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Research Article

Determination of the Relationship Between the Number of Clusters and Primary Bud Size in *Vitis vinifera* and *Vitis labrusca* Grapevines by Histological Sectioning Method

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Abstract

This study was conducted to reveal the relationship between the number of clusters and primary buds size in dormant grapevine winter buds. In addition, it has been tried to determine how the size of the primary bud and the cluster primordials in it change according to the position on one-year-old shoots. In this study, *Vitis vinifera* L. cv. 'Karaerik' and *Vitis labrusca* L. cv. 'Ülkemiz' varieties was used. Primary buds are separated from other shoot beds (secondary and tertiary buds) with scalpel, fixation, vacuuming, paraffin impregnation, paraffin embedding, freezing, sectioning and tissue staining were performed, respectively. The stained samples were examined microscopically, images were taken and these images were transferred to the computer and histological analyzes was made. In the study, it was determined that bud sizes including three clusters were statistically different ($p < 0.05$) from other bud sizes (without clusters, one cluster and two clusters of buds). It was determined that there were statistical differences between cultivars in terms of both bud size and the number of cluster. In addition, it was determined that the middle (0.77) buds of the 'Karaerik' variety contained more clusters than the basal (0.66) and apical (0.59) buds, whereas the apical (1.19) buds of 'Ülkemiz' variety contained more clusters than the basal (0.88) and middle (0.98) buds. It is very important to know the structural features of the buds in order to make the right pruning application suitable for the variety.

Keywords: Bud histology, grapevine, primary bud, cluster number

Asmalarda Salkım Sayısı ile Primer Tomurcuk Büyüklüğü Arasındaki İlişkinin Histolojik Kesit Alma Yöntemiyle Belirlenmesi

Öz

Bu çalışma dormant haldeki asma kış gözü içerisindeki primer tomurcukların büyüklüğü ve salkım sayıları arasındaki ilişkiyi açıklamak için yapılmıştır. Ayrıca primer tomurcuk büyüklüğü ve salkım taslaklarının bir yaşlı sürgün üzerindeki pozisyonlarına göre nasıl değiştiği belirlenmeye çalışılmıştır. Çalışmada *Vitis vinifera* L. cv. 'Karaerik' ve *Vitis labrusca* L. cv. 'Ülkemiz' çeşitleri kullanılmıştır. Primer tomurcuklar diğer sürgün yataklarından (sekonder ve tersiyer tomurcuklar) bir bistüri yardımı ile ayrılarak sırasıyla fiksasyon, vakumlama, parafin emdirme, parafine gömme, dondurma, kesit alma ve doku boyama işlemleri yapılmıştır. Boyanmış örnekler mikroskobik olarak incelenip görüntüleri alınmış ve bu görüntüler bilgisayara aktarılarak histolojik incelemeler yapılmıştır. Çalışmada, üç salkım içeren tomurcuk boyutlarının diğer tomurcuklardan (salkımsız, bir salkımlı ve iki salkımlı) istatistiksel olarak farklı olduğu ($p < 0.05$) belirlenmiştir. Çeşitler arasında hem tomurcuk büyüklüğü hem de salkım sayısı bakımından istatistiksel olarak farklılıklar olduğu belirlenmiştir. Ayrıca Karaerik üzüm çeşidinin orta (0.77) tomurcuklarının dip (0.66) ve uç (0.59) tomurcuklara göre daha fazla salkım içerdiği, Ülkemiz üzüm çeşidinin ise uç (1.19) tomurcuklarının dip (0.88) ve orta (0.98) tomurcuklara göre daha fazla salkım içerdiği belirlenmiştir. Çeşide uygun doğru budama uygulamasının yapılabilmesi için, tomurcukların yapısal özelliklerinin bilinmesi oldukça önemlidir.

