Determination Of Resistance To Low Temperatures Of Winter Buds According To Position In Karaerik (*V. vinifera L.*) Grape Cultivar

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Abstract- This experiment was carried out to determine the tolerance level of winter buds according to positions in Karaerik cv. grape cultivar grown in Erzincan province during the winter colds that occurred in 2013/14 and 2014/15 years. For this purpose, frost tolerance levels of the first 4 buds of one-year old shoot and their damaged varability rates by exposure to low winter temperatures have been detected with binocular microscope and lipid peroxidation (MDA) analysis. The winter buds at the 2nd and 3rd shoot were found to be most sensitive to low temperatures with average damage rates of 26.5% and 33.5%, respectively during the years of the research. Additionally, the winter buds at the 1st and 4th nodes according to positions were found to be the most tolerant buds with average damage rates of 18.5% and 19.5%, respectively. On the other hand, the winter buds found on the 2nd and 3rd nodes according to positions were found to be the most sensitive buds with average MDA content of 3.72 and 3.74 nmol/ml respectively. For all that the winter buds in the 1st and 4th nodes according to positions were identified as the least injured buds with average MDA content of 2.92 and 3.12 nmol/ml respectively. Therefore, in pruning this is recommended to reduce yield losses by making it from node position 4th after the severe winter cold. In this way, with standardization of pruning levels for Karaerik grape cultivar can be improved yield and quality.

Index Terms- Erzincan, Karaerik, low temperature tolerance, lipid peroxidation

I. INTRODUCTION

Grapes, due to their wide spreading area on the earth, are one of the temperate fruit crops most frequently damaged by low temperatures (Fennell, 2004). Low temperatures occurring especially in winter limiting the grape cultivation is among the most common environmental stress (Ma *et al.*, 2010). Along with that, regions where vegetation duration is short and which have continental climate are affected more by this stress, which is caused by the low winter temperatures. In these areas with the increase in the intensity of winter temperatures, permanent damage to the tissues and organs of the grapevine occurs without compensation. Such a situation can result in reduced yield and substantial economic losses to grape growers, subsequently impacting fruit wholesalers, distributors, vineries and related industries (Fennell, 2004; Zabadal *et al.*, 2007; Li, 2014).

Tolerance to low winter temperatures in grapevines include a complex set of traits that are influenced by the inherent genetic characteristics and their interaction with the environment. However, the tolerance to low temperatures of the grapevine affects factors such as the degree of low temperature, the rate of fall, the speed of fall, rootstock is grafted onto the vine, altitude and the location of the vineyard, dormant period temperatures, pruning time and method, training shape, crop load and support system, irrigation, fertilization disease and pest control level (Khanizadeh *et al.*, 2005; Çelik *et al.*, 2008; Köse and Güleryüz, 2009). In addition, tolerance to low winter temperatures in grapes varies between species, cultivars and tissues depending on environmental factors and cultural practices. In addition to this, it is not possible to give a definite value for the lowest temperature value of species, cultivars or tissues due to the dynamic nature of the resistance to low winter temperatures. However, varieties of *V. vinifera* L. which provide more than 90% of world grape production are limited to areas where low winter temperatures are above a minimum of -25^{o}C (Fennell, 2004; Mills *et al.*, 2006; Davenport *et al.*, 2008; Ferguson *et al.*, 2011; Ferguson *et al.*, 2014; Keller, 2015).

After the low winter temperatures variety of tissues of *V. vinifera* L. and organs to determine the degree of damage caused in the right way, is extremely important in terms of the adjustment crop load in winter pruning (Ershadi *et al.*, 2016). For this purpose, determination of the degree of damage by looking at the color change (browning of bud tissues) of living and dead tissues in winter buds is one of the most widely used methods (Wolf and Cook, 1992; Rekika *et al.*, 2004; Köse and Güleryüz, 2009; Ershadi *et al.*, 2016). On the other hand, tolerance to low temperatures in grapes usually involves a combination of morphological, physiological and biochemical features which develop by natural selection over very long periods of time. Therefore these features are often associated so that cold hardiness can be determined by testing for change in the relative amounts of particular biochemicals (Zhang *et al.*, 2012). The increase and decrease in the amount of malondialdehyde (MDA) from these biochemicals is a good indicator that the structural integrity of the cellular membranes has deteriorated (Lin *et al.*, 2006; Kaya and Köse, 2016). For this reason, the increase in the proportion of MDA in the grape buds after low winter temperatures can be used as an alternative method for assessing frost tolerance.

This study was carried out to determine the tolerance level of winter buds (1st, 2nd, 3rd and 4th buds) of Karaerik cv. grape cultivar which have a significant share in Erzincan viticulture

during winter colds that occurred in 2013-2014 and 2014-2015 years.

