Stomatal density in various Turkish grape cultivars

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Summary: In this study the number of stomata per unit area and dimensions of the stomata were determined in 15 important Turkish grape cultivars which were 20 years old and grafted on Kober 5 BB rootstock. Number of stomata per mm^2 of area varied from 129 ± 18 to 254 ± 10, stomatal length ranged from 22.6 ± 2.6 μm to 28.3 ± 4.3 μm, and stomatal width from 13.6 ± 2.2 μm to 18.6 ± 3.2 μm among cultivars. The cultivars having minimum and maximum number of stomata per unit area were Balbal and Pembe Gemre; Erenköy Beyazi and Müsküle had minimum and maximum stomata size, respectively. Correlations between the number of stomata per unit area and the stomatal length ($r = 0.100$), and width ($r = 0.184$), and between the stomatal length and width ($r = 0.493$) were not significant among cultivars.

On the other hand, Balbal, Hafizali and Yapincak were found to be more tolerant than the other cultivars.

The characteristics of the cultivars in relation to their drought tolerance and stomatal densities were discussed.

Key words: stoma, leaf, transpiration, water, drought, resistance, variety of vine, Turkey.

Introduction

The vineyards of Anatolia are spread over the dry regions in which the summer rainfall is often insufficient. For this reason vine growers make use of the spring and winter rainfall and give more importance to special cultivation practices. Today more than 1000 grape cultivars (possibly some of them are synonymous) are grown in Turkey. These cultivars are different from each other with respect to their qualitative and physiological characteristics. In the adaptation to different ecological conditions (e.g. drought) both rootstocks' and cultivars' own characteristics are important factors to postpone dehydration. Deep root system, thick cutin, and good stomatal control of transpiration as well as the capacity of protoplasm to endure desiccation are the main factors in drought tolerance (Kramer and Kozlowski 1979).

Root system development is dependent on the rootstock variety. Morphological features of the leaves are also important in this respect. According to Kisselew (cited in Levitt 1980), the small-leaf forms of *Scorzonera* survive a greater loss of water then the broad-leaf forms. Runyon (cited in Kramer and Kozlowski 1979) found that the leaves of creosote bush produced during moist weather are large and easily injured by water deficit, but the leaves produced during dry weather can be dried to a saturation deficit of 50 %. Desiccation tolerance of the leaves also shows a great variance between species. Oppenheimer (cited in Kramer and Kozlowski 1979) reported that the leaves of almond could be dried to a saturation deficit of 70 % and olive to 60 % before injury occurred, but fig dried to only 25 %.

The volume/surface ratio of the cells of drought tolerant plants is low. Iljin (cited in Levitt 1980) concluded that this ratio is 1-2 in xerophyllic mosses; near 20 in sensitive cells; 5-10 in cells of intermediate tolerance. Merieux *et al.* (cited in Levitt 1980) found that *Vitis vinifera* plants grown with low moisture have smaller cells and are more drought tolerant.

High osmotic pressure has also been regarded as an important adaptation to water stress (Kramer and Kozlowski 1979).

The size and frequency of stomata have an importance in the physiology of the plants. The relations between stomatal size and frequency and transpiration rate are still uncertain (Vardar 1972). However, plants native to arid regions and regions with long summer droughts have heavily cutinized leaves and very low transpiration rates after the stomata have closed (Kramer and Kozlowski 1979). Stomatal frequency of the drought resistant xerophyte plants is generally high

Table 1: Stomatal frequency of various grape cultivars

Cultivars	Number / mm^2
Kozak Beyazı	201 ± 23
Siyah Razakı	205 ± 25
Çavuş	187 ± 16
Amasya	197 ± 8
Müşküle	140 ± 9
Alphonse[1)]	179 ± 9
Hafızali	192 ± 10
Sultani Çekirdeksiz	251 ± 9
Tarsus Beyazı	153 ± 9
Yapıncak	192 ± 19
Balbal	129 ± 18
Erenköy Beyazı	151 ± 5
Pembe Gemre	254 ± 10
Öküzgözü	159 ± 18
İskenderiye Misketi	184 ± 24
Buca Razakısı	200 ± 19

1) To be considered only in the calculation of the correlation coefficients

(VARDAR 1972). In addition, some variances have occurred in the structure of stomata of the plants grown under different ecological conditions (MEIDNER and MANSFIELD 1968).