Anahtar Kelimeler: Tomurcuk histolojisi, asma, primer tomurcuk, salkım sayısı

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1. Introduction

Horticultural crops include a diverse array of crops comprising fruits, grapes, vegetables, nuts, flowers, aromatic and medicinal plants. They provide nutritional, medicinal, and aesthetic benefits to mankind for centuries [1-5]. They are genetically very diverse group and including cultivars, accessions, genotypes, types etc. [6-10].

Grapevine dormant buds are quite complex due to their anatomical and morphological structure [11, 12]. Dormant buds various shapes and appearances, but they are generally large and angular [13]. They contain leaf scales, shoots and clusters and take their final shape in autumn [14]. Dormant buds on the shoots are closely related to the yield of vine [15, 16]. Dormant buds occur in leaf apical on summer shoots. The dormant buds that complete their development in the spring and spend the winter resting usually consist of more than one bud [17]. These are called the primary, secondary and tertiary buds. The primary bud is the main fruiting bud for the following year [18].

One of the shoot apical is in the middle and the other two are on the sides. The scales of the middle shoot apical (primary buds) are larger and the organs are better developed. With the beginning of the vegetation period, the shoot apical in the middle develops and forms the summer shoot. In the shoot apical called the secondary bud, the organs are weaker than the primary buds and in some cases they may form clusters. The growth cone, which is called the tertiary bud and located on the upper part of the middle shoot apical, is more primitive than the other two shoot axillaries and does not contain clusters. The growth of the dormant buds (shooting) is seen in March-April in temperate climates and usually as the growth of the primary bud, which is the apical middle shoot that has completed its development [16, 18]. If this primary bud is damaged due to late spring frosts, diseases and pests or mechanical impacts, the secondary bud development, and if secondary bud is damaged, the tertiary bud it begins to develop. However, the most important bud in terms of grape yield in viticulture is the primary buds [19, 20]. In the grapevine, 0-4 cluster primordium can occur in the primary buds. Many studies have shown that the total number of clusters in a shoot apical varies according to the age of the grapevine, the nutritional status, genetic structure and the position of the dormant buds on a year old shoot [21-23].

It is stated that the number of cluster is high in the basal and middle buds in the seeded varieties and in the apical buds in the seedless varieties. In addition, it has been observed that the number of cluster in the middle node is higher than the other nodes in most of the table grape varieties [18, 24]. Bud productivity for grapes is closely related to the growth, development and performance of the vine during the vegetation period one year ago. The main purpose of this study is to reveal the relationship between the size of the primary buds and their productivity. In addition, it has been tried to determine how the size of the primary bud and the cluster primordiums in it change according to the position on a year old shoot. In this study conducted on two grape cultivars, (*Vitis vinifera* L. cv. ‘Karaerik’ and *Vitis labrusca* L. cv. 'Ülkemiz') the

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size of the primary buds and the number of cluster primordiums within the buds were determined by histological examinations. By determining the cluster primordium in the primary buds, it will be possible to make an accurate yield estimation in the early period in the vineyards. Depending on the relationship between the structure of the primary buds and their productivity, the traditional pruning method may need to be updated.

2. Materials and Methods

It is known that there are structural differences between grapevine specie, varieties and cultivars [25-28]. In the study, two different cultivars with high economic value, which differ in terms of genetic, morphological, physiological characteristics and adaptability, were preferred. Samples of the 'Karaerik' grape cultivar of *V. vinifera* with large berries consumed for table (fresh) are taken from within the borders of Üzümlü District of Erzincan Province. In Erzincan, a 25-year-old vineyard at 1190 m altitude in Baran trained system was used. Examples of genotype 'Ülkemiz' belonging to V. labrusca specie, which stands out with its resistance to high humidity and low temperatures [29-31]. It was obtained from Samsun Ondokuz Mayıs University. 'Ülkemiz' grape cultivar were formed with a wired training system at 195 m altitude, shaped like a cord, and were obtained from 15 aged vineyards.