II. MATERIAL AND METHODS

II.1. Plant material

This study focused on a homogeneous plant material consists of a single vine variety of *Vitis vinifera* L. cv. Karaerik. In study, cultivar from 25 years old own-rooted vines grown in Erzincan, were evaluated in the early spring after low winter temperatures in 2013/14-2014/15. All vines were spaced 2.5 m apart in north-south oriented rows that were 2.0 m wide. The vines were spur pruned and Baran system-trained. The height of head was 0.2 m bove ground. Cultural practices such as fertilization, irrigation, and pest control were uniform across the vineyards.

Vines had been pruned to 2nd to 4th node spurs each dormant season before the study but were not pruned before the collection of buds for this experiment. Healthy canes of 4 buds with uniform periderm formation were randomly sampled from the vine canopy for all the cultivar at each evaluation year. From each of the plots occupied by the cultivar, 400 samples of cane were taken of during March 13, 2014 and March 17, 2015. One-year-old shoots, free from structural damage, were taken from vines. Upon collection, samples were placed in plastic bags, immediately brought to the laboratory, and for the enzymatic browning of bud tissues kept at 25°C for 48 hours until testing.
The frost damage and amount of lipid peroxidation of the winter buds of the vines were assessed following the weather conditions described in Table 1 and Table 2. Extremely low temperatures, below –15°C, occurred in January 6, 2014 and January 10, 2015.

II.2. Determination of enzymatic browning in winter buds tissues

Determining low temperature damage in winter buds was estimated according to the method of Odneal (2004). The buds under assessment were cut cross-sections. Winter buds on the first 4 nodes of one-year-old cane were examined by binocular microscopy. The winter buds were considered to be dead if both the main bud (primary bud) and the replacement buds underneath were dark brown. The primary buds that appeared bright and green were considered alive, and those appearing dull, strawcolored or black/brown were calculated as dead (Wolf and Cook 1992, Linden 2002; Karami *et al.*, 2016). The proportion of injured buds was determined as the number of buds injured/total buds.

II.3. Determination of lipid peroxidation in winter buds tissues

Malondialdehyde (MDA), an indicator of lipid peroxidation, was determined by the thiobarbituric acid (TBA) reaction according to the method of Heath and Packer (1968). The bud sample, a 0.2 g sample was mixed with 2 mL trichloroacetic acid and a small quantity of quartz sand in a mortar. The homogenate was centrifuged at 12000 x*g* for 15min at 4°C. The supernatant was mixed with the equal volume of 5% TCA containing 0.5% TBA. The mixture was heated at 90°C for 30 min and quickly cooled to room temperature and then centrifuged at 12000 x*g* for 10 min at 4°C. Absorbance was recorded at 532, and 600 nm. MDA content was calculated using the following formula: MDA (nmol/ml) = $[(A_{532}- A_{600})/155000] X 10^6$ (Heath and Packer 1968; Jaleel *et al.*, 2008).

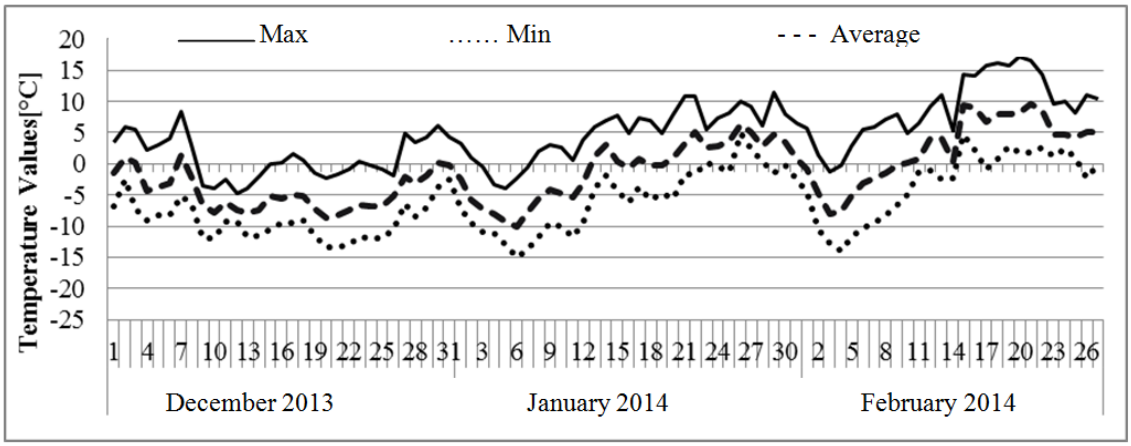
II.4. Statistical analysis

The study was carried out in four replications according to randomized block trial design. The difference between the averages of the variance analysis was compared in the Jamp Packet program according to the LSD multiple comparison test.