According to SMART (cited in WEAVER 1976), stomata closed at a leaf water potential of -13 bars in vines. In some vine cvs at pre-dawn water potentials down to -8 bars, assimilation and especially transpiration were reduced (DÜRING and KLINGENMEYER 1987). However, stomatal control of transpiration rate varies among the species (KRAMER and KOZLOWSKI 1979). JOHNS and LAZENBY (cited in LEVITT 1980) stated that stomatal control of senescing leaves may be ineffective leading to their death, whereas young leaves are uninjured. Cv. Riesling, considered drought tolerant, has low stomatal conductance resulting in high water use efficiency (ratio CO_2 uptake/H_2O loss) and low hydraulic conductance (DÜRING 1988).

The stomatal frequencies of some grape species, hybrids and cultivars have long been studied (DÜRING 1980; SCIENZA and BOSELLI 1981; DÜZENLI and ERGENOGLU 1988). Many factors can affect the frequency and/or size of stomata such as nutritional conditions (BALO *et al.* 1986), K fertilization (BOSELLI and SCIENZA 1983), N fertilization (RÜHL and IMGRABEN 1985), fungicide applications (SEKERA 1983), vine vigor, leaf position (FORLANI *et al.* 1983), leaf maturity (DÜRING 1980), shading (KRAMER and KOZLOWSKI 1979), rootstocks and training systems (DÜZENLI and ERGENOGLU 1988).

In this study stomata size, density and drought tolerance of some Turkish grape cultivars were determined and discussed.

Materials and methods

The following cultivars were used: Kozak Beyazi, Siyah Razaki, Cavus, Amasya, Müsküle, Hafizali, Sultani Cekirdeksiz (Sultanina), Tarsus Beyazi, Yapincak, Balbal, Erenköy Beyazi, Pembe Gemre, Öküzgözü, Iskenderiye Misketi (Muscat d'Alexandrie) and Buca Razakisi. The cultivars were 20 years old and trained to Guyot and Cordon systems. All vines were grafted on Kober 5 BB rootstock.

Stomatal counts were made on leaves taken from the 3rd-5th nodes after the leaves had reached full maturity. Nail polish, diluted in some degrees with acetone, was spread on the mid portion of upper and lower surface of the leaves and later removed. These moulds were used for countings and measurements. In total, 135 microscopic view fields were considered in the countings. Stomata length and width were measured on 30 randomly selected stomata.

Drought tolerance of the cultivars was determined on plants obtained from rooted cuttings. For this purpose, cuttings of the cultivars were collected just before spring, prepared with one bud and set in boxes containing perlite, then placed in the greenhouse. Temperature was held between 25 and 28 °C. Cuttings leafed out and rooted in this environment and were watered and fertilized with HOAGLAND nutrient solution (HOAGLAND and ARNON 1950). After the shoots had 4-5 leaves, watering was interrupted and the young plants were exposed to water stress. Plants did not receive water during a period of 27 d. At the end of this period, plants were rewatered. The cultivars were then evaluated for injury from water deficit. Wilting of the leaves was recorded since it could be used for indirect estimates of the water status (KRAMER and KOZLOWSKI 1979) and drought tolerance of the vine cvs (DÜRING 1988). In addition, yellowing of the leaves and the plants completely injured from water deficit was also recorded. In total, 30 plants (3 x 10) of each cultivar were used in the experiment.

Results

Stomatal density and size of stomata

No stomata were observed on the upper surface of the leaves. However, stomatal frequency on the lower surface of the leaves revealed great variance among the cultivars (Table 1). Minimum and maximum stomatal frequencies (number/mm^2) were determined in Balbal (129 ± 18) and Pembe Gemre (254 ± 10) cvs. The frequencies of other cultivars were between these two values.

Mean stoma length and width of the cultivars are shown in Table 2. Some differences also existed among the cultivars in this respect. Mean stoma length ranged from 22.6 ± 2.6 to 28.3 ± 4.3 μm, and mean stoma width from 13.6 ± 2.2 to 18.6 ± 3.2 μm. Müsküle and Erenköy Beyazi cvs have maximum and minimum values of both stoma length and width, respectively.

