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ORIGINAL ARTICLE

Defoliation alleviates cold-induced oxidative damage in dormant buds of grapevine by up-regulating soluble carbohydrates and decreasing ROS

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Abstract
The number of pre-bloom, bloom and fruit-set source leaves are the most important determinant of tolerance of buds to low temperatures during winter. In this study, we tested whether pre-bloom (LR-PB), bloom (LR-FB) and fruit-set (LR-FS) hand defoliation are effective in interacting the cold hardiness and biochemical parameters in dormant buds (basal-medium and apical buds) of a high-yielding cultivar Karaerik during period of 2-year field study. In the LR-PB contrary to control (C) was found that basal-medium buds showed high-temperature exotherm (HTE) values and low-temperature exotherm (LTE) values at high temperatures than apical buds; therefore, basal-medium buds (1st, 2nd, 3rd, 4th and 5th) had less tolerance to low temperature than apical buds (6th, 7th, 8th, 9th and 10th). Additionally, the contents of the soluble carbohydrates increased in buds apical after the LR-PB treatment, while malondialdehyde (MDA), hydrogen peroxide (H_2O_2) and superoxide radical ($O_2^{\cdot-}$) were decreased. The results from defoliation indicated that the LTE values are positively correlated with water content, MDA, H_2O_2 and $O_2^{\cdot-}$ and are negatively correlated with soluble carbohydrates content. Particularly, LR-PB treatment played the key role to explain the difference of cold hardiness between basal-medium and apical buds. Therefore, since LR-PB treatment does not have a negative effect on basal bud survival and increases the survival of apical buds in cool climatic regions, it could be used as a powerful technique for grape cultivars with poor basal buds fruitfulness.

Keywords *Vitis vinifera* L. · Early leaf removal · Cold hardiness · Karaerik grape cultivar

Introduction

Due to the short growing season and cool climate in Eastern Anatolia region of Turkey, grape producers face with major challenges when attempting to grow high quality grapevine. As it is well known, in the high-yielding grape cultivars grown in cool and/or cold climates conditions both crop load adjustment and cultural practices have great importance in order to ensure a balanced vine management (Poni et al. 2009; Chalfant 2012; Hickey and Wolf 2018; Hickey et al. 2018; Alessandrini et al. 2018). From this point of view, various practices have been widely used to achieve an optimal balance between the reproductive and vegetative structures of the vines. In particular, canopy management techniques such as fruit zone leaf thinning are used to improve fruit composition and microclimate conditions. Moreover, it has been reported that properties such as yield, berry growth and composition, grape carotenoids and aroma composition, flavonoid and anthocyanin synthesis, bud survival, phenolics and wine sensory profile are affected by defoliation treatments (Hickey et al. 2018; Poni et al. 2006; Tardaguila et al. 2010; Palliotti et al. 2011; Bubola et al. 2017; Sivilotti et al. 2016). Fruit-zone leaf removal is a traditional practice between pre-bloom and veraison, commonly used in cool climate vineyard regions to improve spray coverage, cluster composition, fruit exposure and bunch microclimate and to decrease canopy density, disease pressure and cluster compactness (Hickey and Wolf 2018; Tardaguila et al. 2010; Bubola et al. 2017; Intrieri et al. 2008; Sabbatini and Howell 2010; Gatti et al. 2012; Hed et al. 2015; Hed and Centinari 2018). Therefore, adjustment of canopy management with defoliation treatments is vital for quality grape production in the vineyards, especially under cool climate conditions.

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Crop yield reduction has to be applied in many grape cultivars because regulation of crop yield is playing key role on vine size and cold hardiness, in turn causing a reduction of both quality of the fruit and cold hardiness of buds. For this reason, crop yield reduction is commonly adopted in cool and/or cold wine regions (Dami et al. 2005; Ferree et al. 2003; Kurtural et al. 2006). The leaf removal practices in the early season (i.e. from two weeks before bloom to the pea-size phenological stage) seemed an effective way to reduce cluster compactness, fruit set and yield, while improving fruit quality (Hickey and Wolf 2018; Sabbatini and Howell 2010). When leaves are removed from the basal of the shoots during pre-bloom, bloom and fruit-set, a great amount of leaf area reduction occurs in the shoots (Risco et al. 2014; Frioni et al. 2019). Indeed, Frioni et al. (2019) reported that the removal of 10 basal leaves at bloom caused a reduction of about 44% of the whole canopy leaf area available per each inflorescence, as compared to untreated vines. Basal leaves in shoot are the major source of assimilate substances at flowering stage and the primary determinant of fruit-set (Poni et al. 2006; Caspari et al. 1998). Actually, the mature leaves which are able to synthesize/release sugars to the sinks are the main source organs during grapevine flowering (Lebon et al. 2008). As such, early leaf removal significantly changes the source-sink balance in shoots, causing a reduction of sugar supply to inflorescences and, consequently, of cluster compactness (Frioni et al. 2018). Therefore, restriction of carbohydrates production as a result of decreasing photosynthetic leaf area during flowering time induces a reduced fruit set (Candolfi-Vasconcelos and Koblet 1990). It is, however, still unknown how carbohydrates source-sink manipulation from defoliation might affect the storage of soluble carbohydrates that play a critical role in vine cold hardiness. Potential effects of defoliation on vine susceptibility to winter minimum temperature damages are a major consideration for vineyard regions that experience low winter temperatures capable of damaging buds of grapevine cultivars.