During the winter of 2016-2017 samples taken from Erzincan and from Samsun were transported to the laboratory in polyethylene protection bags for histological testing. In the study carried out on two different cultivars, dormant buds in three different positions (basal nodes, middle nodes and apical nodes) were examined. The study was planned as three repetitions for each position and three samples for each repetition. In the study, 1st, 2nd and 3rd nodes are considered basal buds, 4th, 5th and 6th nodes are considered as middle buds, and 7th, 8th and 9th nodes are considered as apical buds. Efficiency values in different buds in grapevine can be determined using different methods such as binocular microscopy, sectioning from dormant buds with microtome, shooting test, counting the somacs by forcing single-bud cuttings, or determining the somacs on the long pruned annual shoots [32, 33]. In this study, we used the microtome sectioning method for histalogical examination.

2.1. Histological studies

Since the number of cluster primordiums in the buds changes according to the position of dormant buds on one year old shoot [15, 34], primary buds were taken from different positions. Histological examination of the cluster primordiums on the primary buds was made according to Odabas [15] (Figure 1).

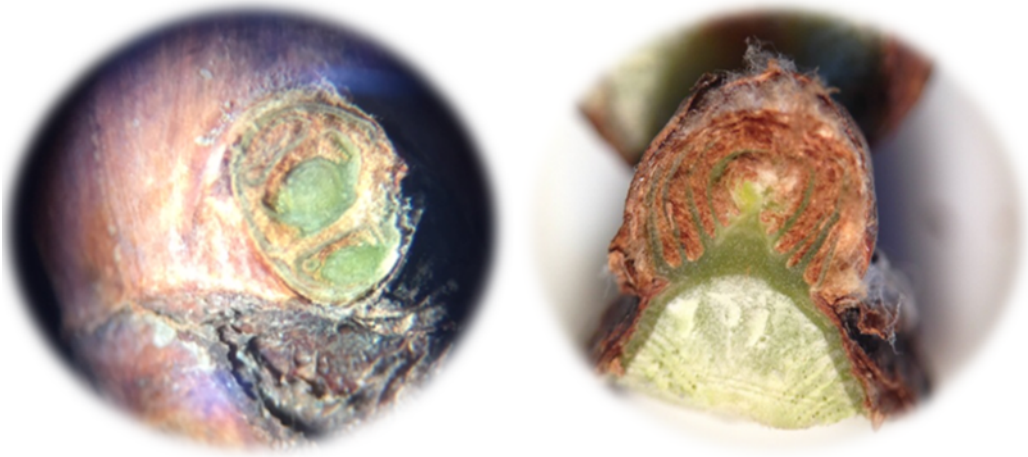

Figure 1. Cross-sectional view of the primary bud (original).

Primary buds separated from secondary and tertiary buds in the dormant buds with the help of a scalpel were placed in separate bottles. In order to soften the primary bud tissue, 2 mL of fixation liquid consisting of 5 mL formaldehyde, 5 mL glacial acetic acid, 90 mL 70% alcohol was added on each sample and placed in the desiccator and vacuuming was performed at certain intervals. According to Odabas (1976) samples was kept in a solution consisting of 50 mL of water + 40 mL of ethyl alcohol (95%) + 10 mL of tertiary butyl alcohol for two hours. After the fixation process was completed, paraffin was added to completely cover the samples taken into the empty bottles, and paraffin impregnation was performed by keeping them in the oven at 60 °C for 24 hours. Samples removed from the oven at the end of 24 hours (through liquid paraffin) were embedded in paraffin. The paraffin blocks in which the samples are embedded were kept in the freezer at -20 ºC for 24 hours. Samples taken out of the freezer are stored at room temperature (22-24 ºC) for 5 minutes. After waiting for a period of time, longitudinal sections were taken with the help of a rotary microtome in thicknesses varying between 8-12 microns depending on the fragmentation of the tissues. Since the buds are at different depths in the buds, care has been taken to take sections from different depths. The sections taken were laid in a gelatin-poured hot water bath (30 ºC).

