seo and Koshiba 2011), leaf removal could affect

Correspondence to: Dr. H. Sarikhani, Department of Horticultural Sciences, Bu-Ali Sina University, Hamedan 6517833131, Iran. E-mail: sarikhani@basu.ac.ir

abscisic acid content, and winter cold hardiness. Since a limited number of studies are available, more investigations are highly required to understand the influence of inducing factors affecting the acclimation stage in grapevine plant.

Therefore, the current research was aimed at inquiring about the effect of defoliation time on cold hardiness in grapevine of 'Sultana'. 'Sultana' ('Bidaneh Sefid') is one of the most widely planted grapevine cultivars in Iran for fresh consumption and raisin production.

Material and Methods

Plant materials and treatment: Based on preliminary results obtained during the 2013-2014 winter, an experiment was designed in 2017-18. This research was conducted from autumn to winter 2017-18 on grapevines of 'Sultana' (syn. 'Bidaneh Sefid') cultivar grown at Malayer Grape Research Station (34.2635 °N, 48.8025 °E) affiliated to Hamedan Agricultural and Natural Resources Research Center and Department of Horticultural Science, Bu-Ali Sina University. A randomized complete block design with three treatments was established. Vines were mechanically defoliated 10 and 25 d after harvest on Oct. 1 (early-Oct.) and Oct. 15 (mid-Oct.), and were compared with the control (natural leaf fall: NF) in three replications (using one vine per replicate). The grapevine bushes were subjected to natural temperature conditions (Fig. 1). Sampling was done in December and February in similar conditions from the middle nodes of one-year-old canes. After marking, the specimens were sealed in plastic bags separately for each replication and were immediately transferred to the laboratory using a container box. To remove surface contamination, the canes were completely washed with distilled water. In the laboratory, the samples were divided into two lots. The first lot was used to investigate the features such as proline content, soluble carbohydrate, water content, and the amount of abscisic acid. The second lot was employed to evaluate the cold hardiness in cane and bud using a freezing chamber at a controlled temperature by electrolyte leakage and tetrazolium stain test.

Proline concentration: Proline measurement was conducted in bud samples only according to the method of Paquin and Lechasseur (1979). At first, 0.5 g of the tissue was completely ground with liquid nitrogen in a mortar. Then, 10 mL of 3 % sulfosalicylic acid was added to the crushed sample before being mixed. The mixture was then centrifuged for 15 min at 12,000 rpm. Afterward, 2 mL of the extract was mixed with 2 mL of glacial acetic acid and 2 mL of ninhydrin reagent (1.52 g ninhydrin + 30 mL glacial acetic acid + 20 mL phosphoric acid 6 mM), and kept in a bain-marie at 90 °C for 1 h. After immediate cooling, 4 mL toluene was added to each tube and the tubes were shaken vigorously until formation of a brick-color in the toluene phase. The absorbance of the brick colored solution at 518 nm was read in a spectrophotometer (Cary 100, Varian, USA). The proline concentration was determined by comparing with the standard curve of different proline concentrations, and then calculated as $mg \cdot g^{-1}$ FW.

Soluble carbohydrate concentration: First, the bud and cane samples were dried in an oven (70 °C for three days) and then milled (Chow and Landhäusser 2004). To extract soluble carbohydrates, 0.5 g of powder was crushed in a mortar applying 5 mL of 95 % ethanol, and then the upper phase of the solution was separated. The procedure was repeated twice with 5 mL of 70 % ethanol. After centrifugation (15 min at 6,000 rpm), the upper phase of the solution was used to measure the soluble carbohydrates according to Yemm and Willis (1954) method with slight modifications. Briefly, 0.1 mL of the obtained alcoholic extract was mixed with 3 mL of freshly prepared anthrone (150 mg of anthrone + 100 mL of 72 % Sulfuric acid). To begin the staining reaction, the tubes were placed in a bain-marie at 90 °C for 10 min. After cooling, the absorbance of the samples was read with the spectrophotometer at 625 nm. The concentration of the soluble carbohydrates was determined according to the standard curve of glucose and expressed in $mg \cdot g^{-1}$ DW.