When taking into account the cultivars together, the correlation coefficients between the stomatal frequency and stoma length and width were found to be non-significant ($r = 0.100$ and

Table 2: Mean stoma length and width of various grape cultivars

Cultivars	Stoma length (μm)	Stoma width (μm)
Kozak Beyazı	26.0 ± 3.5	18.4 ± 3.4
Siyah Razakı	27.2 ± 4.7	17.9 ± 3.0
Çavuş	25.7 ± 3.6	17.6 ± 2.6
Amasya	24.1 ± 3.4	17.2 ± 2.3
Müşküle	28.3 ± 4.3	18.6 ± 3.2
Alphonse 1)	24.6 ± 3.3	18.4 ± 2.6
Hafızali	24.4 ± 3.6	16.4 ± 1.6
Sultan Çekirdeksiz	24.4 ± 3.2	17.2 ± 2.0
Tarsus Beyazı	24.5 ± 3.4	15.5 ± 3.1
Yapıncak	27.1 ± 4.1	15.1 ± 4.4
Balbal	23.0 ± 3.3	16.1 ± 2.4
Erenköy Beyazı	22.6 ± 2.6	13.6 ± 2.2
Pembe Gemre	24.2 ± 3.1	16.3 ± 1.8
Öküzgözü	23.8 ± 3.0	16.6 ± 3.1
İskenderiye Misketi	25.0 ± 3.8	17.4 ± 3.3
Buca Razakısı	26.6 ± 4.2	16.4 ± 2.6

1) To be considered only in the calculation of the correlation coefficients

r = 0.184, respectively). However, the correlation coefficient between stoma length and width was relatively high, though not significant (r = 0.493).

Drought tolerance of the cultivars

The percentages of completely injured plants at the end of the 27 d water stress period are shown in Table 3. The cultivars which suffered the most from water deficit were Cavus, Tarsus Beyazi and Amasya. Balbal, Hafizali, Yapincak and Erenköy Beyazi suffered much less injury than the other cultivars. After rewatering, injury in some of the cultivars continued rapidly. For example, total loss in Erenköy Beyazi increased from 16.6 % to 86.6 %. On the other hand, this increase in Yapincak was only 10 % in the last 7 d of the test period (Table 3).

Injury from water deficit began early and increased rapidly in some of the cultivars (e. g. Amasya, Tarsus Beyazi and Cavus). Some of the cultivars could tolerate drought until a certain time and then collapsed rapidly (e. g. Iskenderiye Misketi, Erenköy Beyazi and Buca Razakisi).

Table 3: Percentages of the completely injured plants

Cultivars	Completely Injured Plants (%) At the end of 27 days of unwatering period	At the end of 7 days after rewatering point
Kozak Beyazı	(No data obtained)	
Siyah Razakı	30.0	56.6
Çavuş	96.6	100
Amasya	100	100
Müşküle	36.6	66.6
Hafızali	13.3	53.3
Sultani Çekirdeksiz	76.6	93.3
Tarsus Beyazı	100	100
Yapıncak	16.6	26.6
Balbal	10.0	43.3
Erenköy Beyazı	16.6	86.6
Pembe Gemre	33.3	66.6
Öküzgözü	23.3	66.6
İskenderiye Misketi	40.0	83.3
Buca Razakısı	33.3	70.0

Commencement of the injury was late and the total loss was low in Balbal and Yapincak (Fig.). Siyah Razaki, Pembe Gemre, Hafizali, Öküzgözü and Müsküle were between the two extremes and showed medium degree of injury.

Injury due to water deficit appeared first as yellowing and wilting of the leaves and was followed by death of the shoots. However, yellowing of the leaves was much less distinct in Cavus and Tarsus Beyazi cvs than in the other ones. Drooping leaves were the first sign in Cavus, at the beginning of injury.

Discussion

There are few studies on the stomatal frequency of Turkish grape cultivars. Stomata were found only on the lower surface of the leaves. This was also observed by DÜRING (1980) in some *Vitis* spp. and cvs. So the cultivars studied were shown to be hypostomatal.