Intact tissues were selected, placed on a slide and labeled. The samples taken on the slide were heat treated in the oven at 60-70 ºC for 1 h, and then the paraffin on them was melted. To remove the melted paraffin from the tissue in the samples taken from the oven, it was kept in xylol, ethyl alcohol and distilled water for 5 minutes, respectively.

After waiting for the water on the slide to dry, a drop of Toluidine blue was dropped on each section sample and after waiting for 5 minutes, the sections were washed with water and allowed to dry. The coverslip was adhered by dropping 1 drop-holding gel on each of the dried sections and the sections were conduct ready for examination under a light microscope.

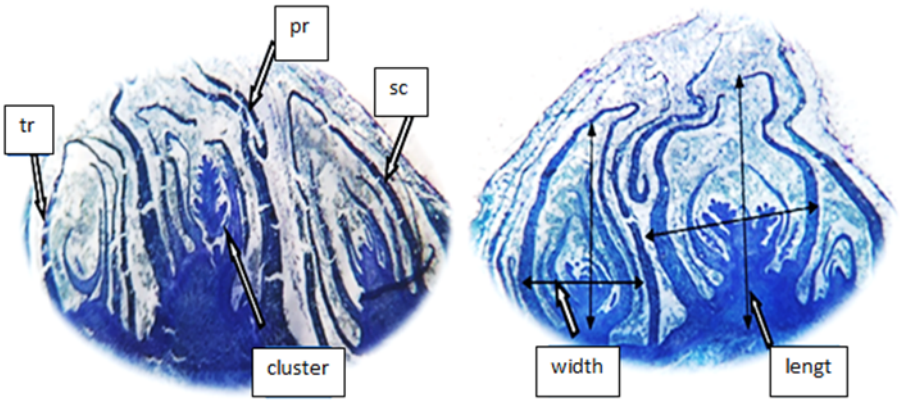

Figure 2. Method of determining the position, width and length of buds in histological observations (pr: primary bud, sc: secondary bud, tr: tertiary bud).

Histological examinations, computer transferred to be imaged with the help of a camera (moticam 480) integrated into the microscope. With the help of the program installed on the computer (motic images 2010), the examinations of the tissues were made. To make examinations, the number of cluster primordial in the primary bud and the size of the buds (width x height). Firstly bud width and length were measured and by using these two values is expressed as the size of the bud in mm^2. While observing the bud and its size, attention has been paid to make these measurements from the widest points of the buds (Figure 2).

2.2. Statistical Analysis

Duncan test was conducted to reveal the relationship between the size and productivity of primary buds in dormant buds. In the study, the number of clusters was divided into four different groups as buds with no cluster and one, two and three clusters in the bud, and whether there was a difference between the sizes of these buds was determined on a variety basis. In addition, the change of bud size and cluster number according to the positions of the genotypes was demonstrated by [35]. In the study, each group was planned to have three repetitions and 20 samples per repetition.

3. Results and Discussion

Many different studies have been carried out to determine the productivity of grape varieties. While determining the productivity of grapevines, methods of maintaining in the greenhouse, maintaining in the vineyard and examining under microscope conditions are applied [36-39]. In this study, these cross-section images were examined under a binocular microscope after the primary buds were sectioned on the microtome.

The Duncan test results, that revealed the relationship between the size of the primary buds and their productivity (number of clusters) in dormant buds is presented in Table 1. when the genotypes are evaluated together, it was determined that there is a statistically significant difference ($p < 0.05$) between the size of the buds that include three clusters and include two clusters, one cluster or without clusters. When Table 1 is examined, it is understood that the

buds without clusters are the smallest, while the buds with three clusters have the largest structure.