To our knowledge, there are only two reports regarding the effect of early leaf removal on cold hardiness of grapevine buds (Chalfant 2012; Smith and Centinari 2019), but in those studies, it has not been investigated the impact of the defoliation on the biochemical parameters of buds such as soluble carbohydrates, malondialdehyde, hydrogen peroxide and superoxide radical. As it is known, cold hardiness of grapevines, such as many other woody plants is a complex process involving a number of biochemical and physiological changes (Wisniewski et al. 2003; Grant and Dami 2015; Rende et al. 2018), including reduction in the water content of the bud tissues, the induction of genes encoding changes in cell wall compositions and lipid, activation of antioxidative mechanisms, increased levels of soluble carbohydrates (Guy 1990; Thomashow et al. 2001; Kaya and Köse 2017). Additionally, some studies indicate that the main target for cold injury is the cell membranes of plant (Levitt 1980; Griffith and Brown 1982; Kaya 2020; Kaya et al., 2018, 2020; Kaya and Kose 2019) Freezing damage may also increase the level of reactive oxygen species and cause an increase in the amount of lipid peroxidation in the membrane of the cell walls, creating serious oxidative damage to the tissues (Griffith and Brown 1982; Stepenkus, 1984), protein degradation (Thomashow et al. 2001; Salzman et al. 1996), membrane deterioration and metabolic function disruption (Lin et al. 2005). The limited carbohydrates caused by defoliation could affected negativelythe induction of cold hardiness of buds, but, currently, it is unclear how defoliation influences relationship between bud death and physiological parameters during low winter temperatures.

The objectives of the study are: (1) to determine the effects of (LR-PB, LR-FB and LR-FS) early leaf removal on the vine cold hardiness of Karaerik grape cultivar; (2) to assess the relationship between bud death (LTE_{50}) and soluble carbohydrates, water content, malondialdehyde, hydrogen peroxide and superoxide radical of dormant buds.

Materıals and methods

Plant material and experimental design

This study was conducted in 2017 and 2018 on own-rooted Karaerik cl.18 vines (*Vitis vinifera* L.) at a commercial vineyard in Erzincan, Turkey (39° 36′ N, 39° 75′ E. 1309 m asl). The vines were grown and planted in 2002. The vine spacing was 2.0 m within rows and 2.5 m between rows and trained to a bilateral low cordon training system. Winter pruning was performed retaining 14 nodes in six spurs with two count nodes each (i.e. 28 buds in total for each vine). Shoot number was adjusted to an average of 14 shoots per meter of cordon on 2 June 2017 and 5 June 2018 when shoots reached growth stage E–L 15 (Eichhorn and Lorenz 1977). Hedging was performed the third week of August. Insect and disease control practices applied in the vineyard. A base fertilizer 5 kg ha^{-1} K_2SO_4, 6 kg ha^{-1} TSP, 5 kg ha^{-1} $ZnSO_4$, 25 kg ha^{-1} $MgSO_4$ were applied in autumn close to plant roots with the 50-60 cm distance and 15 cm depth with rotovator, to sustain the normal growth of vine. Additionally, before bud break stage; 32 kg ha^{-1} from 10–20–20– (N P K) 6AS + 1Zn fertilizer, in flowering stage 14 kg ha^{-1} from 33% ammonium nitrate and in grain size stage; 16 kg ha^{-1} from 33% ammonium nitrate were applied. Drip irrigation in vineyard was done with two pressure-compensated emitters of 2.4 L/h located at 60–65 cm on each side of the vines. Irrigation application in vineyard was 142 and 126 mm in 2017 and 2018, respectively, by the end of the season.

Treatments were set up in a randomized complete block design with four replicates (six vines per rep.), with treatments re-randomized each growing season. Defoliation was performed at three times: LR-PB, LR-FB, LR-FS, corresponding to growth stage E-L 19 (first cap fallen) (LR-PB), EL-23 (full bloom) (LR-FB) and EL-27 (berries about 2 mm diam) (LR-FS), according to Eichhorn and Lorenz (1977) respectively; non defoliated vines were considered as control (C). The defoliation consisted of manual removal of five basal leaves. In leaf removal treatments were also removed all the lateral shoots from the basal five nodes of primary shoots.

