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DETERMINATION OF STOMATAL MORPHOLOGY IN SOME *CITRUS* SPP. SPECIES

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Abstract
Citrus spp. fruits belonging to the Rutaceae family include sour and bitter oranges, sweet oranges, pomelo, grapefruit, citrons, lemons, oranges, bergamots, mandarins, limes, etc, consists of a wide variety of hybrids and hybrid individuals. In this study, it was aimed to determine the stomatal morphology of 6 different Citrus spp. species (C. lemon L., C. paradisi L., C. aurantium L, C. sinensis L., C. bergamia L., C. reticulata) grown in the ecological conditions of Hatay/Türkiye. Stoma width: 16.66±2.31 ('Citrus paradisi L.')-24.32±1.75 ('Citrus bergamia L.') µm; stoma length: 18.84±2.39 ('Citrus paradisi L.')-29.59±3.27 ('Citrus bergamia L.') µm; stoma density varied between 1007±83.56 ('Citrus reticulata L.') and 1172±84.67 ('Citrus paradisi L.') stoma/mm². As a result of the correlation matrix analysis, a positive correlation was found between stoma width and stoma length ($r=0.90$, $p<0.05$). According to principal component analysis, the first three components explain 96.8% of the total variation. According to the heatmap analysis, Citrus reticula L. formed a group, while other Citrus spp. species formed a group. Stoma morphology was divided into two groups. Stomatal density, stomatal shape coefficient and pore width were included in one group, while other morphological criteria were included in another group.

Keywords: Citrus spp., Hatay, multivariate analysis, stomata morphology

1. INTRODUCTION

Citrus spp., which originates from Asia, belongs to the *Rutaceae* family and has almost 152 genera and 2100 species (Kubitzki et al., 2010). Citrus is the most traded product in the world (Hussain et al., 2021). Although it starts producing crops at the age of 3, it produces crops economically from the age of 8-10 (Dönmez and Başol, 2022). On average, it maintains its economic life until the age of 90 (Tezel, 2020). In Turkey, most *Citrus* spp. production is done in the Mediterranean region provinces such as Mersin, Adana and Hatay (Dönmez and Başol, 2022).
Plant yield depends on many factors (Gregoriou et al., 2007). The most important of these are leaves, which are the photosynthetic organ where stomata, chlorophyll and carotenoid pigments are located and accordingly photosynthesis takes place (Proietti and Famian, 2002). The photosynthetic capacity of leaves is directly related to the yield under different environmental conditions (Tunç et al., 2023). Stomata are of vital importance because plants lose 85-90% of their water through stomatal pores as a result of transpiration (Sümbül, 2022). Therefore, stomata provide the

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connection between the plant and the environment (Montoro et al., 2016) and detailed stomatal morphology provides information about the environmental conditions in which the plant grows and the physiological state of the tree (Batos et al., 2010). The location of stomata on leaves varies depending on plant species. Stomata can be found on the bottom (hypostomatic), on the top (epistomatic) and on both surfaces of the leaves (amphistomatic) (Richardson et al., 2020). Stomata are on the lower surface of the leaf in *Citrus* spp. (hypostomatic) (Aina and Malik, 2013).
The first stoma studies in *Citrus* spp. species began in the early 1930s (Hirano, 1931) and these studies have continued until today (Costa et al., 2021). However, *Citrus* spp. very detailed stomatal morphology studies are lacking. With this study, very detailed stomatal morphology, which is an important deficiency in *Citrus* spp., will be added to the literature. This study will guide researchers who will investigate similar issues in different *Citrus* spp.

2. MATERIALS AND METHODS

Materials

Leaf samples of *Citrus* spp. species collected from the producer garden in Erzin district of Hatay province were brought to the pomology laboratory of Hatay Olive Research Institute Directorate and stomatal analyzes were performed there. In the research, 6 different *Citrus* spp. species *(C. limon* L., *C. paradisi* L., *C. aurantium* L, *C. sinensis* L., *C. bergamia* L., *C. reticulata* L.) were used. The *Citrus* spp. species were 15 years old and planted at 7 × 7m. In addition, cultural maintenance operations such as irrigation, pruning, fertilization and soil cultivation were carried out regularly. The garden has an airy, permeable, clay loam soil structure. The typical Mediterranean climate prevails in the region, with warm and rainy winters and hot and dry summers.

