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Research Article

Determination of Freezing Points of Secondary Buds in *Vitis vinifera* and *Vitis labrusca*

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Abstract. This study was carried out to determine ability of secondary buds to winter freezing in two *Vitis* species viz. *Vitis vinifera* L. and *Vitis labrusca* L. In 2016 and 2017, one genotype of *V. vinifera* and one genotype of *V. labrusca* were sampled at 3 different periods during winter dormant period. The one-year-old shoots obtained from vineyards in winter and the secondary buds on 4th, 5th and 6th nodes on shoots has been used for freezing test. For freezing test, DTA (Differantial Thermal Analyses) methods has been used and test was started at +4 °C and temperature has been decreased and terminated at -30 °C. According to test results, the highest and the lowest temperatures to start freezing of secondary buds of the genotype belongs to *V. vinifera* was -16.48 °C and -19.49 °C, respectively. For *V. labrusca* genotype, these values were -15.77 °C and -20.99 °C, respectively. The short period of exotherm has been occurred in deep dormancy period for both species. As a result, freezing points of secondary buds varied among species as well years and physiological status of plants. Determining of freezing points of secondary buds in grapes, it is possible to make better prediction of winter injury.

Vitis vinifera and *Vitis labrusca*'da Sekonder Tomurcukların Donma Noktalarının Belirlenmesi

Anahtar kelimeler:
Asma, DTA, sekonder tomurcuk, soğuk zararı.

Özet. Bu çalışma, asma kış gözlerinde ikincil sürgün yatağı olarak bilinen sekonder tomurcukların kış donlarına dayanım kabiliyetlerini tespit etmek amacıyla yapılmıştır. 2016 ve 2017 yıllarında *Vitis vinifera* L. ve *Vitis labrusca* türlerine ait birer genotip üzerinde yürütülen çalışmada örnekler üç farklı dinlenme döneminde alınmıştır. Bağdan alınan bir yaşlı dalların 4., 5. ve 6. boğumlardaki kış gözleri içerisinden sekonder (ikincil) tomurcuklar ayrılarak teste tabi tutulmuştur. Don testi DTA (Diferansiyel Termal Analiz) yöntemi ile yapılmış ve +4 °C'de başlatılan testte sıcaklık kademeli olarak düşürülmüş ve -30 °C'de test sonlandırılmıştır. Yapılan test sonucunda, *Vitis vinifera* türüne ait genotipin sekonder tomurcuklarının donmaya başladığı en yüksek sıcaklığın -16,48 °C, en düşük sıcaklığın ise -19,49 °C'de olduğu, *Vitis labrusca* türüne ait genotipin sekonder tomurcuklarının ise donmaya başladığı sıcaklıkların -15,77 °C ile -20,99 °C'de arasında gerçekleştiği belirlenmiştir. Her iki yılda da *Vitis vinifera* türüne ait sekonder tomurcukların donmaya başladığı en düşük sıcaklıkların dayanıklılığın kazanıldığı derin dinlenme döneminde, *Vitis labrusca* türüne ait sekonder tomurcukların ise dinlenmenin henüz başına tekabül eden aklimasyon döneminde olduğu belirlenmiştir. Ekzoterm süreleri bakımında da her iki tür için de en kısa süreli ekzotermlerin derin dinlenme döneminde gerçekleştiği tespit edilmiştir. Sonuç olarak sekonder tomurcukların donma noktasının türler arasında farklılık gösterdiği gibi, yıldan yıla ve bitkinin içerisinde bulunduğu fizyolojik döneme göre de değişebildiği tespit edilmiştir. Sekonder tomurcukların donma noktalarının belirlenmesi ile asma kış gözlerinde daha doğru don hasarı tespitinin yapılabilmesinin mümkün olacağı kanaati oluşmuştur.