III. RESULTS AND DISCUSSION

The air temperature values for the 2013-2014 and 2014-2015 winter period in which the study was conducted are given in Figure 1 and Figure 2. (Anonymous., 2017). Generally, the lowest winter temperatures in our region occur in December, January and February (Kaya, 2011). For this reason, the climate data of these months have been taken into account in the study. The minimum air temperatures were determined as -15.1°C on January 6, 2014 and -16.3°C on January 10, 2015 for the second year. As a matter of fact, the tolerance for low winter temperatures of the *V. vinifera* L. range varies by varieties and this value is from -15°C to -25°C in December, January and February (Andrews et al., 1984; Fennell, 2004). It has been determined that the temperature decreases among these values, which are considered as critical temperatures for the grape winter buds in both years of the study (Figure 1, 2).

Figure 1. Daily temperature values for December 2013 - January - February 2014 period (Anonymous., 2017).



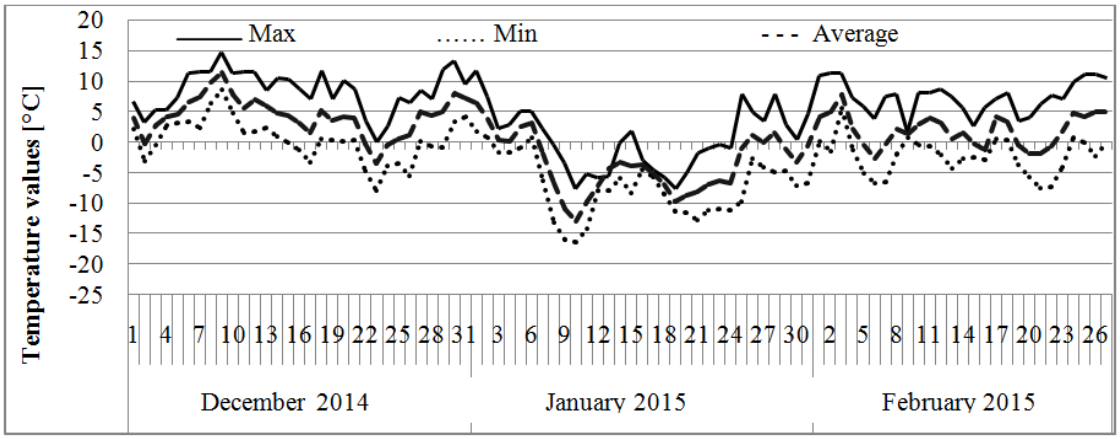


Figure 2. Daily temperature values for December 2014 - January - February 2015 period (Anonymous., 2017).

Frost damage, expressed as the percentage of dying buds exposed to low temperatures during winter, in Karaerik (*V. vinifera* L.) grapevine cultivar (Table 1). After the winters of 2013/2014 and 2014/2015, when the minimum temperature dropped to -15.1°C and -16.3°C respectively, 'Karaerik' primary buds suffered with average damage rates of 22.25% and 26.75% respectively. The damage was more severe than after the much colder winter of 2014/2015. However, this damage rate in the primary buds varied considerably depending on the position on the shoots of the buds. Indeed, the 3rd winter buds on the shoots have been the buds most affected by low winter temperatures with a 35% damage rate during the 2013-2014 working year. On the other hand, the 1st and 4th winter buds were the least damaged buds with 17% damage rate in the same year and they were found more tolerant to low temperatures. Similarly, winter buds on the 2nd and 3rd nodes on the shoot were the most susceptible to frost with 32-33% damage rates respectively, winter buds on the 1st and 4th nodes on the shoot were found to be more tolerant with 20-22% respectively damage rates in 2014/2015 years (Table 1). Differences in the tolerances of the winter buds to low temperatures according to their positions are confirmed by many researchers (Howell and Shaulis, 1980; Köse and Güleryüz, 2009; Buztepe, 2016). The winter buds on the 1st, 2nd and 4th nodes on the shoot of 15 different grape varieties were evaluated for tolerance to low temperatures and 1st winter buds were determined to have the highest tolerance (Çelik *et al.*, 2008).Wolpert and Howell, (1986b) conducted a study on Concord grape variety, they found that basal buds (node positions 3 to 7), were able to withstand freezing stress at lower temperatures than middle buds (node positions 8 to 12), then apical buds (node positions 13 to17). Similarly basal buds(node positions 2 to 4), were able to withstand freezing stress at lower temperatures than middle buds (node positions 6 to 8), then apical buds (node positions 10 to12). While basal buds were generally more freezing tolerant compared to the other node positions, 'Couderc 3309' basal buds were the most 'Cabernet Franc' followed by 'Concord' and 'Cabernet Franc' basal buds were the least freezing tolerance (Grant, 2012).

