



The Relationship Between Bud Size and Exotherm Formation in Dormant Buds of Grapevine

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ABSTRACT: This study was conducted to determine the relationship between bud size and low temperature exotherm (LTE) formation in dormant period of grapevines. For this purpose, primary and secondary buds of Karaerik (*Vitis vinifera* L.) and 53 Pazar 01 (*Vitis labrusca*) genotypes which are known to have differences between the bud structure and size, were examined separately. In the study carried out in 2016-2017 and 2017-2018 dormant periods, low temperature exotherms were determined by Differential Thermal Analysis (DTA) method in order to determine the freezing point of the buds. Dormant buds of grapevine size were determined by microscopic imaging of the sections taken after histological procedures. It was demonstrated by the DTA test that the buds with different histological structures had different character and number of LTE. In this study, it was determined that bud samples belonging to *Vitis vinifera* L. form exotherm at lower temperatures than bud samples belonging to *Vitis labrusca* species. For both genotypes, it was determined by the histological determinations that the primary buds had a larger structure than the secondary buds. In the study, it was found that the buds with an area less than 0.010 mm² did not produce LTE. As a result of the study, it was found that the bud structure and size were effective on LTE formation of grapevine dormant buds.

Keywords: Bud structure, Cold tolerance, Low temperature exotherms, Grapevine

Asma Kış Gözlerinde Tomurcuk Büyüklüğü ile Düşük Sıcaklık Ekzotermi Arasındaki İlişki

ÖZ: Bu çalışma asma kış gözlerinde tomurcuk büyüklüğü ile düşük sıcaklık ekzotermi oluşumu arasındaki ilişkiyi ortaya koymak amacıyla yapılmıştır. Bu amaçla tomurcuk yapıları ve büyüklükleri bakımından farklı olduğu bilinen Karaerik (*Vitis vinifera* L.) ve 53 Pazar 01 (*Vitis labrusca*) genotiplerinin primer ve sekonder tomurcukları ayrı ayrı incelenmiştir. 2016-2017 ve 2017-2018 kış dinlenme döneminde yürütülen çalışmada, tomurcukların donma noktasını belirlemek için kullanılan düşük sıcaklık ekzotermi diferansiyel termal analiz (DTA) yöntemi ile belirlenmiştir. Asma kış gözlerinde dormant tomurcukların büyüklükleri, histolojik işlemlerden sonra alınan kesitlerin mikroskopik olarak incelenmesi ile belirlenmiştir. Yapılan DTA testi ile farklı histolojik yapıya sahip tomurcukların farklı sayıda ve karakterde ekzoterm meydana getirdikleri görülmüştür. Çalışmada *Vitis vinifera* L. türüne ait örneklerin *Vitis labrusca* türüne ait örneklerle kıyasla daha düşük sıcaklıklarda ekzoterm oluşturdukları tespit edilmiştir. Yapılan histolojik tespitlerde her iki genotipin primer tomurcuklarının sekonder tomurcuklarından yapısal olarak daha büyük olduğu görülmüştür. Çalışmada 0.010 mm²'den daha küçük alana sahip tomurcukların ekzoterm meydana getirmediği tespit edilmiştir. Çalışmanın sonucunda asma kış gözlerinin ekzoterm oluşturması üzerine tomurcuk yapısı ve büyüklüğünün etkili olduğu belirlenmiştir.

Anahtar Kelimeler: Tomurcuk yapısı, Don toleransı, Düşük sıcaklık ekzotermi, Asma

INTRODUCTION

In addition to the many benefits of grape on human health, it has a multifaceted effect on human life as it has alternative evaluation opportunities. Climatic conditions are important for an economic viticulture. Although grapevine is a warm-temperate plant, it can be grown in cooler climates due to its high adaptation capability (Çelik et al., 1998). It is known that the grapevine, which is spread over a wide geography (Wample et al., 1991), is significantly damaged by low winter temperatures in areas with continental climate (Zhang et al., 2012). The low temperatures experienced during the winter months cause damage to the dormant buds, one-year

old shoots and even the older trunks of the vines during the dormant period (Fennel, 2004). Low temperatures below freezing point often cause severe damage to the dormant buds of the grapevine.

Due to these damages, yield and quality losses are experienced, and even the complete death of the plant may occur (Linden, 2002). Low temperatures in winter are important stress factors that affect the yield and quality of the vine as well as determine the distribution of species on the earth and limit their spread (Khanizadeh et al., 2005; Zhang et al., 2012).