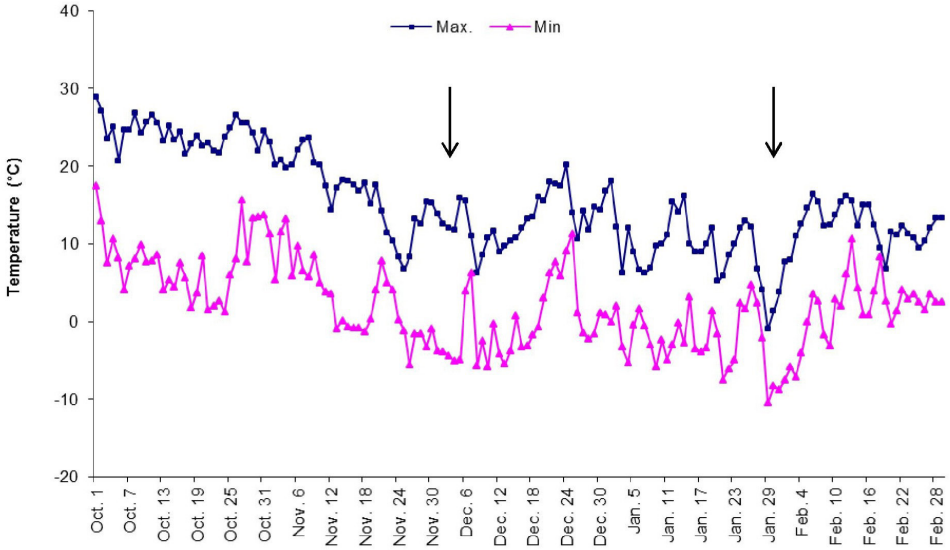

Fig. 1: Daily maximum (■) and minimum (▲) air temperatures recorded in Malayer Weather Station during the experiment. Arrows show December and February sampling time.

Water content (WC): To measure the WC, the canes were used immediately after preparing from vineyard. From each treatment, 5 one-centimeter pieces of cane sample and 5 bud sample were prepared separately in three replications. The cane and bud samples were weighed immediately after preparation for fresh weight (FW) in laboratory. To determine the dry weight (DW), the samples were placed in the oven (70 °C for 3 d). The WC was then calculated using [WC (%) = (FW-DW) × 100/FW] equation.

ABA concentration: ABA concentration in bud tissues of NF and defoliation at early October. was measured based on Kelen *et al.* (2004) method. Briefly, two grams of bud tissue were homogenized with 70 % (v/v) methanol and stirred overnight at 4 °C. The extract was filtered through a Whatman filter (No. 2) letting the methanol evaporate under vacuum. The aqueous phase was adjusted to pH 8.5 with 0.1 M phosphate buffer and then partitioned with ethyl acetate 3 times. After removal of the ethyl acetate phase, the pH of the aqueous phase was adjusted to 2.5 with 1 N HCl. The solution was partitioned with diethyl ether 3 times, and then passed through anhydrous sodium sulfate. After that, the diethyl ether phase was evaporated under vacuum and the dry residue containing hormones was dissolved in 2.0 mL of methanol and stored in vials at 4 °C. ABA content in the samples was quantified using a Smartline HPLC instrument (Knauer, Germany) equipped with a quaternary pump and a UV-VIS detector (D-14163 model). The mobile phases used were acetonitrile-water (26:74 %; 30:70 %; v/v) with a flow rate of 0.8 mL·min^{-1}. An injection volume of 20 µL was used for each analysis. Reverse phase chromatography separation was performed with a C18 Eurospher-100 (5 µm particle, 125 mm × 4 mm) column with a time limit of 30 min. The signal of the compounds was monitored at 265 nm for ABA and expressed as ng·g^{-1} FW.

Sample preparation and freezing procedure: To measure the amount of cold-induced damages, the samples (one-year-old canes) were kept in the freezing chamber (CRP-Z200, Kimia-Rahavard, Tehran, Iran) for artificial cold investigation with special temperature and time control. The treatments included the temperatures of -8, -12, -15, -18, -21 and -24 °C. The initial temperature was chosen equal to the field average temperature at sampling date (5 °C for December and 2 °C for February samples) and the device program was adjusted so as to slowly reach the temperature of -8 °C for about 4 h, which was the first freezing-treatment temperature. The freezing chamber was programmed with rate of 3 °C·h^{-1} reduction in temperature and 3 h in each temperature-treatment (Sarikhani *et al.* 2014). Samples of each temperature-treatment were brought out of the freezing chamber after each treatment and were used to evaluate the low-temperature damages by electrolyte leakage and tetrazolium examinations.