INTRODUCTION

In Turkey, frost events cause great damage to the vineyards for some years, facing producers to significant economic losses. Frost events can be classified into 3 sections: spring frosts, winter frosts and autumn frosts (Uzun, 1996; Çelik *et al*., 1998). However in particular transit regions where the continental climate is dominant vineyards are damaged mostly by low winter temperatures (winter frosts). Winter frosts can cause damage to the winter buds, one-year branches and even the old trunks of the grapevines during the dormant period in the winter months (Küpe and Köse, 2016). Frost resistance or cold hardiness in grapevine is affected mainly by genetic characteristics of the species and varieties, morphological and physiological status of the plant, environmental conditions and cultural practices applied are also affects frost resistance (Eriş, 1982; Rogiers, 1999; Grant *et al.,* 2009). Frost resistance is not constant; even in the same grape variety varies throughout the year or between years and regions (Seyedbagheri and Fallahi, 1994; Sivritepe *et al*., 2001). In the formation of frost damage, the rate of decrease in temperature, the degree of low temperature, the duration of stay at this temperature and the rate of rise of the temperature following frost are effective. In the xylem tissues and dormant buds of most of the deciduous fruit species, water begins to freeze at temperatures lower than 0 ºC and this event is called deep super cooling (Salisbury et *al*., 1992). The degree of freezing of intracellular water, ie temperatures at which deep super cooling occurs, is possible by determining the low temperature exotherms (LTE) at this point. (Andrews *et al*., 1984). LTE works by diagnosing the heat that occurs in ice core formation in the winter buds. As in many woody species, in order to determine the tolerance of tissues to low temperatures in grapevines, natural frost conditions are imitated and controlled frost tests are carried out in the laboratory.

The dormant buds in grapevine are referred to as "compound" buds, as they contain three, internal buds. These are called the primary, secondary and tertiary buds. The primary bud is the main fruiting bud for the following year and generally contains 2-3 inflorescence primordia and 6-12 leaf primordia by the time of winter dormancy, depending on the variety and species. Unfortunately, it is also the least cold hardy of the three buds. The secondary bud may or may not be fruitful, the extent of which is also determined by the variety and species, and may contain 4-6 leaf primordial by the time of winter dormancy. The tertiary bud is vegetative [Keller, 2015; Küpe and Köse, 2019]. The primary bud is less cold hardy becausethe larger organs and more differentiated (specialized) cells reduce its ability to super-cool and heal in response to damage. Secondary buds, which can form efficient summer shoots in case of primary buds in grapevine damaged in winter month for any reason (low winter temperatures, bud necrosis, etc.), are very important for viticulture. These secondary buds are important not only because they contain the final vitality of the grapevine but also contain a certain amount of bunch (Fidan, 1985; Ağaoğlu, 1999; Keller, 2015; Küpe and Köse, 2019).

The main purpose of this study is to determine the freezing points of secondary buds of 2 *Vitis* species and to determine whether there are differences in freezing points between dormant periods among them.

MATERIAL AND METHOD

Plant Material

This study determined the freezing points of secondary buds of genotypes belonging to *Vitis vinifera* and *Vitis labrusca* at long-term low temperatures occurring in different dormant periods (acclimation, resistance and deaclimation) in 2016-2017 and 2017-2018. In the study, a genotype belonging to these species, which exhibit different characters in terms of low temperature tolerance, was preferred. For this purpose, grapevine shoots (cv. Karaerik belongs to V. *vinifera*) was obtained from 25 old vineyards established with the Baran system in Erzincan (Üzümlü) province, which exhibits microclimate in the Eastern Anatolia Region. 53 Pazar 01 genotype of V. *labrusca* specie, which stands out with its resistance to moisture and fungal diseases, was taken from a 15-year-old vineyard, which was trained with a wire training system in Samsun Ondokuz Mayıs University Faculty of Agriculture Application and Research Orchard located in the Black Sea Region. A total of 30 (one year old shoots) including buds were obtained in acclimation, resistance and deaclimation period and were transferred quickly to laboratory.

DTA Testing

Secondary buds are separated from other buds with sharp scalpel and conductive paste is applied to their lower surfaces and placed in the Thermo electric module (TEM) tables for DTA testing. DTA test was started by placing the samples in the TEM tables in the temperature controlled cabin (at +4 ºC) quickly. Samples were tested at a temperature drop rate of 4 ºC hour^{-1}. During the DTA test of all samples, the electrical voltage outputs obtained from TEMs were recorded on the computer (50.000 data sec^{-1}) and the exotherm temperatures were determined by using to the temperature value recorded by a thermocouple in each TEM tray. Test ending temperature was determined as -30 °C (Mills *et al.,* 2006). A total 360 secondary buds each genotype were used for analysis. During the DTA test, the samples were placed on the trays with 1 bud sample in each well.