It was determined that there is a statistically significant difference ($p < 0.05$) between genotypes in terms of the size of the primary buds. In the 'Karaerik' grape variety, the buds containing two and three clusters were placed statistically in the same group ($p > 0.05$) and they were differed from the buds without clusters and one cluster in terms of bud size. 'Karaerik' grape variety, it has been determined that there are buds without clusters, buds with one cluster, buds with two clusters and buds with three clusters in order from the smallest to the largest in terms of the size of the buds. It was determined that there is a statistically significant difference ($p < 0.05$) between the buds with three clusters in the 'Ülkemiz' grape variety and the buds without clusters, one cluster and two clusters. Similar to the 'Ülkemiz' grape variety, it was determined that there are buds without clusters, buds with one cluster, buds with two clusters and buds with three clusters in order from the smallest to the largest in bud size (Table 1).

Table 1. The number of clusters and bud size in the grape varieties.

Cluster Number	Primary bud size (mm^2)		General Mean
	'Karaerik'	'Ülkemiz'	
	*	*	***
Without cluster	0.065±0.028 ^{b}	0.073±0.031^{b}	0.069±0.029 ^{B}
One cluster	0.070±0.028 ^{b}	0.078±0.030 ^{b}	0.074±0.029 ^{B}
Two clusters	0.078±0.028 ^{ab}	0.084±0.037 ^{b}	0.081±0.034 ^{B}
Three clusters	0.095±0.014 ^{a}	0.114±0.034 ^{a}	0.105±0.026 ^{A}

a, b: Mean values of cultivars with different superscripts are significantly different;
A, B: General mean values with different superscripts are significantly different;
*: $p < 0.05$, ***: $p < 0.001$

The Duncan Test, which was made to determine whether the number of clusters changes according to the position of the primary buds in dormant buds, is presented in Table 2. When the genotypes were evaluated together, no statistically significant difference was found between the basal, middle and apical buds in terms of cluster number. It was determined that there is a statistically significant ($p < 0.05$) difference between the genotypes in terms of the number of clusters according to the position. The average number of clusters per bud in the 'Ülkemiz' grape variety is higher than the 'Karaerik' grape variety.

In 'Karaerik' grape variety, it was determined that the basal and middle buds are statistically in the same group in terms of the number of clusters and they are differed from the apical buds. It was determined that the highest number of clusters in the 'Karaerik' grape variety was in the middle buds and the least number of clusters in the apical buds. In the 'Ülkemiz' grape variety, it was determined that the apical buds and middle buds were statistically in the same group in terms of the number of clusters and they were different from the basal buds. It was revealed that in the 'Ülkemiz' grape variety, the highest number of clusters was in the apical buds and the least number of clusters was in the basal buds.

Table 2. The position of the primary buds and the number of clusters in grape varieties

Position	Cluster numbers		General Mean
	'Karaerik'	'Ülkemiz'	
	*	*	ns
Basal buds	0.660±0. 668 ^{ab}	0.876±0.804 ^{b}	0.823±0.761
Middle buds	0.765±0.720 ^{a}	0.981 ±0.759 ^{ab}	0.848±0.742
Apical buds	0.588±0.698 ^{b}	1.192±0.768 ^{a}	0.893±0.791

a, b: Mean values of cultivars with different superscripts are significantly different;
ns: non-significant, *: $p < 0.05$

In order to determine whether there is a relationship between the position of primary buds and their size in dormant buds, Duncan test was conducted in Table 3. When the genotypes were evaluated together, it was determined that the primary buds in the apical buds were statistically different from the primary buds in the basal and middle buds in terms of bud size. It was understood that the largest primary bud in terms of bud size was in the middle buds and the smallest primary bud in the basal buds. It was determined that there is a statistically significant difference between the genotypes in terms of the size of the primary buds. When Table 3 is examined, it has been determined that the primary buds of the 'Ülkemiz' grape variety are larger than the primary buds of the 'Karaerik' grape variety.