Cold hardiness of the buds

The canes were collected in mid-winter (30 Jan 2017 and 25 Jan 2018). Approximately 200 canes with 10–11 dormant buds were cut in the vineyard, and transferred to the laboratory in a container. The canes were then separated, and randomly assigned to six sets for each treatment. Equal number of dormant buds was taken from each node in order to determine the effects of bud position on both biochemical parameters and cold hardiness levels. In the differential thermal analysis (DTA) and the other analyzes were used 1st, 2nd, 3rd, 4th and 5th for basal-medium bud and 6th, 7th, 8th, 9th and 10th for apical bud. Primary bud cold hardiness analyses or LTE were determined using the DTA (Mills et al. 2006). One-year-old canes were excised from nodes 1–5 with approximately 2 mm of intact surrounding tissue for apical buds and from nodes 6 through 10 with approximately 2 mm of intact surrounding tissue for basal-medium buds. Then, buds were placed on a thermo-electric module (TEM), inside a Tenney Junior Environmental Test Chamber (model TU-JR, Thermal Product Solutions, Williamsport, PA), equipped with a temperature controller, (Partlow MIC 1462, The Partlow West Company, New Hartford, NY). Up to four trays, each containing nine modules, were placed in the freezer for a maximum of 36 thermo-electric modules (TEMs) loaded per run (45 buds). The freezer chamber was programmed to hold at 4 °C for 1 h, then the chamber temperature was set to decrease from 4 to − 40 °C at a rate of 4 °C h^{-1} (Fig. 1). The heat released at the moment of supercooling in bud tissues or the temperature at which 50% of primary buds died was recorded as voltage spikes by the thermo-electric modules (Wolf and Pool 1987).

Soluble carbohydrates content of the buds

Soluble carbohydrates of both in apical and basal-medium buds were measured by anthrone method (Yemm and Willis 1954). Dormant buds taken from the first 10 nodes (1st, 2nd, 3rd, 4th and 5th for basal-medium buds and 6th, 7th, 8th, 9th and 10th for apical buds) of the cane were oven-dried at 80 °C for 72 h. Then samples were ground in a grinder,

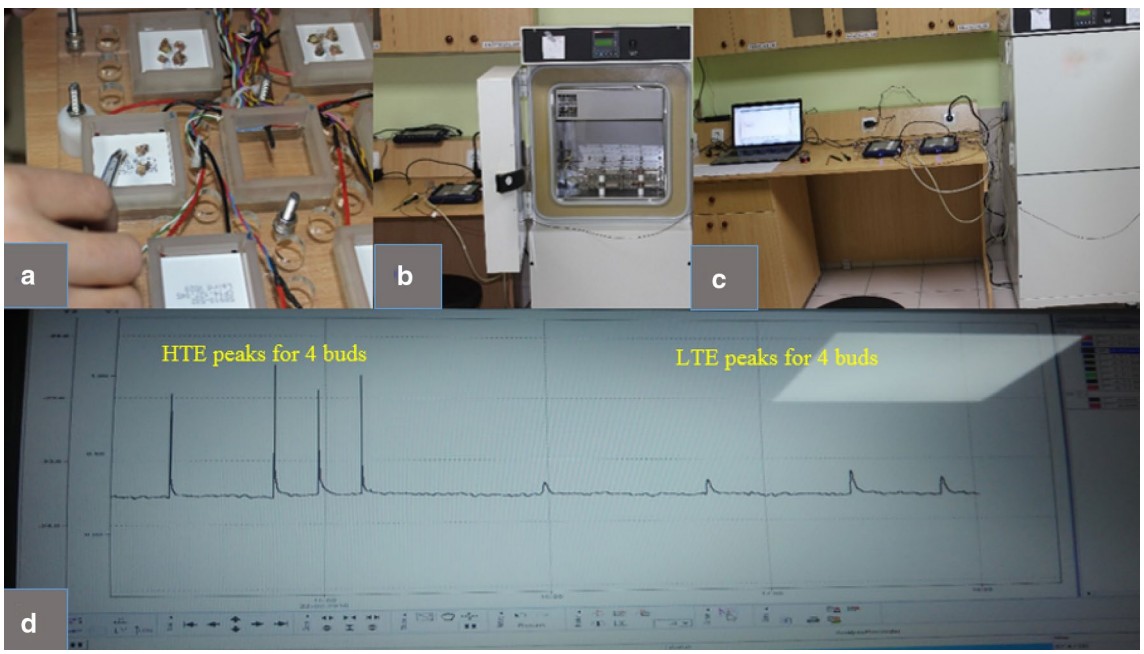

Fig. 1 The stages of differential thermal analysis profile for buds. Samples were prepared in laboratory for the differential thermal analysis test (**a**). Thermoelectric module plates were placed in the programmable test cabinet (**b**). The programmable test cabinet operated (**c**). The high-temperature exotherm (extracellular freezing is considered nonlethal) and the low-temperature exotherm (intracellular freezing is considered lethal) peaks of the buds were determined (**d**)

and were stored in lightless condition until analysis. Soluble carbohydrates of buds both in apical and basal-medium position 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 was 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 apical and basal-medium position was eventually calculated by using a standard glucose curve and expressed as mg g^{-1} dry weight (DW).