Grapevine tissue and organs after frost damage show differences in their ability to tolerate low

temperatures. In addition to the differences between tissues and organs, seasonal differences make it difficult to explain the mechanism of frost tolerance. In many studies on grapevine winter buds, buds capable of forming shoots have been reported to be ranked as primary, secondary and tertiary buds from the most sensitive to the most resistant in terms of sensitivity to low temperatures. In many studies on vine winter buds, it has been reported that the most sensitive to most resistant buds are primary, secondary and tertiary buds, respectively (Fennell and Hoover, 1991; Hemstead and Luby, 2000; Wample et al., 2001; Grant and Dami, 2015).

Different frost tests have been developed in order to determine the factors affecting frost damage, to understand the frost tolerance mechanism of plants and to make accurate damage estimation (Yadava et al., 1978; Bolat, 1997; Küpe, 2012). The usually method used today for deciduous and super cooling species is Differential Thermal Analysis (DTA) (Quamme, 1986; Wample et al., 1990; Fennel, 2004; Mills et al., 2006; Ferguson et al., 2011; 2014; Gao et al., 2014; Salazar-Gutierrez et al., 2014).

In their studies in grapevine winter buds, some researchers concluded that the first exotherm may have been the result of death of the primary bud because the frost tolerance of the primary buds was lower than the secondary and tertiary buds. However, no studies have been conducted to confirm these assumptions. In addition, Wolf and Cook (1994) in their study on the assumption that the primary bud exotherms are larger in vine buds and occur at higher temperatures, they tried to separate primary bud exotherms from secondary bud exotherms. Moreover, the researchers stated that the first and large exotherms determined in the buds with primary bud were suspected that they could belong to the secondary bud and that the exotherms could be misleading.

In this study, after determining the starting temperatures of LTE in primary and secondary buds in grapevine dormant buds, it was studied to determine whether the bud size had an effect on exotherm formation. In this way, it is aimed to contribute to a certain extent to explain the freezing mechanism that occurs in grapevine winter buds and is known to be influenced by many factors. In addition, it can be possible to correctly select the one-year old shoots and buds to be left according to the growth force and size during pruning in the vineyard, especially in hard winter regions.

MATERIAL AND METHOD

Material

In this study, LTE observed in dormant buds of two different genotypes by thermal analysis method were associated with bud size. In the study, one

genotype belonging to two species which exhibited different characters in terms of low temperature tolerance was preferred. Karaerik (*V. vinifera*) grape variety with partially low frost tolerance and type of 53 Pazar 01 (*V. labrusca*) with medium-high frost tolerance were used (Odabaş 1976). Karaerik grape variety shoots were obtained from 25 years old own-rooted vines grown in Erzincan. All vines were spaced 2.5 m apart in north-south oriented rows that were 2.0 m intervals. The vines were spur pruned and Baran system-trained. The height of head was 0.2 m above ground. The samples belonging to type 53 Pazar 01 (*V. labrusca*) (Çelik et al., 2008) selected by selection from the Black Sea Region are supplied from Samsun province. This vineyard consists of 15-years-old vines, which are formed by a trellising system and grown own roots. The height of head was 1 m above ground. In the study, the 4th, 5th and 6th nodes (middle buds) of an elderly shoots from the bottom were used.

Method

Samples were carried to the laboratory as soon as possible in polyethylene bags to prevent moisture loss (Kovacs et al., 2002; Mills et al., 2006; Salazar-Gutierrez et al., 2014). Stronger developing primary buds were obtained by removing the secondary and tertiary buds of the full bud from the middle part of shoots by scalpel. Secondary buds, which are smaller in structure than the primary bud, were obtained by removing the primary and tertiary buds of a full winter bud taken from the middle part of shoots. Prepared samples were placed in thermoelectric module (TEM) trays, 1 sample for each box (bud) was applied to the bottom of the thermal conductive paste and then made ready for testing. Samples were tested at a temperature drop rate of 4 °C per hour. During the DTA test, the electrical voltage outputs obtained from TEMs were recorded to the computer (50000 data / sec) and the exotherm temperatures were determined by means of the temperature value recorded by a thermocouple in each TEM tray. Test end temperature was -30 °C (Mills et al., 2006).

After the DTA tests, each of the samples were taken into separate bottles and 2 ml of fixation liquid (5 ml formaldehyde, 5 ml glacial acetic acid, 90 ml 70% alcohol) were placed on the desiccator and vacuuming was performed periodically. Paraffin impregnation process (24 hours at 60 °C) was performed on the samples after fixation was completed. Samples removed from the oven after 24 hours (through liquid paraffin) were embedded in paraffin so that longitudinal sections could be taken. The paraffin blocks where the specimens were buried were stored for 24 hours in a -20 °C freezer. Samples removed from the freezer were stored at room temperature (22-24 °C) for 5 minutes. Afterwards,

longitudinal sections of thicknesses ranging from 08-12 microns were taken by rotary microtome (Odabaş, 1976). After the sections were kept in water bath, they were placed on slide and kept in oven at 60 °C for one hour. The samples were passed through a series of xylol, alcohol and distilled water to remove the dissolved paraffin from the tissue. A drop of Toluidine blue was dropped onto each slide. After waiting 5 minutes, the sections were washed with water and the coverslip was made ready for examination. In histological examinations, images were transferred to the computer by a microscope integrated camera (Made in Hong Kong in 2002, moticam 480 model device). Measurements were made on photographs taken by a computer program (Motic Images Plus 2.0 program made in Hong Kong in 2010). During measurements, bud width, length and areas were determined while taking these measurements from the widest points.