RESULTS AND DISCUSSION

Table 1 and 2 shows 1st and 2nd year freezing test results of both species. When the secondary buds of Karaerik grape variety sampled in 1st year and were examined, it is seen that the temperatures at which the buds begin to freeze are found to be between -17.14 °C and -19.49 °C. While the highest temperature (-17,14 °C), where the buds started to freeze, was observed in the acclimation period and the lowest temperature (-19.49 °C) was seen in the resistance period. The exotherm duration from the highest to the lowest occurred in the periods of acclimation (2 min), deacclimation (1.42 min) and resistance (1.08 min) (Table 1). In 2nd year the highest temperature where the buds started to freeze, was observed in the deacclimation period (-13.75 °C) and followed by acclimation (-14.04 °C) and resistance period (-18.36 °C), respectively. In 2nd year of experiment, the exotherm duration close the each other of periods and the highest exotherm duration was occurred in deacclimation period (1.33 min) while it was the shortest in resistance period (0.18 min), respectively (Table 1).

Table 2 indicated freezing test results of *V. labrusca* cv. 53 Pazar 01 genotype in 2016 and 2017 years. According to 1st year results, the highest temperatures at which the buds begin to freeze was seen deacclimation period (-15.77 °C) while the lowest temperature was seen at acclimation period (-20.99 °C), respectively. At resistance period, secondary buds formed to start exotherm at -16.52 °C. The longest exotherm duration were obtained from acclimation period (1.30 min), and followed by deacclimation (1.03 min.) and resistance period (0.50 min), respectively (Table 2). In 2nd year the highest temperature where the buds started to freeze, was observed in the deaclimation period (-15.78 °C) and the lowest temperatures were seen in resistance period (-19.67 °C), respectively. In 2nd year of experiment the longest exotherm duration was recorded at acclimation period (1.37 min) while it was the shortest in resistance period (1.02 min), respectively (Table 2).

By evaluating the periods and years together, the temperature values of the secondary buds of the two genotypes, which are formed by exotherm, were given in Table 3. When Table 3 is examined, the exotherms in the secondary buds of the Karaerik grape variety start at -16.93 °C and end at -17.13 °C, and the average exotherm duration is 1.28 min, and the exotherms start at -18.02 °C in the 53 Pazar 01 genotype, it was observed that it ended at -18.15 °C and the average exotherm duration was 1.19 min.

It was observed that the exotherms that took place at the lowest temperature in both years of the study in the Karaerik grape variety were in the deep dormant period. Indeed, in order to plant survive in cold ecologies dominated by the continental climate, freezing is expected to occur at lower temperatures in the deep dormant period, which coincides with the coldest period in winter (Lewitt 1980; Eriş 1995; Ashworth 1998; Mittler 2006). When the secondary buds of the Karaerik grape variety were examined in terms of exotherm durations, similar results were obtained in both study years, and it was understood that the lowest exotherms coincided with the deep dormant period. It is thought that the water capacity decreases as a result of some physiological and metabolic events occurring within the grapevine due to the decrease in temperatures, and accordingly, exotherms are seen at lower temperatures. It has been stated in literature that the water in the tissue varies according to the periods, the water in the grapevine has not reached the lowest level in the acclimation period, the grapevine prepares to budburst in the deacclimation period and the water content increases accordingly (Ağaoğlu, 1999; Çelik, 2008). Buztepe (2016) reported that the maximum tolerance levels of winter buds in Karaerik grape variety occur until the end of November and the first week of February.