Methods and stoma analysis

In order to determine the stomatal morphology of citrus species in detail, a total of nine leaves from each species were taken at the end of the winter harvest period (Gregoriou et al., 2007). Leaves were taken from the 3th and 4th nodes of annual shoots (Gözel, 2018). In order to see the stomata clearly, the leaves were polished four times with nail polish. The stoma molds were glued to the surface of a square ocular slide (Marienfeld brand) and placed under a trinocular microscope (Soif Optical Instrument; 40 × magnification) for counting (Tunç et al., 2023). Very detailed stoma morphology was determined according to Tunç et al. (2023) (Table 1). Stoma length, stoma width, pore length and pore width were measured with the ImageJ© (ImageJ©, 2024) program (Petrik et al., 2020) (Figure 1).

Statistical analysis

The differences between the varieties were measured by quantitative analysis method in the research, which was established according to the random plots trial decotation. The parameters were subjected to variance analysis (One-Way Anova Test) and correlation analysis. Analyzes were performed with the LSD Multiple Comparison Test JMP® Pro 17.0.0 (JMP®, 2024) program and compared at a 5% significance level. As a result of the LSD comparison, the groups were obtained and lettered according to the degree of importance. Principal component analysis (PCA), correlation matrix analysis, and heatmap analysis were performed with the Origin Pro® 2024 (OriginLab®, 2024) statistical program.

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Table 1. Very detailed stoma morphologies, used formulas and units

Very detailed stoma morphology	Abbreviations	Description and formulas	Unit
Stoma length	SL	The length of the stoma was measured from end to end	µm
Stoma width	SW	The width of the stoma was measured from end to end	µm
Stomata density	SD	Number of stomata per square millimeter	stoma/mm²
Stomata index	SI	SL÷SW	
Stomata pore surface	SPS	(SL×SW×π)÷4	µm²
Stomatal shape coefficient	SSC	(SW÷SL)×100	
Potential conductivity index	PCI	[(SL)²÷SD]×100	
Relative stomatal surface	RSS	(SPS×SD×100) ÷1000000	%
Pore width	PW	Pore length measured end to end	µm
Pore length	PL	Pore width measured end to end	µm

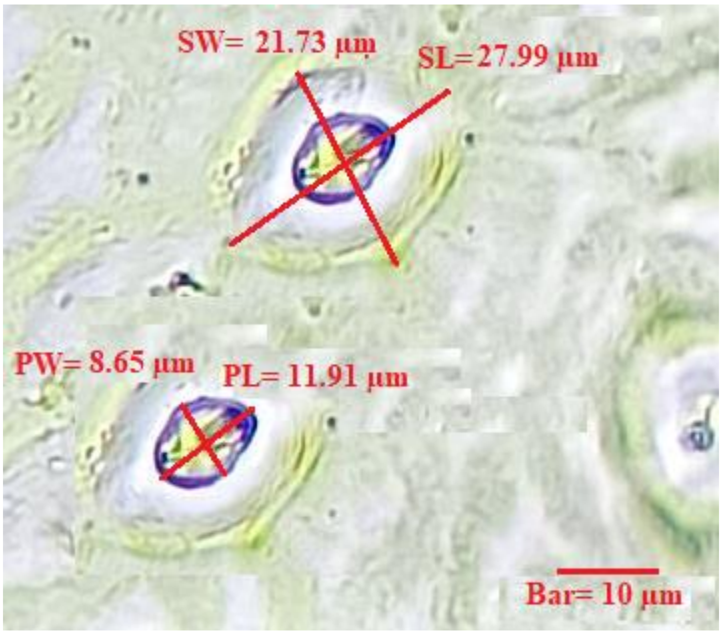

Figure 1. Stoma length, stoma width, pore length and pore width measurement with ImageJ© program

3. RESULTS AND DISCUSSIONS

The very detailed stomatal morphology of *Citrus* spp. species is presented in detail in Table 2. The findings of researchers working on stoma morphology are presented in detail in Table 3. When our research findings and the researchers' findings are evaluated together, there are similarities and differences in stoma morphological features. The reason for the differences is that stomatal morphological features depend on many different environmental and physiological factors such as plant species and varieties, wind speed, annual rainfall, biotic and abiotic stress status, light intensity, and CO_2 concentration (Bertolino et al., 2019).