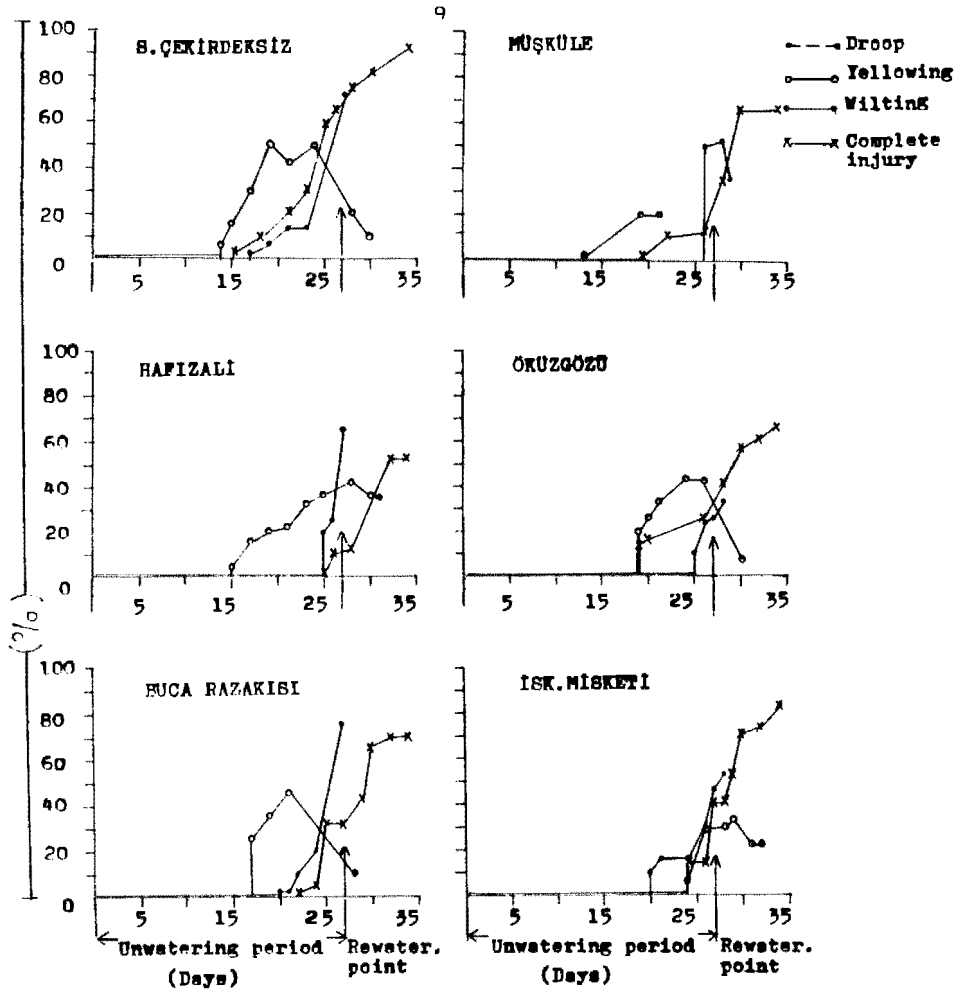
Stomatal frequency of the cultivars varied from 129 ± 18 to 254 ± 10, which was generally between the values found by other researchers in some *Vitis* spp. and cvs (DÜRING 1980; DÜZENLI and ERGENOGLU 1988). The stomatal frequency of Sultani Cekirdeksiz found by DÜZENLI and ERGENOGLU (1988) is very close to our result. But the value found by the same researchers for Tarsus Beyazi (227-233) is different from our finding. This variance can be attributed to

environmental and other factors (Boselli and Scienza 1983; Forlani *et al.* 1983; Rühl and Imgraben 1985; Balo *et al.* 1986; Düzenli and Ergenoglu 1988).

Stomata length and width of the cultivars were found to be different. In general, stomata lengths determined in this study, at least in some cultivars, were close to the value given for *V. vinifera* (29.7 μm) by Kramer and Kozlowski (1979). However, the size of stomata can also be changed by K (Boselli and Scienza 1983), fungidice applications (Sekera 1983), and possibly other factors.