Electrolyte leakage: Electrolyte leakages of the cane and bud samples were measured according to the method proposed by Lutts (1995), with some slight changes. After cold treatment, the canes were taken out of the freezing chamber, placed outside for 4 h at 4 °C, and then at 20 °C for 2 h. The cane samples were later immersed individually in 70 mL cans containing 40 mL distilled water. The cans were placed on the shaker at room temperature for 120 h at 120 rpm. Afterward, their electrical conductivity (ECf) was measured utilizing a pH/Cond 720 EC-meter (WTW InoLab, Weilheim, Germany). The cans containing the samples were autoclaved at 121 °C for 20 min. After cooling, their total electrical conductivity (ECt) was measured, and finally the electrolyte leakage (EL) was obtained from the [EL (%) = (ECf / ECt) ×100] equation. Andrews *et al.* (1984) method was used to calculate the lethal temperature (LT), at which 50 % of total electrolyte leakage took place, in the cane and bud tissues (EL-LT_{50}).

Tetrazolium staining test: To evaluate the cold damages in tissues under artificial freezing treatment, tetrazolium staining test method (Okamato *et al.* 2000) was used. After cold treatment, as the electrolyte leakage examination method, the cane specimens were taken out of the freezing chamber. They were gradually incubated for 4 h at 4 °C and later for 2 h at 20 °C. The temperature-treated cane samples (n = 5 nodes per treatment and n = 4 canes per treatment) were soaked in 5 mL of 1.0 % (w/v) 2, 3, 5-triphenyl-tetrazolium chloride before being located in a dark place for 24 h at 24 °C. Formation of red color in the primary, secondary, and tertiary buds was chosen as the criterion for evaluating the viability. It was detected using Leica binocular (MS5, Heerbrugg, Switzerland).

Freezing induced damages in cane and bud tissues were evaluated by the tetrazolium stain methodology. Using a sharp razor blade, each bud (n = 12 per treatment) was cut in half horizontally and investigated for surviving tissues under the binocular. Odneal (1983) method was employed to classify the microscope surviving buds in which the values of 0.66, 0.33, and 0.01 were ascribed to primary, secondary, and tertiary buds; respectively. Cane injuries (n=15 per treatment) were quantified based on an enhanced version of the method of Mills *et al.* (2006) according to a 1 to 4 scale as follows: 1 = no injury, 2 = small phloem injury, 3 = 50 % phloem injury, and 4 = 100 % phloem injury. The lethal temperature at which 50 % of the cane and bud tissues died estimated by tetrazolium staining (T-LT_{50}) was eventually calculated by fitting response curves (Fiorino and Mancuso 2000).

Statistical analysis: The PROC GLM procedure in the SAS software (version 9.1; SAS Institute, 2003) was applied to statistical analysis of all experimental data by one-way analysis of variance. Means were compared using Duncan's multiple range tests at 5 % level of significance ($P \leq 0.05$). The PROC CORR procedure was applied to statistical analysis of correlation between traits (n = 18 for all traits).

Results

Proline concentration: According to the statistical analysis, a significant difference ($P < 0.01$) was observed among defoliation treatments in terms of proline concentration in December and February sampling (Tables of analysis of variance are not given). The highest and lowest concentrations were observed in the buds of NF and early-Oct. treatments, respectively. In the February sampling, a similar trend was observed for the buds proline

Table 1

Effects of defoliation time on the cane and bud proline, soluble carbohydrate concentrations, and water content in 'Sultana' in December 2017 and February 2018

Defoliation time	Cane		Bud	
	December	February	December	February
Proline concentration (mg·g^{-1} FW)				
Natural leaf fall (control)	-	-	13.03 ± 0.25a	13.71 ± 0.21a
Early-Oct.	-	-	11.06 ± 0.41b	12.86 ±1.61a
Mid-Oct.	-	-	9.60 ± 0.55c	11.09 ± 0.72b
Soluble carbohydrate concentration (mg·g^{-1} DW)				
Natural leaf fall (control)	22.06 ± 0.52a	24.63 ± 0.94a	18.86 ± 0.91a	19.66 ± 1.34a
Early-Oct.	22.50 ± 1.63a	24.30 ± 0.43a	16.66 ± 1.51b	17.96 ± 1.29b
Mid-Oct.	18.20 ± 1.02b	20.28 ± 0.71b	16.30 ± 0.52b	17.35 ± 0.57b
Water content (%)				
Natural leaf fall (control)	52.53 ± 3.28b	48.37 ± 2.42b	33.51 ± 1.69b	29.40 ± 0.68b
Early-Oct.	55.18 ± 2.04b	50.60 ± 0.70b	35.17 ± 0.64b	30.17 ± 1.24b
Mid-Oct.	60.09 ± 1.13a	56.72 ± 1.41a	38.10 ± 0.74a	33.43 ± 0.90a

Each value is a mean of three replications (n = 6). Mean values with the same letter in each column show no significant difference at $P \leq 0.05$ by Duncan's multiple range tests.

concentration. There was no significant difference between NF and leaf removal treatments at mid-Oct. (Tab. 1).