Table 3. The position and size of the primary buds in grape varieties

Position	Primary bud size		General Mean
	'Karaerik'	'Ülkemiz'	
	**	***	***
Basal buds	0.062±0.021 ^{b}	0.080±0.027 ^{a}	0.071±0.026 ^{A}
Middle buds	0.073±0.031 ^{a}	0.083±0.036 ^{a}	0.078±0.034 ^{A}
Apical buds	0.059±0.020 ^{b}	0.061±0.016 ^{b}	0.060±0.018 ^{B}

a, b: Mean values of cultivars with different superscripts are significantly different;
A, B: General mean values with different superscripts are significantly different;
**: $p < 0.01$, ***: $p < 0.05$

It was determined that the bud size of 'Karaerik' grape variety at the middle buds was statistically different from basal and apical buds. It has been revealed that the largest primary buds in the 'Karaerik' grape variety are in the middle buds and the smallest primary buds are in the apical buds. In the 'Ülkemiz' grape variety, it was determined that the bud size in apical buds was statistically different from the basal and middle buds. In the 'Ülkemiz' grape variety, it was determined that the largest buds were in the middle buds and the smallest buds in the apical buds, as in the 'Karaerik' grape variety.

The results showed relationship between the bud size and dormant bud productivity on the genotype of 'Karaerik' grape variety (*V. vinifera)* and 'Ülkemiz' (*V. labrusca*). [40], also stated that the growth power is one of the most important factors affecting the cluster and flower formation. The results also clearly indicated that the buds included 3 clusters were different than buds include one cluster, two clusters or without cluster. In terms of size of primer buds, there were statistically differences among genotypes. The biggest and the smallest buds were obtained from buds included 3 clusters and buds without cluster in both genotypes, respectively. [41], reported that very strong growth reduced bud productivity in grapevine. In general, it is known that the yield of one year later is negatively affected when the vegetative and generative development of the grapevine is not in balance [42]. [17], stated that very strong vegetative growth delay the formation of cluster primordium in dormant buds and decrease the number of clusters / bud ratio, and the growth power is one of the most important factors affecting the cluster and flower formation in grapevine. On the other hand, [43] reported that the relationship between shoot diameter and cluster number/bud was statistically insignificant in Kalecik Karası variety. In addition, [44] stated that one of the factors that show the strength of shoot development is the carbohydrate level in the bud tissues differ according to varieties, climatic conditions and cultural practices. There was no study that directly correlated the bud size with the number of cluster primordiums.

Since the grape yield obtained from the vineyards can vary depending on the position of the dormant buds on the 1-year-old shoot, it is of great importance to be able to determine the dormant bud productivity in different positions (node) [33, 45]. It was determined that the number of clusters was high in the middle buds of the 'Karaerik' grape variety and in the middle and apical buds in the 'Ülkemiz' grape variety. There is a statistically significant ($p < 0.05$) difference between the genotypes in terms of the number of clusters according to the position, and the average number of clusters per bud in the 'Ülkemiz' grape variety is higher than the 'Karaerik' grape variety. As a matter of fact, [24] investigated the maximum productivity of the buds in five different wine grape varieties and found that the maximum productivity was obtained at 4th, 5th and 6th buds in the Hasandede grape variety, the 5th and 6th buds in the Papazkarası grape variety, the 1st and the 7th in the Kalecik grape variety, 6th buds in the Öküzgözü grape variety and 5th buds in the Furmint grape variety, respectively. In addition, [23] in their study, in which they determined the productivity of 37 grape varieties grown in Tokat region according to the positions of the first 10 internodes, reported that the maximum productivity varied between the 3rd and 10th nodes, and the first nodes were inefficient in some varieties. [17], stated that the number of buds and clusters in an bud varies according to the position of the buds, and although it varies according to the varieties, generally, the buds at the base and apical buds contain less buds than the middle part. The results obtained in terms of the average number of clusters per dormant buds are similar to the findings of some researchers regarding the differences between grape varieties [16, 22, 24]. Considering the averages obtained, the difference seen in the primary bud productivity values on the 1-year old shoot on the basis of genotypes, mainly affected by genetic structure, yearly cultivation treatments, number of buds left in pruning, training method and climate factors, etc.

