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Research Article

# Determination of Stoma Characteristics and SPAD Values of Some Local and Commercial Grape Varieties Cultivated in Kayseri Ecological Conditions

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**Abstract**

This study was carried out to determine the leaf stoma characteristics and SPAD value of total 18 grape varieties (10 local and 8 commercial) grown in the Kayseri region. Among the varieties SPAD value ranged from 29.23 (Çekirdeksiz) to 42.26 (Kara Burcu), and the stoma frequency (stoma/mm$^2$) ranged from 85.99 (Kara Burcu) to 136.83 (Tarsus Beyazı). Although there was a positive correlation between stoma width and stoma length, these parameters did not show a statistical correlation with stoma frequency. Stoma frequency and stoma surface showed a high positive correlation with both potential conductance index (r=0.76 and r=0.53, respectively) and relative stoma frequency (r=0.75 and r=0.65, respectively). Stoma shape coefficient showed a negative correlation with potential conductance index (r=-0.28). In this study, it was determined that the effect of the grape varieties on leaf stoma characteristics and SPAD value was important statistically. This result shows that leaf stomatal characteristics and SPAD value are genetically controlled.

**Keywords:** *Vitis vinifera* L., Stoma, Stoma characteristics, SPAD value, Chlorophyll

# Kayseri Ekolojik Koşullarında Yetiştirilen Bazı Yerel ve Ticari Üzüm Çeşitlerinin Stoma Özellikleri ve SPAD Değerlerinin Belirlenmesi

**Öz**

Bu çalışma, Kayseri bölgesinde yetiştiriciliği yapılan toplam 18 üzüm çeşidinin (10 yerel ve 8 ticari) yaprak stoma özellikleri ve SPAD değerini belirlemek için yapılmıştır. Çeşitler arasındaki SPAD değeri 29.23 (Çekirdeksiz) – 42.26 (Kara Burcu), stoma sıklığı (stoma/mm$^2$) ise 85.99 (Kara Burcu) – 136.83 (Tarsus Beyazı) arasında değişmiştir. Stoma eni ile stoma boyu arasında pozitif korelasyon olmasına rağmen, bu parametreler stoma sıklığı ile istatistiksel bir korelasyon göstermemiştir. Stoma sıklığı ve stoma yüzeyi, gerek potansiyel iletkenlik indeksi (r=0.76 - r=0.53) gerekse göreceli stoma sıklığı (r=0.75 - r=0.65) ile pozitif yönde yüksek korelasyon göstermiştir. Stoma şekil katsayısı ise potansiyel iletkenlik indeksi (r=-0.28) ile negatif bir korelasyon göstermiştir. Çalışmada çeşitlerin yaprak stoma özelliklerine ve SPAD değerine etkisinin istatistiksel olarak önemli olduğu tespit edilmiştir. Bu sonuç yaprak stoma özellikleri ve SPAD değerinin genetik olarak kontrol edildiğini göstermektedir.

**Anahtar Kelimeler:** *Vitis vinifera* L., Stoma, Stoma özellikleri, SPAD değeri, Klorofil

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## 1. Introduction

It is extremely important for the future of viticulture to produce solutions against drought and global climate change, which increased impact in recent years. The most important solution against these problems will be to benefit from local grape varieties and genotypes adapted to biotic and abiotic stress conditions of the region. Although the vine is among the moderately drought-resistant species [1], its adaptability to hot and dry conditions varies according to the species and variety [2]. The adaptation abilities of vines depend on transpiration and photosynthesis events in their leaves. Transpiration and photosynthesis events that ensure the continuity of the life of plants occur in stomata and chlorophyll.

Stoma is the vital transition point between inside and outside environment in plants [3]. Stomata play a vital role in transpiration and photosynthesis by regulating the carbon dioxide ($CO_2$), oxygen ($O_2$) and water balance between leaf and atmosphere. The location of stomata in leaves may differ according to plant species. Stomata can be found on both surfaces (amphistomatic), only the lower surface (hypostomatic) and only the upper surface (epistomatic) of the leaves [4]. In vines, stomata are located on the lower surface (hypostomatic) of the leaves [5]. 85-90% of plant water losses occur in stomata as a result of transpiration [6]. For this reason, it is important to know the number of stomata, the stomata sizes and the position of the stomata on the leaf in order to control the plant-water balance in plants. The number and anatomy of stomata in leaves can vary according to plant species, varieties and even environmental and maintenance conditions [4,7,8,9].