Soluble carbohydrate concentration: Defoliation treatments significantly ($P < 0.01$) affected bud and cane soluble carbohydrate concentration at both sampling dates. In December, the highest concentration of soluble carbohydrate was observed in cane of vines defoliated at mid-Oct, but it was not significantly different from NF canes. The lowest value was found after leaf removal treatment in early-Oct. (Tab. 1). A similar trend was observed in the February sample although the soluble carbohydrate content in canes was slightly higher in comparison with December sampling.

In December, a significant higher soluble carbohydrate concentration was found in buds of NF samples in comparison with the defoliation treatments. However, there were no significant differences between the two dates of leaf removal. A very similar trend was observed for bud samples collected in February (Tab. 1).

Water content: The WC of plant buds and canes were found to be significantly different ($P < 0.01$) between the December and February samples. In December, the lowest cane and bud WC was recorded for NF samples with 52.53 % and 33.51 %, respectively, but was not significantly different for samples of mid-Oct. leaf removal treatment. The highest WC was observed in samples of the defoliation treatment of early-Oct. A similar trend was observed for the WC of buds and canes tissues collected in February (Tab. 1).

ABA concentration: The concentration of ABA was higher in the buds of NF plants in comparison with the defoliated plants in Oct. 1 (Fig. 2).

Electrolyte leakage: The defoliation treatments affected significantly the EL-LT_{50} values of buds and canes sampled in December ($p < 0.01$). The results indicated that buds were less tolerant to low temperatures in

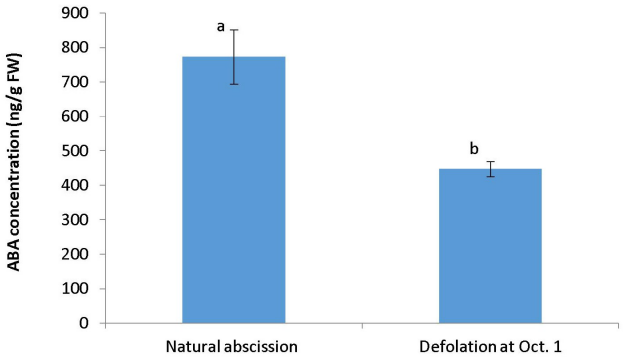

Fig. 2: ABA concentration (ng·g^{-1} FW) in the bud samples of natural abscised plant (NF) and defoliation at Early-Oct. Each column represents the means of three replications. Mean values followed by the same lower-case letter show no significant difference at $P \leq 0.05$ by Duncan's multiple range test.

comparison with the canes. The highest estimated EL-LT_{50} values were found for the canes of the NF treatment. Early defoliation decreased the plant hardiness, and the lowest EL-LT_{50} values were recorded for the canes sampled on vines defoliated on Oct. 1. A similar trend was observed in bud samples. The highest EL-LT_{50} values were recorded for buds of NF samples. Early defoliation caused a reduction of bud cold hardiness, and the lowest EL-LT_{50} value was recorded for the treatment of defoliation on Oct. 1 (Tab. 2). Similar trends were observed between treatments for bud and cane samples of February (Tab. 2).

Tetrazolium stain test: Damages induced by freezing temperatures in buds and canes are illustrated in Fig. 3. In December and February samples, cane and bud T-LT_{50} values differed significantly among treatments ($p < 0.01$). In December, cold hardiness was significantly higher (lower T-LT_{50}) for canes of the NF treatment, in comparison to defoliation treatment of mid-Oct., and ear-

Table 2

Effects of defoliation time on EL- LT_{50} and T-LT_{50} values of canes and buds estimated by electrolyte leakage and tetrazolium staining test in 'Sultana' in December 2017 and February 2018