In grape, the tolerance of winter buds to low temperatures is directly related with maturation cane (Wolpert and Howell, 1986b; Fennell, 2004). Bud dormancy is initiated at the base of the cane and continues towards the apical buds (Fennell and Hoover, 1991). According to our results the reason to be more tolerant of winter buds at the 1st node may be related to earlier maturation and decreased water content. In addition, the amount of carbohydrate stored in the basal buds may affect this situation. Carbohydrate concentrations in bud tissues were not always uniform throughout the cane, and basal buds often had higher concentrations of raffinose compared to middle or apical buds, indicating differences in hardiness progression (Fennell, 2004; Grant and Dami, 2015).

On the other hand, it was determined that the winter buds at the 4th node on the shoot showed tolerance to low temperatures as much as the winter buds in the 1st node. Indeed vines trained with baran training system occurs a bending in the 4th node on the shoot during the vegetation period. In these buds 4th node may have caused the accumulation of carbohydrate substance for this bending at the 4th node on the shoot and therefore it is thought to be enhanced the tolerance of the winter buds at 4th node. In fact, this view supports to be more sensitive to low temperatures of the winter buds at 3rd node on the shoot in both working years.

Table 1.The damage rate of primary buds of winter buds according to node positions in the Karaerik (*V. vinifera* L.) grapevine cultivar.

Node position	The damage rate of primary buds of winter buds according to node positions (%)		
	2013/2014 years	2014/2015 years	*Mean*
Buds in the 1 node	17 **c**	20 **b**	*26.5*
Buds in the 2 node	25 **b**	33 **a**	*33.5*
Buds in the 3 node	35 **a**	32 **a**	*18.5*

Buds in the 4 node	17 **c**	22 **b**	*19.5*
Mean	*22,25*	*26.75*	*24.5*
F test	$p \leq 0,01$	$p \leq 0,01$	
CV	*8.3*	*3.7*	

Cold tolerance in grapevines usually involves a combination of morphological, physiological and biochemical features (Keller, 2015). These features are often associated with cold tolerance so that cold tolerance can be screened by testing for change in the relative amounts of particular biochemicals and thus, change in malondialdehyde (MDA) can be associated with the tolerance of many grape the winter buds to low temperatures (Zhang et al., 2012).

Table 2. MDA content of winter buds according to node positions in the Karaerik (*V. vinifera* L.) grapevine cultivar (nmol/ml)

Node position	MDA content of winter buds according to node positions (nmol/ml)		
	2013/2014 years	2014/2015 years	*Mean*
Buds in the 1 node	3.92 **c**	2.03 **b**	*2.97*
Buds in the 2 node	4.29 **a**	3.16 **a**	*3.72*
Buds in the 3 node	4.14 **b**	3.34 **a**	*3.74*
Buds in the 4 node	3.98 **c**	2.26 **b**	*3.12*
Mean	*4.08*	*2.69*	*3.38*
F test	$p \leq 0,01$	$p \leq 0,01$	
CV	*1.2*	*6.29*	

According to our results significant differences have been determined in the MDA content of winter buds according to node positions after lower winter temperatures (Table 2). MDA content of winter buds according to node positions were determined as average 4.08 nmol/ml in 2013/14 and average 2.69 nmol/ml in 2014/15. At the same time of the winter buds in the 1st and 4th node MDA contents were determined at the lowest rate with values of 3.92-3.98 and 2.03-2.26 nmol/ml, respectively in both 2013/14 and 2014/15. On the contrary, winter buds in the 2nd and 3rd node MDA contents were found at higher levels with values of 4.29-4.14 and 3.16-3.24 nmol/ml, respectively in both years results. These results show that there is a linear relationship between tolerance to low temperatures with the change in MDA content in winter buds. Indeed, our observations verified those of Kaya (2011) who reported that *V. vinifera* bud tissues had the highest MDA content after low temperatures. In China, 64 accessions of 18 wild Chinese *Vitis* species and 9 accessions of 7 wild American *Vitis* species have been reported to have a positive correlation with cold tolerance of MDA content (Zhang *et al.*, 2012).

IV. CONCLUSION

Here, we provide evidence for cold resistance of winter buds according to node positions in Karaerik (*V. vinifera* L.) grapevine cultivar after winter cold in 2013/14 and 2014/15. In this study, it was determined that the winter buds at the 4th node on the shoot showed tolerance to low temperatures as much as the winter buds at the 1st node. In general the Karaerik grape variety is spur pruned (node position 2nd) according to node positions. This situation causes an increase in yield losses when spur pruned after years of experiencing the severe winter cold. Therefore, in pruning this is recommended to reduce yield losses by making it from node position 4th after this years. In this way, with standardization of pruning levels for Karaerik grape cultivar can be improved yield and quality. On the other hand change in the rate of MDA contents in the winter buds can be used as indicator of the degree of winter hardiness.

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