Water content of the buds

Dormant buds taken from the first 10 nodes of canes were excised and were weighed immediately with precision scales and placed in an oven for 2 days at 85 °C. Water content of buds both in apical and basal-medium position was determined as percent of fresh tissue weight by using the following formula:

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

MDA, H_2O_2 and $O_2^{\cdot-}$ contents of buds

Superoxide anion content of buds was measured as 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 $O_2^{\cdot-}$. The hydrogen peroxide of buds was measured by monitoring the absorbance at 410 nm wavelength in titanium reagent (He et al. 2005). Lipid peroxidation of dormant buds was measured as described by Jalel et al. (2007) using the thiobarbituric acid test, which defines malondialdehyde as a final product of lipid peroxidation. Absorbance was recorded at 600 and 532 nm. MDA content in dormant apical and basal-medium buds was calculated using the following equation:

$$\text{MDA (nmol/ml)} = \left[\left(A_{532} - A_{600}\right)/155000\right] \times 106.$$

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 test parameters which were significantly different between basal-medium and apical buds within sampling time with a level of significance $p \leq 0.01$. Each measurement parameter was presented as the mean ± standard error with a minimum of four experiments. The obtained averages were compared by one-way analysis of variance and Duncan's multiple range test at the 1% level of significance. Additionally, correlations among MDA, H_2O_2 and $O_2^{\cdot-}$ contents and LTE values of buds were determined by Pearson index, and they were significant per $p \leq 0.01$ (*) (R as reported).

Results

mHTE and mLTE values of buds

On the basis of standard DTA tests, HTE and LTE results of buds from each defoliation treatment and from both years were similar. In 2017, for the control treatment, there were not significant differences in the basal node positions both for HTE and LTE in relation to the defoliation treatments, and in 2018, there were significant differences in the basal-medium node positions for HTE which was similar to LR-FB and lower than LR-PB or LR-FS treatments (Table 1). At the control, HTE and LTE values per bud were the highest in apical and the lowest in basal-medium. On the contrary, at LR-PB treatments, HTE and LTE values per bud were the highest in the basal-medium and the lowest in apical buds. For apical buds every year, LR-PB vines had higher bud cold hardiness than LR-FB, LR-FS and the C. Further, the LTE values of apical buds occurred at higher temperatures compared to LTE values of basal-medium buds in both years; the LTE values of apical buds were 1.63 °C (2017) and 1.29 °C (2018) lower than that of the basal-medium buds. However, LTE values of basal-medium buds in control were lower in 2017 (1.78 °C) and 2018 (3.98 °C) compared with the apical buds (Table 1).

Biochemical parameters of buds

The soluble carbohydrates of basal-medium and apical buds were variable in C and LR-PB treatment, but not differences were found LR-FB and LR-FS treatments. The soluble carbohydrates of basal-medium buds for the LR-PB treatment was 2.63 mg g^{-1}DW (2017) and 2.39 mg g^{-1}DW (2018) lower than that of the apical buds. The soluble carbohydrates of apical buds for the control were 0.89 mg g^{-1} DW (2017) and 2.03 mg g^{-1} DW (2018) lower than that of the basal-medium buds. In both years, the soluble carbohydrates of apical buds were the highest in LR-PB treatment and the lowest in C, while not significant differences in basal-medium buds were observed among treatments. On the other hand, the water content

Table 1 Effects of treatment on mean high-temperature exotherms and low-temperature exotherms for basal-medium and apical buds of Karaerik vines

Year	Exotherm temperatures	Node position	Control	Pre-bloom	Bloom	Fruit-set	Significance[a]
2017	mHTE (°C)	Basal-medium	− 9.76 ± 0.06	− 9. 51 ± 0.09	− 9.21 ± 0.13	− 9.31 ± 0.13	ns
		Apical	− 8.03 ± 0.05C[c]	− 10. 21 ± 0.12A	− 9.34 ± 0.08B	− 9.28 ± 013B	*
		t test	*	*	ns	ns	
	mLTE (°C)	Basal-medium	− 15.26 ± 0.05	− 15.32 ± 0.08	− 15.35 ± 0.08	− 15.43 ± 0.07	ns
		Apical	− 13.48 ± 0.11D[c]	− 16.95 ± 0.03A	− 15.66 ± 0.08B	− 15.11 ± 0.04C	*
		t test	*	*	*ns*	ns	
2018	mHTE (°C)	Basal-medium	− 9.16 ± 0.08b[b]	− 10.35 ± 0.12a	− 9.33 ± 0.15b	− 10.23 ± 0.16a	*
		Apical	− 8.12 ± 0.09C[c]	− 11.54 ± 0.23A	− 10.39 ± 0.02B	− 10.08 ± 021B	*
		t test	*	*	*	ns	
	mLTE (°C)	Basal-medium	− 16.43 ± 0.07	− 16.53 ± 0.05	− 16.47 ± 0.07	− 16.35 ± 0.06	ns
		Apical	− 12.45 ± 0.04D[c]	− 17.82 ± 0.06A	− 16.76 ± 0.02B	− 16.43 ± 0.04C	*
		t test	*	*	ns	ns	