Defoliation time	Cane		Bud	
	December	February	December	February
	EL- LT_{50}			
Natural leaf fall (control)	-23.03 ± 0.92a	-21.26 ± 1.23a	-19.83 ± 0.64a	-18.10 ± 1.21a
Early-Oct.	-20.29 ± 0.31b	-20.55 ± 0.28b	-16.84 ± 0.12b	-16.57 ± 0.82b
Mid-Oct.	-18.32 ± 0.25c	-17.51 ± 0.80c	-14.93 ± 0.23c	-15.19 ± 0.47c
	T-LT_{50}			
Natural leaf fall (control)	-29.08 ± 1.36a	-28.65 ± 1.24a	-21.27 ± 1.35a	-20.06 ± 1.14a
Early-Oct.	-27.31 ± 1.21b	-26.40 ± 1.10b	-19.80 ± 0.98b	-18.36 ± 0.54b
Mid-Oct.	-24.99 ± 1.35c	-24.04 ± 1.42c	-18.31 ± 0.38c	-17.72 ± 0.48c

Each value is a mean of three replications (n = 6). Mean values with the same letter in each column show no significant difference at $P \leq 0.05$ by Duncan's multiple range tests.

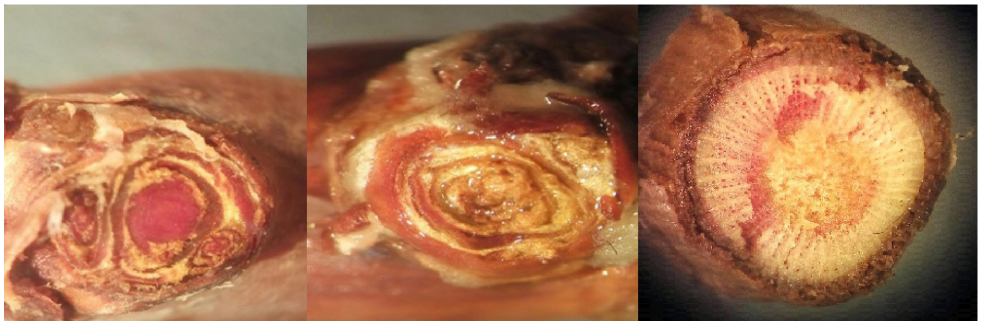

Fig. 3: Comparison of the viability of grapevine bud or cane using tetrazolium stain test after cold treatment. Left: Bud staining belongs to the natural leaf abscission without remarkable damage by -18 °C cold treatment, Middle: No bud staining belongs to the defoliation on Early-Oct. showing damages at -18 °C, Right: the cane texture in this treatment has a defective stain indicating cane injuries.

ly-Oct. The same treatment effects were detected for buds sampled in December, and for canes and buds sampled in February (Tab. 2).

Correlation between biochemical traits and cold hardiness parameters: High and significant correlations were observed ($p < 0.01$ or $p < 0.05$) between proline concentration, soluble carbohydrate concentration, and WC in buds and canes on one hand, and estimated LT_{50} based on ion leakage and tetrazolium stain examinations, on the other hand (Tab. 3).

Discussion

According to the present results defoliation resulted in a higher cold-sensitivity of canes and buds, estimated both in December and February with electrolyte leakage or tetrazolium staining tests. Defoliation treatments resulted in less negative LT_{50} values.

The methods based on electrolyte leakage or tetrazolium staining tests have been previously used to evaluate the cold hardiness in different grape cultivars and other trees (Sarikhani *et al.* 2014, Karimi 2017). Our study confirms the close correlations found between the cold hardiness measured by electrolyte leakage and tetrazolium tests. Proline is one of the plant osmotic regulators, which participates to acclimation and survival of plants under environmental stresses (Ashraf and Fooland 2007). During plant stress proline contributes to the stabilization of subcellular structures, modification of free radicals, and redox potential (Kaur and Asthir 2015). Considering the conditions induced by cold and freezing temperatures, proline prevents the exit of water from the cell through osmotic regulation of the cytoplasm, hinders extracellular ice formation, and protects membrane proteins from oxidative stress damage (Ashraf and Fooland 2007). Based on previous studies, cold-tolerant grapevine cultivars produce higher levels of proline (Ershadi *et al.* 2016). For this reason, proline has been recognized as one of the most important physiological indices in identifying cold-tolerant cultivars (Dionne *et al.* 2001). Overall, a high correlation has been reported between cold hardiness and proline concentration in grapevine (Zhang *et al.* 2012, Sarikhani *et al.* 2014, Beheshti-Rooy *et al.* 2017). From our results, the lower proline concentration in buds for the defoliated vines could be a parameter to explain the decrease of winter cold hardiness.