The correlation between the size of stomata (as length and width) and the frequency was not significant. A negative correlation between both characters has been observed in some plant species (Kramer and Kozlowski 1979). The correlation between the length and width of stomata was fairly high ($r = 0.493$).

There were important differences among the cultivars with respect to their tolerance to water deficit. The following relative order of the cultivars could be given: Cavus, Amasya, Tarsus Beyazi



The curves of droop, yellowing and wilting of the leaves and completely injured plants. (Continued overleaf.)

and Sultani Cekirdeksiz are least tolerant; Yapincak and Balbal are most tolerant, and other cultivars range between the two extremes. But these moderately tolerant cultivars have also shown different degrees of tolerance.

The relations between stomata frequency and drought tolerance are uncertain. For example, Tarsus Beyazi and Sultani Cekirdeksiz, which are considered to be sensitive cultivars, have 153 ± 9 and 251 ± 9 stomata/mm^2 of area, respectively. The same pattern is also true for the tolerant cvs

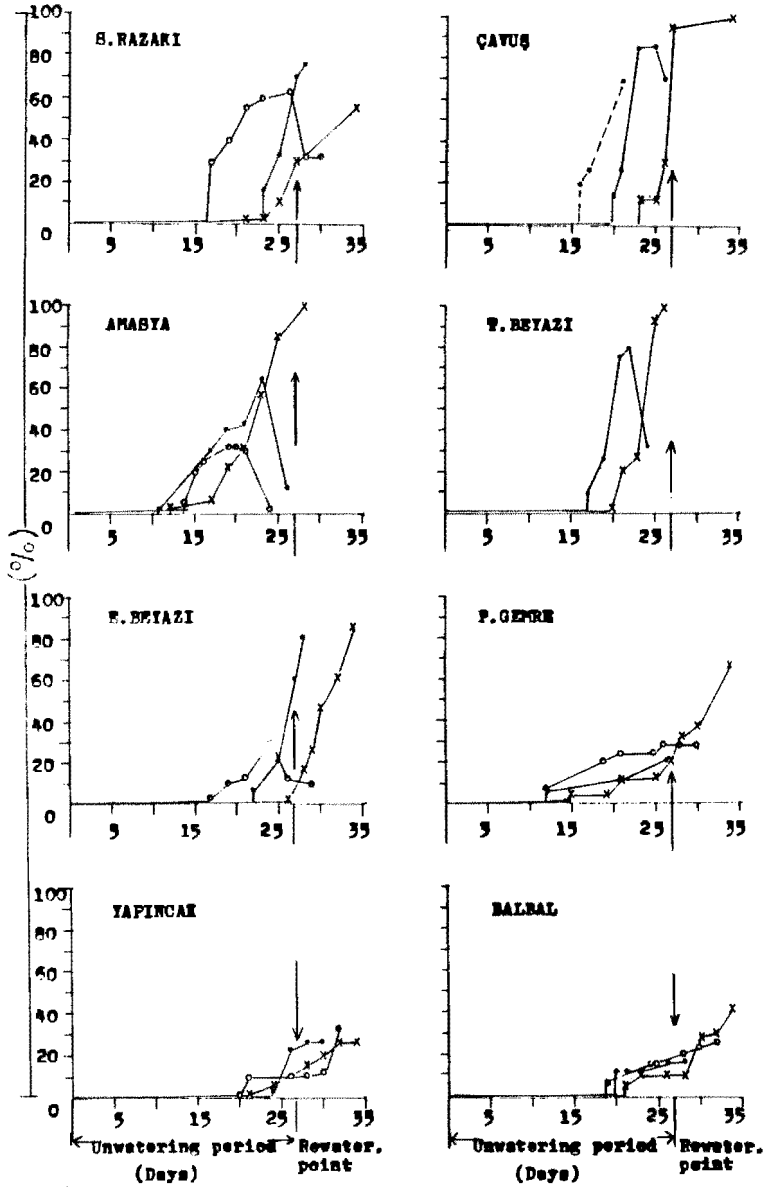


Fig. (continued).