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Table 2. Very detailed stomatal morphology of Citrus spp. species

Citrus spp.	SW (µm)	SL (µm)	SD (stoma/mm²)	SI	SPS (µm²)	SSC
C. aurantium L.	21.00±2.41 b	24.80±3.03 b	1121±56.28 ab	1.19±0.17 ab	410.21±79.76 b	85.49±13.71 ab
C. bergamia L.	24.32±1.75 a	29.59±3.27 a	1073±80.82 bc	1.22±0.15 ab	564.67±57.93 a	83.04±9.73 ab
C. lemon L.	16.78±3.41 c	21.96±2.93 bc	1008±38.82 c	1.33±0.18 ab	294.69±94.50 c	76.09±9.42 ab
C. paradisi L.	16.66±2.31 c	18.84±2.39 c	1172±84.67 a	1.14±0.10 b	248.99±65.14 c	88.62±8.91 a
C. reticulata L.	18.31±1.80 bc	22.50±1.43 bc	1007±83.56 c	1.23±0.11 ab	324.66±42.66 bc	82.48±8.13 ab
C. sinensis L.	17.63±1.89 c	23.85±3.08 b	1012±68.33 c	1.35±0.12 a	331.46±56.96 bc	74.64±7.49 b
Mean	*19.12±3.40*	*23.24±4.22*	*1065±91.06*	*1.23±0.15*	*359.95±12.21*	*83.22±10.31*

Citrus spp.	PCI	RSS (%)	PW (µm)	PL (µm)	PI
C. aurantium L.	70.16±19.22 b	46.16±10.50 b	9.95±1.61 a	10.78±1.40 b	1.11±0.25 d
C. bergamia L.	95.01±23.96 a	60.58±7.96 a	5.61±2.09 bc	11.91±1.53 a	2.27±0.57 a
C. lemon L.	48.89±11.21 bc	29.43±8.39 c	4.82±9.30 c	10.34±1.58 b	2.19±0.43 ab
C. paradisi L.	41.56±80.17 c	28.85±5.77 c	7.17±2.55 b	9.98±1.70 c	1.51±0.47 cd
C. reticulata L.	51.68±70.22 bc	32.73±5.63 c	5.58±1.17 bc	11.07±1.84 ab	2.11±0.78 a-c
C. sinensis L.	58.15±14.62 bc	33.48±5.93 c	6.62±1.15 bc	10.09±1.53 bc	1.56±0.36 b-d
Mean	*60.10±23.07*	*38.30±13.60*	*6.61±2.29*	*10.70±1.61*	*1.79±0.63*

Table 3. Literature summaries on the subject

Stomatal morphology	Unit	Lower-Uplower limits	Reference	Subject studied
SL	µm	18.73-27.52	Çimen et al., 2016	Citrus
SW	µm	22.00-27.45	Avcı and Aygün, 2014	Hazelnut
SD	stoma/mm²	807-1042	Costa et al., 2021	Citrus
SI		1.29-1.70	Çimen et al., 2016	Citrus
SPS	µm²	230.30-475.74	Tunç et al., 2023	Olive
SSC		51.57-71.31	Sümbül, 2022	Grape
PCI		28.4-78.5	Wang et al., 2012	Ligustrum quihoui
RSS	%	9.58-32.49	Tunç et al., 2023	Olive
PW	µm	2.62-7.37	Bongi et al., 1987	Chestnut
PL	µm	17.34-19.22	İkinci et al., 2022	Jujube

The correlation matrix formed by stomatal morphology features in *Citrus* spp. species is shown in Figure 2. As a result of the correlation matrix analysis, a positive correlation was found between stoma width and stoma length (r=0.90, $p<0.05$). Our finding of correlation between stoma length and stoma width is similar to the findings of Tunç et al. (2023). In general, the correlation between stoma density and other variables was found to be statistically insignificant.