Basal-medium = nodes one to five; Apical = nodes six to 10, *t* test was performed between basal-medium and apical buds

Data are the mean of 9 replicates ± SE

[a]Data were analyzed by one-way ANOVA (*ns* not significant; $*p \leq 0.01$) and when differences were significant, mean separation was performed with Fisher's LSD test ($p \leq 0.01$)

[b]Treatment means followed by different capital letters within a lines are significantly different ($p \leq 0.01$)

[c]Treatment means followed by different lowercase letters within a lines are significantly different ($p \leq 0.01$)

of basal-medium and apical buds according to the node position did not differ among treatments in both years.

No difference in MDA, H_2O_2 and $O_2^{\cdot -}$ were observed between node position in LR-FB and LR-FS treatments in both years. However, there was a significant difference in MDA, H_2O_2 and $O_2^{\cdot -}$ between node positions in LR-FB and C vines. In control, MDA, H_2O_2 and $O_2^{-\cdot}$ were lower in basal-medium buds compared to apical buds, unlike LR-FB treatment. Furthermore, not significant differences among treatments were found in the content of MDA, H_2O_2 and $O_2^{\cdot -}$ of the basal-medium buds. On the other hand, MDA, H_2O_2 and $O_2^{-\cdot}$ contents of the apical buds were significantly affected by the LR-PB, LR-FB and LR-FS treatments in comparison to C vines. In both years, LR-PB vines had lower MDA, H_2O_2 and $O_2^{\cdot -}$ contents than LR-FB, LR-FS and the C vines. Defoliation, either applied at LR-PB, LR-FB and LR-FS treatments, consistently reduced the MDA, H_2O_2 and $O_2^{\cdot -}$ contents of buds caused by cold injury (Table 2).

There were high correlations between mLTE values and soluble carbohydrates, water content, MDA, H_2O_2 and $O_2^{\cdot -}$ contents of buds in all defoliation treatments and control. Negative correlations were found between mLTE and soluble carbohydrates of buds in all defoliation treatments and control; the highest correlation was seen in LR-PB treatmentin either year. Additionally, positive correlations were found between mLTE and water content, MDA, H_2O_2 and $O_2^{\cdot -}$ contents of buds in all defoliation treatments and control; the highest correlations were observed in water content, MDA, H_2O_2 and $O_2^{\cdot -}$ contents of buds in LR-PB treatment in both years (Table 3).

Discussion

The primary objective of this work was to understand how leaf removal at LR-PB, LR-FB and LR-FS impact physiological parameters and tolerance to winter temperature of Karaerik grape cultivar under cool-climate conditions. Temperature exotherms in our investigations showed a systematic pattern for basal-medium and apical buds amongst all treatments in both years. There were significant differences in between freezing of extra- and intra-cellular water (mHTE and mLTE values) of basal-medium and apical buds, with the exception of LR-FB and LR-FS treatments. mHTE values that are nonlethal were generally changed between − 9.16 and − 10.35 °C in basal-medium buds, while occurring between − 8.03 and − 11.54 °C in apical buds (Table 1). Typically, the freezing temperature of extracellular regions in the buds has been reported to be between − 5 and − 16 °C (Andrews et al. 1984; Badulescu and Ernst 2006), and our findings are consistent with previous results.

There was not significant effect between mLTE values of basal-medium and apical buds at the LR-FB and LR-FS treatmens, with the exception of LR-PB and C vines. In control vines, basal-medium buds had higher mLTE values compared to apical buds, while apical buds had higher mLTE values compared to basal-medium buds in LR-PB

Table 2 Effects of treatment on mean soluble carbohydrate, water content, MDA, H_2O_2 and $O_2^{-\cdot}$ for basal-medium and apical buds of Karaerik vines