Chlorophylls are the pigments in which photosynthesis takes place. Chlorophylls act as catalysts in the conversion of light energy into chemical energy in the photosynthesis process [5]. The amount of chlorophyll of leaves affects the photosynthesis intensity and carbohydrate production of plants [10,11]. Chlorophyll content of leaves is directly related to plant stress and nutrient deficiencies [12,13]. Therefore, the leaf chlorophyll content affects the yield and quality of grapes. Determination of chlorophyll level using chlorophyll meter is a method used to determine various stresses [14,15]. The SPAD value, which can be measured with a portable chlorophyll meter, can be used to determine the chlorophyll of content in the leaves [16].

The aim of this study is to determine the SPAD value which shows the leaf chlorophyll content, and stomatal characteristics of some local and commercial grape varieties grown in Kayseri conditions.

## 2. Material and Methods

### 2.1. Plant materials

This study was carried out in the growers vineyard in Yeşilhisar district of Kayseri province. A total of 10 local (Kara Dimrit, Mor Şahabu, Beyaz Buludu, Mor Şabu, Mor Buludu, Çekirdeksiz, Parmak Üzümü, Kara Burcu, Siyah Şabu, Gül Üzümü) and 8 commercial (Öküzgözü, Horoz Karası, Tarsus Beyazı, Emir, Yalova İncisi, Kalecik Karası, Victoria, Red Globe) grape varieties widely cultivated in the Kayseri region were used in the study. Grape varieties grown as grafted on the Emir variety are 8 years old. The vineyard was established with 1.5 m x 3 m planting distance in a T-shaped wire training system. There is a drip irrigation system in the vineyard. In addition, all cultural and technical practices (irrigation, fertilization, tillage, etc.) are carried out routinely.

## 2.2. Stoma analysis

In order to determine the stomata characteristics of the leaves, three vinestocks were determined for each grape variety and 10 healthy leaf samples were taken from each vinestock. Leaf samples were taken from the 7th and 8th nodes of shoots exposed to sunlight and with similar growth rates. Leaf samples were taken in the morning hours (08:00 – 10:00) when stomata were open and the leaves were transported to the laboratory in ice boxes to prevent water loss. The stomata density in grapevine leaves can vary according to different parts of the leaf [17]. Therefore, stomata molds were taken from 3 different points (tip slice, upper lateral slice, and lower lateral slice) in leaf samples. The molding process was done with the nail polish method [18,19]. Flormar brand transparent nail polish was applied to the area where stoma molds on the leaves would be taken. After waiting 5-10 minutes for the nail polish to dry, the stoma molds were removed with a transparent tape and attached to the slide. Photographs of stoma molds were taken under SOIF brand light microscope (10x40 magnification) with MShot brand camera. Stoma measurements were made in the computer program of the MShot brand camera. Stoma width and stoma length were calculated as micrometers, and stoma frequency was calculated as the number of stomata in 1 $mm^2$ area. In addition, the stomata surface (SS) (1), the stomata shape coefficient (SSC) (2), the potential conductivity index (PCI) (3) and the relative stomata surface (RSS) (4) [20] reported according to the formulas.

Stomata Surface = [(Stomata length * Stomata width*π)] / 4 (1)

Stomata Shape Coefficient = (Stomata width / Stomata length) * 100 (2)

Potential Conductance Index = $(\text{Stomata length})^2$ * Stomata frequency * $10^{-4}$ (3)

Relative Stomata Surface =Stomata surface * Stomata frequency * 100 (4)

## 2.3. Chlorophyll analysis

Chlorophyll content of grape varieties was determined by the SPAD value, which can be measured with a portable chlorophyll meter (Minolta SPAD-502, Osaka, Japan). Leaf chlorophyll measurements were carried out as 4 replications on a total of 100 leaves. Chlorophyll measurement was carried out from healthy leaves on the sun-exposed parts of the vinestocks.