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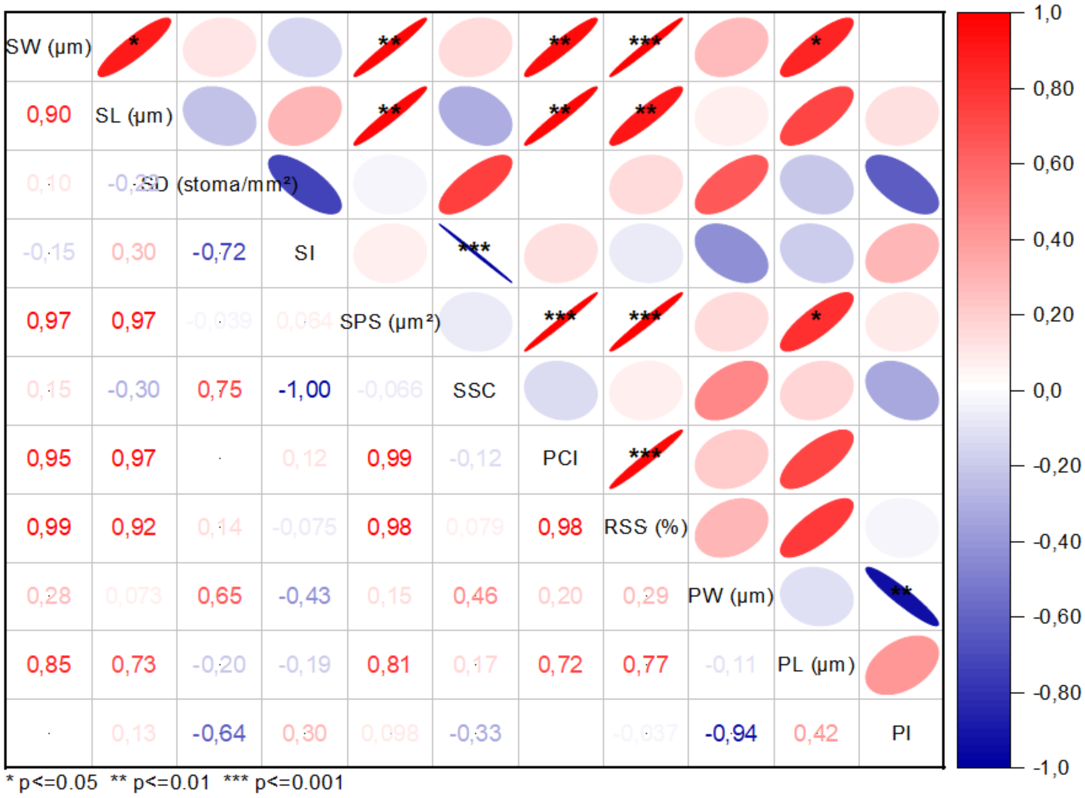

Figure 2. Correlation matrix data of Citrus spp. species

The location graph corresponding to the correlation values as a result of the principal component analysis of *Citrus* spp. species and morphological features is presented in Figure 3. Accordingly, The first (PC_1), second (PC_2) and third (PC_3) principal components represent 50.5%, 32.7% and 13.6% of the total variance, respectively. The first three principal components constitute 96.8% of the total variation. Our principal component analysis findings are higher than the findings obtained by Zhang et al. (2023) in their study on citrus fruits in China. It is estimated that the reason for this difference is due to the different environmental factors in which citrus species are grown. Principal component analysis divides complex data into groups by minimizing the complex data consisting of dependent and independent variables (Yıldız et al., 2023). The high variance analysis results of PC_1, PC_2 and PC_3 in the research findings explain that the classification of *Citrus* spp. species was made successfully.