Year	Biochemical parameters	Node position	Control	Pre-bloom	Bloom	Fruit-set	Significance[a]
2017	Soluble carbohydrate (mg g^{-1} DW)	Basal-medium	4.12 ± 0.04	4.18 ± 0.06	4.24 ± 0.11	4.32 ± 0.08	ns
		Apical	3.23 ± 0.05C[a]	6.81 ± 0.06A	4.28 ± 0.09B	4.41 ± 0.07B	*
		t test	*	*	ns	ns	
	Water content (%)	Basal-medium	32.35 ± 0.13	32.45 ± 0.16	31.33 ± 0.16	31.25 ± 0.12	ns
		Apical	32.64 ± 0.11	32.62 ± 0.18	32.48 ± 0.25	32.03 ± 0.21	ns
		t test	ns	ns	ns	ns	
	MDA (nmol g^{-1} FW)	Basal-medium	134.18 ± 0.62	135.13 ± 0.28	133.56 ± 0.35	134.28 ± 0.26	ns
		Apical	165.21 ± 0.91A[a]	120.16 ± 0.69C	134.14 ± 0.46B	135.03 ± 0.58B	*
		t test	*	*	ns	ns	
	H_2O_2 (µmol g^{-1} FW)	Basal-medium	0.65 ± 0.01	0.64 ± 0.04	0.64 ± 0.07	0.66 ± 0.06	ns
		Apical	0.84 ± 0.03A[a]	0.39 ± 0.04C	0.65 ± 0.08B	0.67 ± 0.05B	*
		t test	*	*	ns	ns	
	$O_2^{-\cdot}$ (µmol g^{-1} FW)	Basal-medium	6.31 ± 0.05	6.43 ± 0.15	6.45 ± 0.28	6,38 ± 0.15	ns
		Apical	8.51 ± 0.29A[a]	4.29 ± 0.13C	6.43 ± 0.37B	6.42 ± 0.26B	*
		t test	*	*	ns	ns	
2018	Soluble carbohydrate (mg g^{-1} DW)	Basal-medium	4.72 ± 0.02	4.19 ± 0.07	4.39 ± 0.05	4.69 ± 0.01	ns
		Apical	2.69 ± 0.09C[a]	6.58 ± 0.08A	4.58 ± 0.07B	4.72 ± 0.06B	*
		t test	*	*	ns	ns	
	Water content (%)	Basal-medium	33.42 ± 0.22	31.38 ± 0.06	32.61 ± 0.55	33.21 ± 0.09	ns
		Apical	34.94 ± 0.09	32.51 ± 0.15	33.63 ± 0.06	34.23 ± 0.27	ns
		t test	ns	ns	ns	ns	
	MDA (nmol g^{-1} FW)	Basal-medium	146.76 ± 0.73	147.25 ± 0.62	146.72 ± 0.43	146.86 ± 0.33	ns
		Apical	172.23 ± 1.24A[a]	131.91 ± 2.93C	148.63 ± 0.62B	147.30 ± 1.71B	*
		t test	*	*	ns	ns	
	H_2O_2 (µmol g^{-1} FW)	Basal-medium	0.77 ± 0.02	0.76 ± 0.03	0.74 ± 0.03	0.78 ± 0.03	ns
		Apical	0.96 ± 0.01A[a]	0.42 ± 0.01C	0.73 ± 0.01B	0.79 ± 0.09B	*
		t test	*	*	ns	ns	
	$O_2^{-\cdot}$ (µmol g^{-1} FW)	Basal-medium	7.20 ± 0.08	7.57 ± 0.24	6.86 ± 0.56	7.32 ± 0.11	ns
		Apical	9.47 ± 0.31A[a]	5.34 ± 0.08C	6.55 ± 0.33B	6.96 ± 0.04B	*
		t test	*	*	ns	ns	

Basal-medium = nodes one to five; Apical = nodes six to 10, *t* test was performed between basal-medium and apical buds

Data are the mean of 4 replicates ± SE. [a]Data were analyzed by one-way ANOVA (*ns* not significant; *$p \leq 0.01$) and when differences were significant, mean separation was performed with Fisher's LSD test ($p \leq 0.01$)

[a]Treatment means followed by different lowercase letters within a lines are significantly different ($p \leq 0.01$)

vines. Our findings obtained from C vines corroborate those of Fennell (2004), Buztepe et al. (2017) and Badulescu and Ernst (2006) who reported that grape basal buds had the lowest mLTE values. However, leaf removal did not significantly affect the mLTE values of basal-medium buds. Indeed, it was determined that LR-PB reduced bud mortality as compared with non defoliation vines, and early season leaf removal (pre-bloom) had not negative impact on low temperature exotherms of buds (Chalfant 2012; Smith and Centinari, 2019). In all treatments, leaf removal significantly affected mLTE values of apical buds. Moreover, mLTE value of apical buds in LR-PB treatment occurred at lower temperatures than apical buds of LR-FB, LR-FS treatments and C vines in both years. (Table 1). This was likely related to an increase in the development of laterals from the apical part of the main shoot after defoliation, a quite common consequence of early leaf removal (Kaya, 2019). This, in turn, could have induced an increase of photosynthetic efficiency and carbohydrates accumulation in apical buds (Reynolds and Wardle 1989; Hunter and Roux 1992). In previous studies, however, it remains unclear how the biochemical contents and mLTE values of apical and basal buds are affected by leaf removal treatments, because these changes related to a combination of several differentinternal factors that may be physiological, biochemical, genetic or morphological. It has usually been shown that in buds of different