## 2.4. Data analysis

The data obtained in the study were subjected to variance and correlation analysis by using JMP Pro 14 (SAS Institute Inc., Cary, NC, USA) statistical package program. Differences between the means for stomata characteristics and chlorophyll contents were compared at the 5% significance level using the LSD multiple comparison method.

## 3. Results and Discussion

Data regarding SPAD values and leaf stoma characteristics of grape varieties are shown in table 1. The effect of grape varieties on leaf stomata characteristics and SPAD value was determined statistically significant ($p<0.05$).

The SPAD value showing leaf chlorophyll content was found to be higher in local grape varieties compared to commercial grape varieties. The SPAD value varied between 29.23 (Çekirdeksiz) and 42.26 (Kara Burcu) in local grape varieties, and between 30.75 (Horoz Karası) and 40.81 (Kalecik Karası) in commercial grape varieties. The average SPAD value was determined as 37.98 in local grape varieties and 36.19 in commercial grape varieties.

Chlorophyll is parameter that affects yield, quality, bunch characteristics and disease resistance in grapes [21]. Therefore, the chlorophyll content of the leaves is important. In this study, the SPAD value showing the leaf chlorophyll content varied between 29.23 and 42.26. Our study results are similar to the findings detected in different grape varieties and rootstocks. [22] reported that SPAD values ranged from 20.62 to 30.19 in 13 different American grapevine rootstocks. [23] reported that the SPAD values of 28 different local grape varieties grown on their own roots in the Bitlis region ranged from 27.48 to 45.78. The amount of leaf chlorophyll can vary according to the life forms of plants, season and light conditions [11].

Stoma frequency, which expresses the number of stomata in 1 $mm^2$, was determined higher in commercial varieties than in local grape varieties. The average stomata frequency was determined as 106.99 stoma/$mm^2$ in local grape varieties and as 111.87 stoma/$mm^2$ in commercial grape varieties. The stoma frequency was determined in lowest Kara Burcu (85.99 stoma/$mm^2$) and highest in Tarsus Beyazı (136.83 stoma/$mm^2$) in all grape varieties.

Stoma width and stoma length showed similar values in local and commercial grape varieties according means. However, stoma width and stoma length values showed differences between grape varieties. In the study, stomata width was determined the highest in Mor Buludu (18.06 µm) and lowest in Yalova İncisi (14.00 µm), while stoma length was determined the highest in Çekirdeksiz (28.43 µm) and lowest in Emir (23.43 µm).

The stomata surface between varieties was determined to be relatively higher in local grape cultivars compared to commercial varieties. The stomata surface of grape varieties varied between 389.25 $\mu m^2$ (Çekirdeksiz) and 267.85 $\mu m^2$ (Yalova İncisi). The average stomata surface was determined as 336.36 $\mu m^2$ in local grape varieties and as 334.02 $\mu m^2$ in commercial grape varieties.