In both years of the study, it was seen that the exotherms that occurred at the lowest temperature in the 53 Pazar 01 were in the period of acclimation (entering to dormancy). When the exotherms formed by the secondary buds of the genotype 53 Pazar 01 were examined in terms of their duration, similar results were obtained in both study years, and the shortest term exotherms were found to be in the deep dormant period. It is expected that the shorter exotherm periods in the deep dormant period depend on the low amount of water in the buds. Indeed, water content is known to be inversely related to low temperature resistance (Wolpert and Howell, 1985; Zhang *et al*., 2012). Grant and Dami (2015) found that the tolerance of low temperature in the grapevine buds was maximum in January and decreased in March and April. It is determined that the 53 Pazar 01, which we included in the study, considering that it has higher resistance to colds, generated exotherms at lower temperatures than Karaerik grape variety as expected. It is thought that one of the most important reasons of this situation, which is identified among genotypes, may be due to climate difference in the regions where samples are provided. Considering the periods during which the samples were taken during the year, water content of the sample tissues may be different. Previous studies have demonstrated that the water, carbohydrates, proteins, enzymes, fats and plant nutrient content of plants are effective in the frost resistance level in the frost resistance process (Howell and Shaulis, 1980; Wolpert and Howel, 1985). When the physiological mechanism that is effective on the basis of frost resistance is examined, it is seen that there is a correlation between frost resistance and the water content of the tissues and the form of water (Wolpert and Howel, 1985). When the sample groups evaluated, there were differences between the years and periods of study as well as between genotypes in terms of exotherms starting and ending temperatures in secondary buds and exotherm durations as well. It is thought that this situation may be largely due to genetic structure, but climatic factors (effective temperature sum, precipitation, exposure to sun etc.) and cultural treatments (irrigation, fertilization, pruning, etc.) may also have an effect on bud development. Wolf and Pool (1987), found that exotherms occur between -9 ºC and -16 ºC on the Chardonnay grape variety in order to determine the factors that may affect the determination of exotherms in the DTA method. Also, Clark et *al*. (1996) used two different grape varieties of *Vitis labrusca*, reported that low temperature exotherms occurred up to -23.4 ºC.

Table 1. Exotherm data of secondary buds in Karaerik grape variety (*V. vinifera).*
Çizelge 1. Karaerik (V.vinifera) üzüm çeşidinde sekonder tomurcukların ekzoterm verileri.

Specie	Year	Period	Average exotherm starting temperature (ºC)	Average exotherm ending temperature (ºC)	Average exotherm duration (min)
Karaerik (*V.vinifera*)	**1st**	**Acclimation**	-17.14	-17.27	2.00
		Resistance	-19.49	-19.50	1.08
		Deaclimation	-18.78	-18.85	1.42
	2nd	**Acclimation**	-14.04	-14.33	1.23
		Resistance	-18.36	-18.77	0.18
		Deaclimation	-13.75	-13.94	1.33

Table 2. Exotherm data of secondary buds in 53 Pazar 01 genotype (*V. labrusca*).
Çizelge 2. 53 Pazar 01 genotipinde (V. labrusca) sekonder tomurcukların ekzoterm verileri.

Specie	Year	Period	Average exotherm starting temperature (ºC)	Average exotherm ending temperature (ºC)	Average exotherm duration (min)
53 Pazar 01 (*V. labrusca*)	**1st**	**Acclimation**	-20.99	-21.10	1.30
		Resistance	-16.45	-16.52	0.50
		Deaclimation	-15.77	-15.82	1.03
	2nd	**Acclimation**	-19.67	-19.75	1.37
		Resistance	-19.41	-19.50	1.02
		Deaclimation	-15.78	-16.02	1.09

Table 3. In secondary buds average exotherm data with years, genotypes and periods evaluated together.
Çizelge 3. Sekonder tomurcuklarda yıllar, genotipler ve dönemlerin birlikte değerlendirilidiği ortalama ekzoterm verileri.

Specie	Average exotherm starting temperature (ºC)	Average exotherm ending temperature (ºC)	Average exotherm duration (min)
Karaerik (*V. vinifera*)	-16.93	-17.13	1.28
53 Pazar 01 (*V. labrusca*)	-18.02	-18.15	1.19

CONCLUSION

Consequently, in this study, if primary buds do not survive for any reason, secondary buds, which are used as secondary shoot beds and which can form efficient summer shoots, were determined by determining low temperature exotherms (LTE) against low temperatures. In addition, by determining the exotherm durations of secondary buds, an idea was obtained about the water contents of the buds periodically. As a result of the study, it was determined that the freezing points of the secondary buds can change according to the genetic structure (species and variety) and the dormant period in which they are located. Although the low-temperature resistance levels of primary buds, known as the main shoot bed in grapevine, have been determined in many studies, no studies have been conducted that demonstrate the frost resistance levels of secondary buds by a reliable method such as the DTA method. In our study, we believe that it will be possible to determine the freezing points of secondary buds and to determine more accurate frost damage in the grapevine winter buds.

CONFLICT OF INTEREST

The author declare that they have no conflict of interest.

DECLARATION OF AUTHOR CONTRIBUTION

Conceptualization, data curation, formal analysis, methodology, visualization, writing original draft writing review and editing by co-author Muhammed Küpe.

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