The grouping of *Citrus* spp. species and their stomatal morphological features in the heat map are presented in Figure 4. Stoma morphological features were divided into two main groups (A and B). These two main groups were divided into 2 subgroups each (A-1, A-2 and B-1, B-2). SW (µm), RSS (%), SL (µm), SPS (µm²), PCI, PL (µm) in A-1 group; SI and PI took part in the A-2 group. SD (stoma/ µm²) and SSC in B-1 group; PW (µm) took part in the B-2 group. *Citrus* spp. species were divided into two main groups (C and D). Here, only group D is divided into two subgroups (D-1 and D-2). *Citrus reticulata* L. is in group C; *Citrus aurantium* L., *Citrus paradisi* L. are in group D-2; all remaining *Citrus* spp. species are in group B-2.

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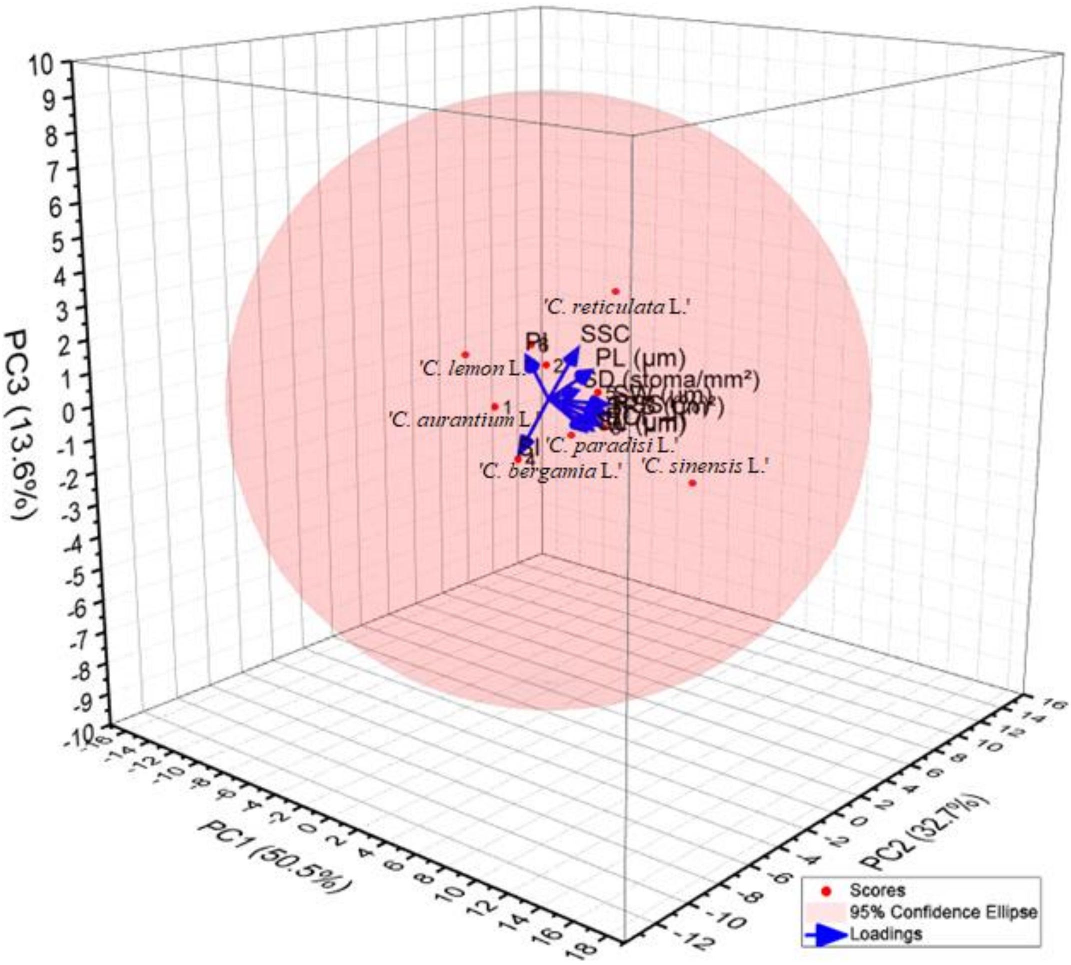