It was determined that the highest number of clusters in the ‘Karaerik grape variety was in the middle buds and the least number of clusters in the apical buds. Because, in a study conducted on grape varieties known to be suitable for short pruning, the productivity values in the dormant buds were found higher in the second buds of Hamburg Muscat and Balbal grape varieties; In Hafızali, Razaki and Çavuş grape varieties, it is 3rd-4th buds [46]. [32], determined that table grape varieties such as Ata Sarısı and Cardinal should be pruned short over 2 buds, Yalova Incisi, Amasya and Italia grape varieties should be pruned short over 2-3 buds, and Uslu grape varieties should be pruned in medium length over 3-5 buds. It was revealed that in the ‘Ülkemiz’ grape variety, the highest number of clusters was in the apical buds and the least number of clusters was in the basal buds. As a matter of fact, [34], conducted their study to determine the variation of bud fruitfulness in Samsun ecological conditions of four different grape types belongs to foxy grape and the productivity in the buds located in the first node of Pazar 3 and Güneysu 3 types was less than 1. These findings are in accordance with the findings determined by different researchers that the productivity increases towards the middle buds but decreases again towards the apical buds [15, 16].

When the genotypes were evaluated together in terms of bud size according to their position, it was found that the primary buds in the base (basal) buds were statistically different from the primary buds in the basal and middle buds. It was found that the largest primary bud is in the middle buds and the smallest primary bud is in the basal buds. It was determined that there is a statistically significant difference between the genotypes in terms of the size of the primary buds. It was determined that the primary buds of the ‘Ülkemiz’ grape variety has larger than the primary buds of the ‘Karaerik’ grape variety. It has been revealed that the largest primary buds are in the middle buds and the smallest primary buds are in the basal buds of the ‘Karaerik’ grape variety. In the ‘Ülkemiz’ grape variety, it was determined that the largest buds were in the middle buds and the smallest buds in the basal buds, as in the ‘Karaerik’ grape variety. We believe that this situation may be related to the aging of an old shoot and related nutrient delivery. As a matter of fact, it is known that an old shoot in the grapevine begins to lignify from the basal to the apical, and accordingly it is known that it is more wooded in the basal nodes and less in the apical nodes compared to the middle nodes [18]. As a matter of fact, [24] reported that the primary buds form structures containing 1-4 bunches, 6-12 nodes and/or leaves, and a few leech drafts in the 7-8 month period from the beginning of the development period to the enter rest. After this date, [47] stated that the buds entered winter dormancy until March of the following year, and then the development of the buds started again, but new organs could not be formed, but the structures that were formed developed. In addition, [48] reported that shoots developed better due to the long vegetation in hot regions and there was a positive correlation between shoot development and bud development.

4. Conclusion

In this study, it was determined that the number of clusters in the primary buds in the vines affects the bud sizes. In the study, it was observed that the size of the bud increased as the number of primordium of clusters in the bud increased in both ‘Karaerik’ and ‘Ulkemiz’ grape varieties (It was determined that buds containing 1 cluster were larger than buds without

clusters, buds containing 2 clusters were larger than buds with 1 cluster, and buds containing 3 clusters were larger than buds with 2 clusters). As a result of the study, it was understood that the basal and middle buds of 'Karaerik' grape variety were larger than the apical buds and the number of cluster primordium of these buds was higher. In 'Ulkemiz' grape variety, although the apical buds are smaller than the basal and middle buds, it has been determined that the number of clusters is higher. It was determined that the base and middle buds of Karaerik grape variety were more productive, so the buds could be left up to the 6th node in pruning. Since the apical buds of 'Ulkemiz' grape variety are more productive, it has been seen that long pruning would be more appropriate. According to the results obtained from this study, in addition to the growth forces of the one-year-old shoots to be preferred in winter pruning, bud structures/sizes should also be taken into account.

Ethics in Publishing

There are no ethical issues regarding the publication of this study.

Author Contributions

Conceptualization, data curation, formal analysis, methodology, visualization, writing-original draft, writing-review and editing

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