During the endodormancy, the degree of cold hardiness depends also on the presence of compounds that prevent the formation of ice cores. It is commonly assumed that an increase of the cellular glucose concentration may lower the

Table 3

Pearson correlation coefficients between EL-LT_{50} and T-LT_{50}, and soluble carbohydrate, proline concentration, water content, and T-LT_{50} in canes and buds of 'Sultana'

Variable	BP	BSC	CSC	BW	CW	B T-LT50	C T-LT50	B EL-LT50
Bud proline (BP)	1							
Bud Soluble carbohydrate (BSC)	-0.60**	1						
Cane Soluble carbohydrate (CSC)	-0.83**	0.51**	1					
Bud Water content (BW)	0.47**	-0.30**	-0.65**	1				
Cane Water content (CW)	0.65**	-0.50**	-0.67**	0.87*	1			
Bud T-LT_{50}	-0.45**	-0.27*	-0.42*	-0.32*	-0.43*	1		
Cane T-LT_{50}	-0.65*	-0.45*	-0.50*	-0.43*	-0.55*	0.72**	1	
Bud EL-LT_{50}	-0.50 **	-0.47**	-0.79**	-0.33*	-0.54*	0.89**	0.88**	1
Cane EL-LT_{50}	-0.67**	-0.60**	-0.83**	-0.47**	-0.65*	0.78**	0.89**	0.93**

n = 18 for each traits. ** and *significant at $P \leq 0.01$ and $P \leq 0.05$, respectively. T, tetrazolium stain test; EL, electrolyte leakage test.

freezing point of the cell content (Palta and Jensen 1982). In grapevine cultivars a positive correlation between soluble carbohydrate concentration and cold hardiness was already reported (Hamman *et al*. 1996, Sarikhani *et al.* 2014, Rende *et al.* 2018). Rising soluble sugar concentration increases the osmotic potential inside the cell and ultimately induces the cold hardiness (Zhang *et al.* 2012). Soluble carbohydrate concentration is highly related to photosynthesis ratio (Poni *et al.* 2006) and starch conversion to the soluble sugars (Jones *et al.* 1999). In grapevine, at the end of the growing season and after harvest, carbohydrates produced by photosynthesis are transferred and stored into canes and buds to withstand cold-induced stresses (Hamman *et al.* 1996, Jones *et al.* 1999). Although delayed harvests do not obligatorily decrease stem carbohydrate storage pool and cold hardiness (Wample and Bary 1992), defoliation treatments were shown to reduce carbohydrate reserve in grapevine roots and trunks (Bennett *et al.* 2005). As we observed that after-harvest defoliation reduced carbohydrate concentration in canes and buds compared to NF, it could explain the negative effects of defoliation on cold hardiness of grapevine.

Under natural conditions, water content of tissues decreases in autumn because of lower absorption of water by roots at low temperature (Levitt 1980) and water evaporation. In general, the water content of cane and bud decreases with progress of cold acclimation (Wolpert and Howell 1985). Our current results confirmed that decreasing cane and bud water content is associated with high winter cold hardiness, as it was previously shown (Wolpert and Howell 1986, Jiang and Howell 2002, Sarikhani *et al.* 2014). The reasons why defoliation resulted in higher cane and bud water content deserve further investigations. Higher leaf area could be effective in reducing tissue water content through evaporation (Nadi 1974). In addition after harvest and during cold acclimation, leaves support the production of adaptive metabolites such as sugars, which reduces free water in the cell and increases freezing resistance (Charrier and Améglio 2011). In the present study, leaf removal decreased the ABA concentration in buds. During cold stress, ABA triggers an array of cellular changes to prepare woody plants for enduring low temperatures during the dormant period (Zheng *et al.* 2015, Liu and Sherif 2019). It has been suggested that this hormone is associated with the regulation of deep dormancy in buds and reaches its maximal concentration when the depth of dormancy increases in mid-winter (Khalil-ur-Rehman *et al.* 2019). In addition, external application of ABA increased cold resistance (Dami *et al.* 2015, Li and Dami 2015). Our work shows that early defoliation reduces ABA content, which could affect bud and cane cold hardiness.

Our study shows that defoliation reduces the winter cold hardiness in grapevine and results in less negative EL-LT_{50} and T-LT_{50} temperatures. In addition, leaf removal exacerbates the occurrence of winter cold injuries. In parallel, it decreases the concentration of soluble carbohydrate, proline and ABA in canes and buds, and prevents the reduction of water content in grapevine tissues. Altogether these results support that cold hardiness may highly depend on the accumulation of these metabolites and of the water status of overwintering plant parts.

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