**Table 1**. Leaf SPAD and stoma characteristics of grape varieties

| Cultivars | SPAD | S.F (stoma/mm$^2$) | S.W (µm) | S.L (µm) | S.S (µm$^2$) | S.S.C | P.C.I | R.S.S (%) |
|---|---|---|---|---|---|---|---|---|
| Kara Burcu | 42.26a | 85.99h | 15.66c-e | 23.54gh | 289.47fg | 66.57a-d | 4.75ı | 2.47f |
| Kara Dimrit | 36.01g | 105.00ef | 15.83b-d | 25.61b-f | 319.53d-g | 61.74d-f | 6.89c-g | 3.34de |
| Çekirdeksiz | 29.23ı | 105.87ef | 17.40a-c | 28.43a | 389.25a | 61.21d-f | 8.58b | 4.13bc |
| Gül Üzümü | 36.46fg | 100.25fg | 16.83a-c | 25.26c-g | 334.55b-f | 66.73a-d | 6.39d-h | 3.34de |
| Mor Şabu | 38.48d-f | 104.79ef | 17.41a-c | 26.77a-d | 368.72a-d | 65.34a-e | 7.48b-f | 3.84b-d |
| Beyaz Buludu | 41.38ab | 122.95bc | 17.02a-c | 24.00f-h | 321.26d-g | 70.88ab | 7.09c-g | 3.94b-d |
| Mor Buludu | 39.16c-e | 89.39gh | 18.06a | 26.50b-e | 376.05a-c | 68.21a-c | 6.28e-h | 3.35de |
| Mor Şahabu | 40.71a-c | 116.50c-e | 16.48a-d | 25.72b-f | 333.39b-f | 64.13c-e | 7.69b-d | 3.86b-d |
| Siyah Şabu | 37.23e-g | 122.68b-d | 16.35a-d | 26.70a-d | 343.45a-e | 61.34d-f | 8.77ab | 4.20bc |
| Parmak Üzümü | 38.93c-e | 116.49c-e | 14.89de | 24.60f-h | 288.01fg | 60.49ef | 7.05c-g | 3.35de |
| **Minimum** | **29.23** | **85.99** | **14.89** | **23.54** | **288.01** | **60.49** | **4.75** | **2.47** |
| **Maksimum** | **42.26** | **122.95** | **18.06** | **28.43** | **389.25** | **70.88** | **8.77** | **4.20** |
| **Mean** | **37.98** | **106.99** | **16.59** | **25.71** | **336.36** | **64.66** | **7.09** | **3.58** |
| Tarsus Beyazı | 39.30cd | 136.83a | 17.17a-c | 26.93a-c | 364.44a-d | 63.69c-e | 9.92a | 4.98a |
| Victoria | 33.10h | 133.74ab | 17.03a-c | 25.01d-h | 335.32b-f | 68.41a-c | 8.45b | 4.50ab |
| Emir | 33.26h | 110.94d-f | 16.72a-c | 23.43h | 308.44e-g | 71.31a | 6.08gh | 3.41de |
| Kalecik Karası | 40.81a-c | 116.49c-e | 16.34a-d | 25.42c-f | 328.48c-f | 64.14c-e | 7.50b-e | 3.79cd |
| Red Globe | 39.80b-d | 102.52f | 17.49ab | 25.59b-f | 352.37a-e | 68.40a-c | 6.70c-g | 3.60cd |
| Horoz Karası | 30.75ı | 103.15f | 17.79a | 27.32ab | 383.26ab | 65.05b-e | 7.76bc | 3.95b-d |
| Yalova İncisi | 37.16e-g | 104.22f | 14.00e | 24.35f-h | 267.85g | 57.51f | 6.19f-h | 2.79ef |
| Öküzgözü | 35.38g | 87.07h | 17.00a-c | 24.81e-h | 332.04b-f | 68.58a-c | 5.34hı | 2.87ef |
| **Minimum** | **30.75** | **87.07** | **14.00** | **23.43** | **267.85** | **57.51** | **5.34** | **2.79** |
| **Maksimum** | **40.81** | **136.83** | **17.79** | **27.32** | **383.26** | **71.31** | **9.92** | **4.98** |
| **Mean** | **36.19** | **111.87** | **16.69** | **25.35** | **334.02** | **65.88** | **7.24** | **3.73** |

The difference between the averages indicated by different letters in the same column is significant ($p < 0.05$).

Stoma shape index, potential conductivity index and relative stomata surface showed differences between varieties. However, commercial grape varieties showed relatively higher values compared to local grape varieties. Stoma shape coefficient, potential conductivity index and relative stoma surface were ranged from 57.51 (Yalova İncisi) - 71.31 (Emir), 4.75 (Kara Burcu) - 9.92 (Tarsus Beyazı) and 2.47 (Kara Burcu) - 4.98 (Tarsus Beyazı), respectively.

In the study, the stomata characteristics of the grape varieties differed between the varieties. The differences in stomata characteristics of varieties grafted on the same rootstock reveal that these characteristics are specific to the variety. [24] reported that stomata characteristics are genetically controlled along with the morphology and anatomy of the leaves.