Balbal and Yapincak which have 129 ± 18 and 192 ± 19 stomata/mm^2, respectively. The relations between stomata size and tolerance are also uncertain. Other characteristics of the leaves (e. g. size, firmness, hair density) may play a role in this respect. As a matter of fact, mature leaves of the sensitive cultivars (Cavus, Amasya and Sultani Cekirdeksiz) are large and soft in structure. On the other hand, Balbal and Yapincak also have relatively large and fairly soft leaves. The lower surface of the leaves of Cavus and Tarsus Beyazi is densely covered with hair.

For the reasons mentioned above, it is difficult to state that there is a close relationship between the leaf morphology and drought tolerance of the cultivars investigated. Stomatal closure at certain water potentials can be useful to determine the drought tolerance (KRAMER and KOZLOWSKI 1979). Stomatal conductance and water use efficiency can also be helpful to understand the differences among cultivars (DÜRING 1988). In addition, field studies are needed in this respect.

Literature

BALO, B.; MUSTARDY, L. A.; HLDEG, É.; FALUDI-DANIEL, A.; 1986: Studies on the effect of chilling on the photosynthesis of grapevine. Vitis **25**, 1-7.

BOSELLI, M.; SCIENZA, A.; 1983: Effects of potassium fertilization on density and morphological characteristics of stomata in grapevines. Vignevini **10** (1-2), 27-32. [Abstr.: Vitis **22**, 263.]

DÜRING, H.; 1980: Stomatafrequenz bei Blättern von *Vitis*-Arten und -Sorten. Vitis **19**, 91-98.

– – ; 1988: Zur Trockentoleranz des Riesling. Wein-Wiss. **42**, 391-399. [Abstr.: Vitis **27**, 2 C 7].

– – ; KLINGENMEYER, W.; 1987: Stomatal control of water use efficiency in two *Vitis vinifera* cultivars. 3e Symp. Intern: Physiologie de la Vigne, Bordeaux, 179-184. [Abstr.: Hort. Abstr. **57**, 7666.]

DÜZENLI, S.; ERGENOGLU, F.; 1988: Studies on the density of stomata of some *Vitis* varieties grafted on different rootstocks trained to various trellis systems. IIIrd Symp. Viticulture of Turkey, 31 May-3 June 1988, Bursa, Turkey.

FORLANI, M.; PASQUARELLA, C.; COPPOLA, V.; 1983: The relationship between stomatal density and vigour in grapevine rootstocks. Riv. Viticolt. Enol. **36**, 117-125. [Abstr.: Hort. Abstr. **53**, 4948.]

HOAGLAND, D. R.; ARNON, D. I.; 1950: The water culture method for growing plants without soil. Calif. Agricult. Exp. Sta. Circ. 347.

KRAMER, P. J.; KOZLOWSKI, T. T.; 1979: Physiology of Woody Plants. Academic Press, New York, San Francisco, London.

LEVITT, J.; 1980: Responses of Plants to Environmental Stress, Vol. II. Water, Radiation, Salt and Other Stress. Academic Press, New York, London, Toronto, Sydney, San Francisco.

MEIDNER, H.; MANSFIELD, T. A.; 1968: Physiology of Stomata. McGraw-Hill, London.

RÜHL, E.; IMGRABEN, H.; 1985: Einfluß der Stickstoffversorgung auf die Stomatazahl und den Blattaufbau von Weinreben (*Vitis vinifera* L.). Wein-Wiss. **40**, 160-171. [Abstr.: Vitis **24**, 4 E 128.]

SCIENZA, A.; BOSELLI, M.; 1981: Fréquence et caractéristiques biométriques des stomates de certains porte-greffes de vigne. Vitis **20**, 281-292.

SEKERA, D.; 1983: The effect of fungicides and fungicide combinations on grape leaf stomata size and aperture. Vinohrad (Bratislava) **21**, 29-31. [Abstr.: Vitis **22**, 410.]

VARDAR, Y.; 1972: Bitki Fizyolojisi Dersleri. I. Ege Ünv. Fen Fak. Kitaplar Ser. 37, Izmir.

WEAVER, R. J.; 1976: Grape Growing. John Wiley and Sons, New York, Chichester, Brisbane, Toronto.