Table 3 Effects of treatment on correlations between mLTE values and mean soluble carbohydrate, water content, MDA, H_2O_2 and $O_2^{-\cdot}$ for basal-medium and apical buds of Karaerik vines

Node position (nodes one to 10)								
Year	Source of variation		mLTE	Soluble carbohydrate	Water content	MDA	H_2O_2	$O_2^{-\cdot}$
2017	Control	mLTE	1	−0.856*	0.765*	0.886*	0.863*	0.843*
	Pre-bloom		1	−0.969*	0.882*	0.983*	0.946*	0.986*
	Bloom		1	−0.778*	0.768*	0.935*	0.875*	0.912*
	Fruit-set		1	−0.865*	0.702*	0.861*	0.872*	0.873*
2018	Control	mLTE	1	−0.945*	0.658*	0.887*	0.961*	0.958*
	Pre-bloom		1	−0.986*	0.870*	0.778*	0.983*	0.970*
	Bloom		1	−0.860*	0.831*	0.944*	0.956*	0.929*
	Fruit-set		1	−0.905*	0.626*	0.762*	0.963*	0.813*

*Significant at $p \leq 0.01$, Values in the table represent the *R*

grape cultivars, carbohydrates correlate with cold hardiness (Rende et al. 2018; Wample and Bary 1992; Stushnoff et al. 1993; Hamman et al. 1996). In the present study, there were distinct changes in the soluble carbohydrates that correlated with the changes in cold hardiness observed in between the basal-medium and apical buds. Additionally, leaf removal enhanced soluble carbohydrates on apical buds, without affecting those on basal-medium buds (Table 2). It was not unpredictable that higher mLTE values were observed in apical buds than in basal-medium buds, as the soluble carbohydrates were highest in apical buds than in basal-medium buds. Increased soluble carbohydrates resulted in higher cold hardiness of buds, it was demonstrated in previous studies (Fennell 2004; Rende et al. 2018; Smith and Centinari 2019; Ershadi et al. 2016). Furthermore, there were negative correlations between the cold injury and the soluble carbohydrates in all treatments in both 2017 and 2018 (Table 3). We therefore propose that the increase in apical bud cold hardiness was strongly related with a rise in the soluble carbohydrates content in the bud.

In the current study, defoliation treatments did not affect bud water content in both years.. It is reported that the buds reduce their water content by 50–85% in the early-winter (Keller 2015). However, there is a strong association between declining water content and cold hardiness in the buds of the vine (Wolpert and Howell 1985, 1986). Indeed, many researchers reported that the change in bud water plays a key role in cold hardiness and there is a negative relationship between bud water content and cold hardiness (Wolpert and Howell 1985, 1986; Kaya and Köse 2017). This inference is supported by the presence of significant negative correlation between cold injury and water content of buds in our findings (Table 3).

Overall, defoliation effects on MDA, H_2O_2 and $O_2^{\cdot-}$ contents were influenced by the node position on the shoot. However, the increase in the tolerance of both basal-medium and apical buds to cold in LR-PB treatment appeared more pronounced compared to LR-FB, LR-FS treatments and C vines. The MDA, H_2O_2 and $O_2^{\cdot-}$ contents of buds was lower in LR-PB treatments compared to other treatments and C vines in both years. Additionally, the MDA, H_2O_2 and $O_2^{\cdot-}$ contents of apical buds was lower in LR-PB treatments applications compared to basal-medium buds in both years (Table 2). As expected, the lower MDA, H_2O_2 and $O_2^{\cdot-}$ contents of LR-PB vines led to a lower cold injury on mLTE values per vine compared to the LR-FB, LR-FS treatments and C vines. Many previous investigations have shown that *V. vinifera* varieties adapt to alterations in source-sink manipulation (Hunter and Visser 1988; Candolfi-Vasconcelos and Koblet 1990; Smith and Centinari 2019). In our study, there were not significant differences in the soluble carbohydrates of the basal-medium buds of vines belonging to all treatments, and it showed similar effect in all treatments including control. Earlier studies have suggested that loss of leaf area due to defoliation is compensated by the increase in photosynthetic efficiency of the remaining leaves on shoots (Hunter and Visser 1988; Smith and Centinari 2019), a delay in leaf abscission and senescence (Candolfi-Vasconcelos and Koblet 1990) and an increase in lateral shoot (Candolfi-Vasconcelos and Koblet 1990; Hunter and LeRoux 1992). Indeed, Frioni et al. (2018) demonstrated that early leaf removal increases the shoot apex sink strength and the destination of carbon to the distal part of the shoot. In our study the ratio of leaves removed, as previously stated, was likely insufficient to induce any stress for basal-medium buds, and thus fruit zone leaf removal did not affect MDA, H_2O_2 and $O_2^{\cdot-}$ bud contents.