It has been reported by many researchers that there are significant differences between Vitis species and varieties in terms of stomata frequency and other stomata characteristics on leaves. The stoma frequency values obtained in our study are similar with the results of previous studies. The stoma frequency limit values for *Vitis vinifera* L. cultivars vary between 100-200 stoma/mm$^2$ [25]. It has been reported that the stoma frequency of different grape varieties and rootstocks varied between 67.42 – 178.03 stoma/mm$^2$ by [26], 150.0 – 262.2 stoma/mm$^2$ by [27], 151.6 – 189.3 stoma/mm$^2$ by [28]. The present findings of other stoma characteristics (stoma width, stoma length, stomata surface, stoma shape coefficient, potential conductivity index and relative stomata surface) in the study are similar to the results of previous studies [27,28,29,30,31].

The stomata characteristics of grapes show vary according to fertilization [32], rootstock and training system [26,33], environmental and soil conditions [34]. This situation explains the differences between our findings and previous studies.

The correlation matrix of leaf SPAD and stomata characteristics of the grape varieties examined in the study are shown in Table 2. The SPAD value showing the chlorophyll content of the leaves showed a negative correlation with stomata length (r=-0.35) and stomata surface (r=-0.32). While stoma frequency showed not correlated with stoma dimensions, it showed a high positive correlation with potential conductivity index (r=0.76) and relative stomata surface (r=0.75). The stoma width showed a positive correlated with stomata length (r=0.52), stomata surface (r=0.90), stomata shape coefficient (r=0.67), potential conductivity index (r=0.28), and relative stomata surface (r=0.54). The stoma length showed a positive correlated with stoma surface (r=0.83), potential conductivity index (r=0.69) and relative stoma surface (r=0.60), and a negative correlated with stoma shape coefficient (r=-0.28). The stomata surface showed a positive correlation with the stomata shape coefficient (r=0.28), potential conductivity index (r=0.53), and relative stomata surface (r=0.65). While the stoma shape coefficient showed a negative correlation with potential conductivity index (r=-0.28), the potential conductivity index showed a positive correlation with the relative stomata surface (r=0.93).

**Table 2.** Correlation matrix of leaf SPAD and stomata characteristics of grape varieties

| **Variables** | SPAD | S.F | S.W | S.L | S.S | S.S.C | P.C.I | R.S.S |
|---|---|---|---|---|---|---|---|---|
| SPAD | 1 | | | | | | | |
| S.F | 0.02 | 1 | | | | | | |
| S.W | -0.22 | -0.07 | 1 | | | | | |
| S.L | **-0.35**** | 0.06 | **0.52***** | 1 | | | | |
| S.S | **-0.32*** | -0.01 | **0.90***** | **0.83***** | 1 | | | |
| S.S.C | 0.05 | -0.12 | **0.67***** | **-0.28*** | **0.28*** | 1 | | |
| P.C.I | -0.21 | **0.76***** | **0.28*** | **0.69***** | **0.53***** | **-0.28*** | 1 | |
| R.S.S | -0.19 | **0.75***** | **0.54***** | **0.60***** | **0.65***** | 0.09 | **0.93***** | 1 |

*, **, ***: Correlations significant at $p<0.05$, $p<0.01$ and $p<0.001$, respectively

Although stoma width and stoma length were show positive correlated, stoma width and stoma length did not show a statistical correlation with stoma frequency. Our present finding is show similar with the results of other studies [28,29]. The stomata surface showed a positive correlation with potential conductivity index (r=0.53) and relative stomatal surface (r=0.65). This result is similar to the results of the study reported by [28].

Although the stomata shape coefficient is controlled by genotypes, it can change under environmental stress conditions [35,36]. Stoma shape coefficient and potential conductivity index (r=-0.28) showed a negative correlation. This result shows that grape varieties are affected by arid climatic conditions [28].

## 4. Conclusion

Stoma characteristics and SPAD values showed differences between grape varieties. Although it has been reported by some researchers that leaf stomata frequency and stoma size play a role in the struggle of plants against drought, no correlation was found between them. In determining the response of grape varieties to drought, it is recommended to evaluate stomata width, stomata length and stomata frequency, together with stomata shape index, potential conductivity index and relative stomata surface. In addition, stomata characteristics of the same grape variety may vary according to different regions and growing conditions. For this reason, it is thought that stoma frequency, stoma width and stoma length alone will not be sufficient for the identification of cultivars. This study will guide further studies as a result of the determination of stomata characteristics and chlorophyll amounts of local varieties that have disappeared due to various reasons.

## Ethics in Publishing

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

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