In this study, some statistical analyses were performed to reveal the relationship between the size

of vine buds and LTE. The study was conducted with 3 replicates for each sample group (primary and secondary buds) and 27 samples per repetition. In a two-year study on two genotypes, a total of 648 dormant buds were evaluated. The relationship between LTE and bud area was tried to be explained by enter regression analysis in the SPSS 20 for Windows (Arkkelin, 2014).

RESULTS

In the study, the relationship between the cold tolerance levels and the size of primary and secondary buds in the grapevine winter (dormant) buds was revealed. In determining this relationship, LTE numbers in the primary and secondary buds of two different genotypes in dormant buds and the temperatures at which these exotherms started to appear were determined (Table 1). The bud width, length and area were determined by histological examinations performed in the same sample groups (Table 2).

Table 1. Average DTA results of genotypes

Genotype	Year	Exotherm Characters	Primary Bud	Secondary Bud
Karaerik (<i>V. vinifera</i>)	First year	Average LTE Start Temperature (°C)	-19.06	-18.47
		Average Number of LTE (pcs)	1.19	1.13
	Second year	Average LTE Start Temperature (°C)	-17.96	-15.38
		Average Number of LTE (pcs)	1.04	1.29
53 Pazar 01 (<i>V. labrusca</i>)	First year	Average LTE Start Temperature (°C)	-14.96	-17.74
		Average Number of LTE (pcs)	1.04	1.29
	Second year	Average LTE Start Temperature (°C)	-16.32	-18.29
		Average Number of LTE (pcs)	1.05	1.18

Table 2. Mean bud sizes of genotypes

Genotype	Year	Pr width (mm)	Pr length (mm)	Pr area (mm ²)	Sc width (mm)	Sc length (mm)	Sc area (mm ²)
Karaerik (<i>V. vinifera</i>)	First year	0.30	0.34	0.102	0.27	0.30	0.081
	Second year	0.27	0.33	0.089	0.25	0.30	0.075
53 Pazar 01 (<i>V. labrusca</i>)	First year	0.30	0.39	0.117	0.24	0.34	0.082
	Second year	0.29	0.36	0.104	0.22	0.29	0.29

Pr: Primary Bud, **Sc:** Secondary Bud

Table 3. Regression analysis of the relationship between bud area and LTE number when years, genotypes and bud types are evaluated together

	Independent variables	Constant value	Regression coefficient	R ²	Model F Value	N
All Sample Groups	Bud area	0.923	4.121**	0.079	55.387**	648

** : 0.01 at level is important

It was found that LTE could not be detected in many tertiary bud structures when tertiary buds were

subjected to DTA test alone. Based on this information, it was thought that the size of the

structure examined could have an effect on exotherm formation. In Table 3, where years and genotypes were evaluated together, enter regression analysis of the relationship between bud area (width x length) and LTE exotherm number is presented. When the Table 3 is examined, it is seen that the relationship

between bud size and low temperature exotherm (LTE) is significant ($p < 0.01$). The exotherm number increased due to the increase in bud size was revealed in 648 samples examined. In addition, the correlation between bud area and low temperature exotherm was found as 28.1% ($r = 0.281$).

Table 4. Regression analysis of the relationship between bud area and LTE number of genotypes

Genotype	Independent variables	Constant value	Regression coefficient	R ²	Model F Value	N
Karaerik (<i>V. vinifera</i>)	Bud area	1.224	2.492**	0.027	8.468**	309
53 Pazar 01 (<i>V. labrusca</i>)	Bud area	0.692	5.398**	0.144	56.855**	339

** : 0.01 at level is important

The relationship between bud area and exotherm number of genotypes is determined separately in Table 4. It was determined that the number of LTE also increased due to the increase in bud area of Karaerik grape variety and this relationship was significant ($p < 0.01$). Genotype of 53 Pazar 01, it is seen that this relationship is positively

important as in the Karaerik grape variety. When the regression coefficients are examined according to Table 4, it is seen that this relationship is stronger in the 53 Pazar 01 genotype. In addition, the correlation between bud area and LTE number was found to be 16.4% in Karaerik grape variety and 38% in 53 Pazar 01 genotype.