In the current study, the soluble carbohydrates content of apical buds for LR-PB and LR-FB, as well as LR-FS treatments, were higher than those of the control for nearly all apical positions (nodes 6–10) along the cane, with values for basal-medium buds (nodes one to five) being very similar to the control. Furthermore, the soluble carbohydrates content was reduced by control by 3.58 mg g^{-1}DW, by LR-FB

treatment by 2.53 mg g^{-1}DW and by LR-FS treatments by 2.40 mg g^{-1}DW compared with the 6.81 mg g^{-1}DW the soluble carbohydrates content recorded in LR-PB treatment in 2017. The soluble carbohydrates content was reduced by control by 3.89 mg g^{-1}DW, by LR-FB treatment by 2.00 mg g^{-1} DW and by LR-FS treatment by 1.86 mg g^{-1}DW compared with the 6.58 mg g^{-1}DW the soluble carbohydrates content recorded in LR-PB treatment in 2018 (Table 2). Increasing the soluble carbohydrates resulted in increased apical buds cold hardiness in Karaerik grape cultivar, so apical buds had an effect on MDA, H_2O_2 and $O_2^{\cdot-}$ contents when vines were already in dormancy after cold stress. Although MDA, H_2O_2 and $O_2^{\cdot-}$ have been frequently studied during the last decade, to our knowledge there are not previous reports about the effect of defoliation treatments on MDA, H_2O_2 and $O_2^{\cdot-}$ contents in grapevine buds. However, it is reported that low temperatures in the grapevine buds generally lead to lipid peroxidation and ROS generation, such as H_2O_2 and $O_2^{\cdot-}$ (Imlay and Linn 1988; Rende et al. 2018; Ershadi et al. 2016; Zhang et al. 2012). There is also evidences that an increase occurred on the amount of MDA, H_2O_2 and $O_2^{\cdot-}$ in tissues in different grapevine varieties exposed to low-temperature stress (Imlay and Linn 1988; Rende et al. 2018; Ershadi et al. 2016; Zhang et al. 2012; Jiang et al. 2014). Interestingly, in current study, LR-PB treatment, which had the lowest MDA, H_2O_2 and $O_2^{\cdot-}$ contents on apical bud, also had the lowest values of mLTE based on the DTA results, and the relationships between the mLTE and MDA, H_2O_2 and $O_2^{\cdot-}$ were significant in both years. There also was a strong negative correlation between MDA, H_2O_2 and $O_2^{\cdot-}$ contents and cold hardiness (Table 3). Indeed, previous studies reported that correlation between H_2O_2, $O_2^{\cdot-}$ and MDA contents of grapevine buds and cold stress is positively correlated while there is a negative correlation between H_2O_2, $O_2^{\cdot-}$, MDA contents and cold hardiness of grapevine buds (Rende et al. 2018; Zhang et al. 2012). We assume that the effect of the soluble carbohydrates content dominated over the effect of node position in the enhancement of cold hardiness, based on the striking differences in H_2O_2, $O_2^{\cdot-}$ and MDA contents between all treatments including control vines.

Conclusions

The findings of this work demonstrated that regulating the time of defoliation in a region characterized by harsh winter, many important biochemical parameters (H_2O_2, $O_2^{\cdot-}$, MDA and soluble carbohydrates content) and cold hardiness of Karaerik grape cultivar buds could be improved and managed. Defoliation treatments, and especially the more effective LR-PB, significantly decreased H_2O_2, $O_2^{\cdot-}$ and MDA contents of apical buds and increased the soluble carbohydrates content of apical buds resulting in the improvement of bud cold hardiness. This is the first study investigating the relations existing among early leaf removal, bud cold hardiness and bud biochemical parameters, according to their node position along the cane. Therefore, we think that the use of LR-PB treatment to increase apical bud cold hardiness of many grape cultivars in cold climates could be a useful technique. We can also recommend that pre-boloom defoliation may be used to improve the adverse effects of low temperatures not only of Karaerik grape cultivar but also other grapevine varieties. To sum up, the evidence that adopting early leaf removal in cool climates does not have any negative carry over effects on basal bud survival, but could instead improve the survival of apical ones is of special interest in relation to those genotypes with poor basal buds fruitfulness, needing cane-pruning.

Author Contribution Statement Author contributions statement OK designed this study and performed the experiments. OK analyzed the data and wrote the manuscript. OK supervised the experiment and reviewed the manuscript. The author read and approved the final manuscript.

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