Figure 3. 3D principal component analysis position plot

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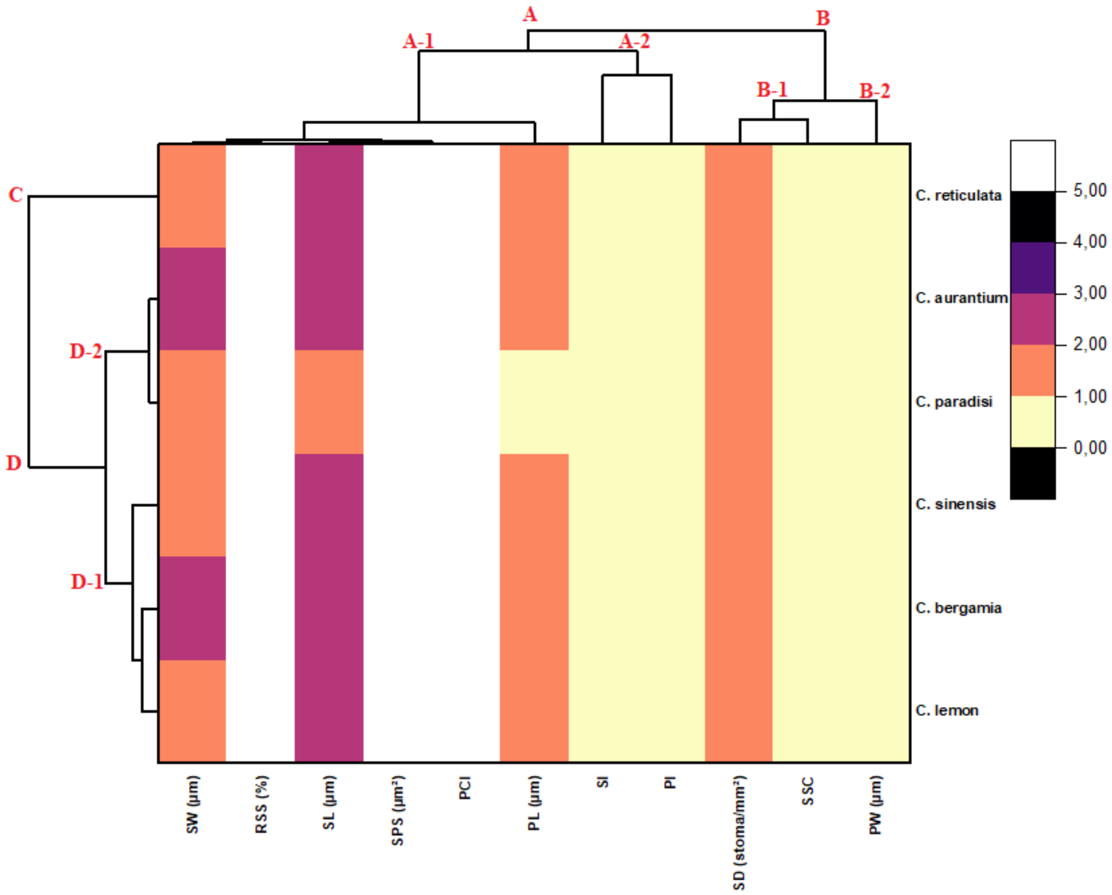

Figure 4. Grouping of Citrus spp. species and stomatal morphological features in the heatmap

4. CONCLUSIONS

As in other fruit species and varieties, stomatal morphology in *Citrus* spp. species varies according to different ecologies and different growing conditions. Therefore, it was concluded that stoma morphology cannot consist only of stoma width, stoma length and stoma density variables. To determine the response of *Citrus* spp. species to drought conditions, different variables such as stomatal index, stomatal shape coefficient, relative stomatal surface, stomatal pore surface, potential conductivity index were added to stomatal morphology. Within the scope of this research, detailed stomatal morphology was determined due to the high production of *Citrus* spp. species in the region. This research serves as a guide for researchers who will work on similar subjects..

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