Table 5. The relationship between the number of buds with an area less than 0.010 mm² and the number of LTEs

	Independent variables	Constant value	Regression coefficient	R ²	Model F Value	N
All Sample Groups	Bud area	2.580	-14.250 ns	0.106	3.435 ns	88

ns: not important

Table 6. The relationship between the number of buds with an area greater than 0.010 mm² and the number of LTEs

	Independent variables	Constant value	Regression coefficient	R ²	Model F Value	N
All Sample Groups	Bud area	1.071	3.563**	0.037	7.459**	560

0.01 at level is important

When years, genotypes and bud types (primary and secondary) were evaluated together, it was shown in Table 3 that the relationship between bud area and LTE number was significant ($p < 0.01$) according to regression analysis. In order to explain this relationship in more detail, the buds with an area less than 0.010 mm² and LTE numbers were subjected to enter regression analysis in Table 5. According to the results of the analysis, it was found that the buds having an area less than 0.010 mm² had no effect on LTE exotherm formation.

When all samples were evaluated together, it was found that there was a statistically significant ($p < 0.01$) relationship between the bud with an area greater than 0.010 mm² and the number of LTE exotherms according to the results of enter regression

analysis. In other words, it is clearly shown that structures with a grapevine bud area greater than 0.010 mm² form exotherms (Table 6).

DISCUSSION AND CONCLUSION

According to DTA test results, low temperature exotherms were observed between -11.72 °C and -24.12 °C in Karaerik grape variety and exotherms formed at -16.63 °C on average. In the genotype of 53 Pazar 01, LTE were observed between -11.62 °C and -20.45 °C and exotherms were realized at -15.89 °C on average. Indeed, Wolf and Pool (1987), Chardonnay grape variety, exotherm determined by the DTA method reported that between -9 °C and -16 °C. Also, Clark et al. (1996) in their study on two different grape varieties belonging to the *Vitis*

labrusca species, LTE were found to be seen up to -23.4 °C.

In our study, contrary to the present literature, genotype of 53 Pazar 01 (*Vitis labrusca*) produced exotherm at higher temperatures generally than genotype of Karaerik (*Vitis vinifera*). We believe that one of the most important reasons for this situation, which is identified among genotypes, may be caused by climate differences in the regions where samples are provided. It is known that frost tolerance of species and varieties may change according to ecology. Indeed, Bordelon et al. (1997), stated that the tolerance of the dormant buds to low temperatures may vary even among the same varieties grown in different regions during the same period. Furthermore, it should not be ignored that plants grown in colder ecologies may have higher frost tolerance than plants grown in hot ecology (Lewitt, 1980; Eriş, 1995; Ashworth, 1998; Mittler, 2006).

According to the two-years data obtained in the study, when all sample groups were evaluated together, the relationship between bud area and LTE number was found to be significant ($p < 0.01$). In order to explain this relationship in more detail, regression analysis was performed to determine the relationship between LTE numbers and buds with an area less than 0.010 mm². According to the results of the analysis, it was found that the buds having area less than 0.010 mm² had no effect on LTE exotherm formation (Table 5). In addition, the fact that the exotherm cannot be detected in these small buds which are quite small in the DTA test of the tertiary buds has also helped us to explain the relationship between bud area and LTE number.

We believe that the detection of exotherms in buds above a certain size may be related to the amount and content of tissue water in the bud. It should not be ignored that the amount of water and bud components of the examined buds may be different. Although it is stated that the amount of water does not change the freezing point, it is known that this applies to pure water. As a matter of fact, when the purity of a substance deteriorates, the freezing point changes and the freezing point decreases with increasing amount of mineral matter in the tissue (Küden et al., 1998). In addition, soluble sugar in tissue structure, proline content and plant growth regulators were found to be effective on the occurrence of freezing (Yang et al., 1982).

As a result, it was determined that the starting temperatures of LTE in primary and secondary buds in vine dormant buds differed according to genotypes. In addition, there was a significant relationship between the exotherm formation and the size of primary and secondary buds in vine dormant buds. In other words, it has been found that the buds

below a certain size do not form exotherm. In this study, it was found that the size of the bud was effective on the frost tolerance mechanism which is known to be influenced by many factors. In the DTA studies to be carried out in dormant buds of grapevine, it should not be ignored that not all exotherm buds are dead and that exotherm formation may be related to bud size. Developing DTA techniques and making more comprehensive researches and revealing all the other factors that may cause exotherm will allow the studies to be more sensitive and reliable.

Statement of Conflict of Interest

The authors declare that they have no conflict of interest.

Authors' Contribution

This article is derived from part of the corresponding author's doctoral work. The second author was the consultant of the doctoral study.

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