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Effects of elevational gradient on stomatal morphology of Mastic tree (*Pistacia lentiscus* L.) in Al-Jabal Al-Akhdar region, Libya

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Abstract
Mastic tree (*Pistacia lentiscus* L.*)* is an evergreen endemic species found naturally in Al-Jabal Al-Akhdar region. *P. lentiscus*, is a dioecious evergreen shrub or small tree in the Anacardiaceae family sclerophyllous and can grow up to 3-5 m tall. The purpose of this study was to compare and analyse the effects of elevational gradients on *P. lentiscus* stomata characteristics, including stomata length, stomata width, stomata size, and stomata density. In four regions of Al-Jabal Al-Akhdar (Karsa, Akfanta, Balagra, and Ras Al-Hilal), where they are growing wild on three distinct terraces at varying elevations (90, 282, 623, and 684 m above sea level, respectively). The findings showed that the stomata's length and width decreased with elevation, hence decreasing their size. On the other hand, as height increases, so does the stomata's density.

Keywords: *Pistacia lentiscus* L., mastic, stomata, elevation, altitude

Introduction

Al Jabal Al Akhdar (Green Mountain) is a small mountain chain that is located in Libya's northeast.

Al-Jabal Al-Akhdar is a hill with three increasingly rising terraces, there is an average height above sea level of no more than 200 m. The second terrace has an average elevation of 460 m above sea level and the third terrace is approximately 880 m above sea level. This level has a chilly winter climate, but most of the summer months are moderate (Azzawam, 1995) [3].

A native of several centuries, *Pistacia lentiscus* L. grows throughout the Mediterranean region and is native to Libya, Tunisia, and Algeria (Bonnier & Douin, 1990) [5]. Dioecious and sclerophyllous, it is an evergreen shrub with separate male and female plants. It is thick, grows one to five meters tall, and has green leaves. Its petite, 4-5 mm black globular drupes are accompanied by pinnate leaves. It has a strong resinous fragrance (Zrira *et al.*, 2003) [15]. The mastic tree, which yields mastic resin, the plant's resinous part, is a highly valued medicinal plant that can occasionally attain tree growth form in more protected and humid locations (Munné-Bosch & Penuelas 2003) [12].

P. lentiscus (Anacardiaceae) is a sclerophyllous and dioecious shrub that can grow up to 3 m tall. In more humid and protected environments, it may even achieve the growth form of a tree. Compared to other evergreen species, this species is extremely tolerant of drought and salinity and is found at low elevations. This species is widespread throughout the Mediterranean Basin (Said *et al.*, 2011) [14]. *P. lentiscus* is used extensively in traditional medicine to cure a wide range of conditions, such as ulcers, eczema, diarrhoea, hypertension, and throat infections, in addition to adding taste and preservation to a range of foods (Amessis-Ouchemoukh *et al.*, 2013, Foddai *et al.*, 2015; Adam *et al.*, 2023) [2, 7].

Stomata are essential anatomical components that most terrestrial plants have and that control gas exchange in photosynthetic tissues (Buckley 2019; Lee & Bergmann 2019; Gupta *et al.*, 2020) [11]. Hundreds of stomata, each consisting of two guard cells surrounding an adjustable aperture or pore on the epidermal surface, can be found on a single leaf (Lucas *et al*., 2006; Bergmann & Sack 2007) [4]. Open pores in stomata allow oxygen, carbon dioxide, and water vapor to freely diffuse between the surrounding air and the internal plant tissue (Lin *et al.*, 2022; Wang *et al.*, 2022).

During drought, pores in stomata must contract to retain water, which inhibits the gas exchange required for photosynthesis. It has been shown that the traits of the leaf epidermis are helpful standards to promote taxonomic research among Pistacia species. Studies utilizing light and scanning electron microscopy (SEM) to examine the properties of the genus Pistacia's leaf epidermis are scarce, nevertheless (Kirschbaum, 2004; Gupta *et al.*, 2020; Hsu *et al.*, 2021).

This study aimed to assess how elevational gradients affected the stomatal morphology of Mastic trees (*Pistacia lentiscus* L.) growing wild on three terraces at different elevations (90, 282, 623, and 684 m above sea level, respectively) in the forested areas of the Al-Jabal Al-Akhdar region (Karsa, Akfanta, Balagra, and Ras Al-Hilal).

Materials and Methods

- **Study region description:**

The study locations are in Al-Jabal Al-Akhdar region of Libya (Karsa, Akfanta, Balagra, and Ras Al-Hilal).

- **Sample collection:**

P lentiscus L. plant samples were collected from the study areas (Karsa, Akfanta, Balagra and Ras al Hilal). Healthy leaves were chosen during the September to December 2023 maturity period for stomata analyses, and some samples were placed in the Silphium herbarium. The specimens were identified using Libyan plants found in the Silphium herbarium and the Flora of Libya books to classify plant samples, at the Faculty of Science, Department of Botany, Omar Al-Mukhtar University, Al-Bayda. Leave samples were brought into the laboratory to be dissected, and their stomata were extracted and then placed on slides for analysis and measurement of various traits.

Stomata of leaves

Tools used to prepare stomatal slides transparent adhesive tape, scissors, transparent nail polish, dye, and slides. Preparation steps:

Leaves were dipped into the dye, then removed and allowed to dry.
The nail polish was then applied to the leaf and allowed to dry.
Next, the sticker was applied to the leaf and carefully peeled off.
The label was then positioned on the slide to allow for a screening of stomata.
In the case of clear stomata, the dye can be used.

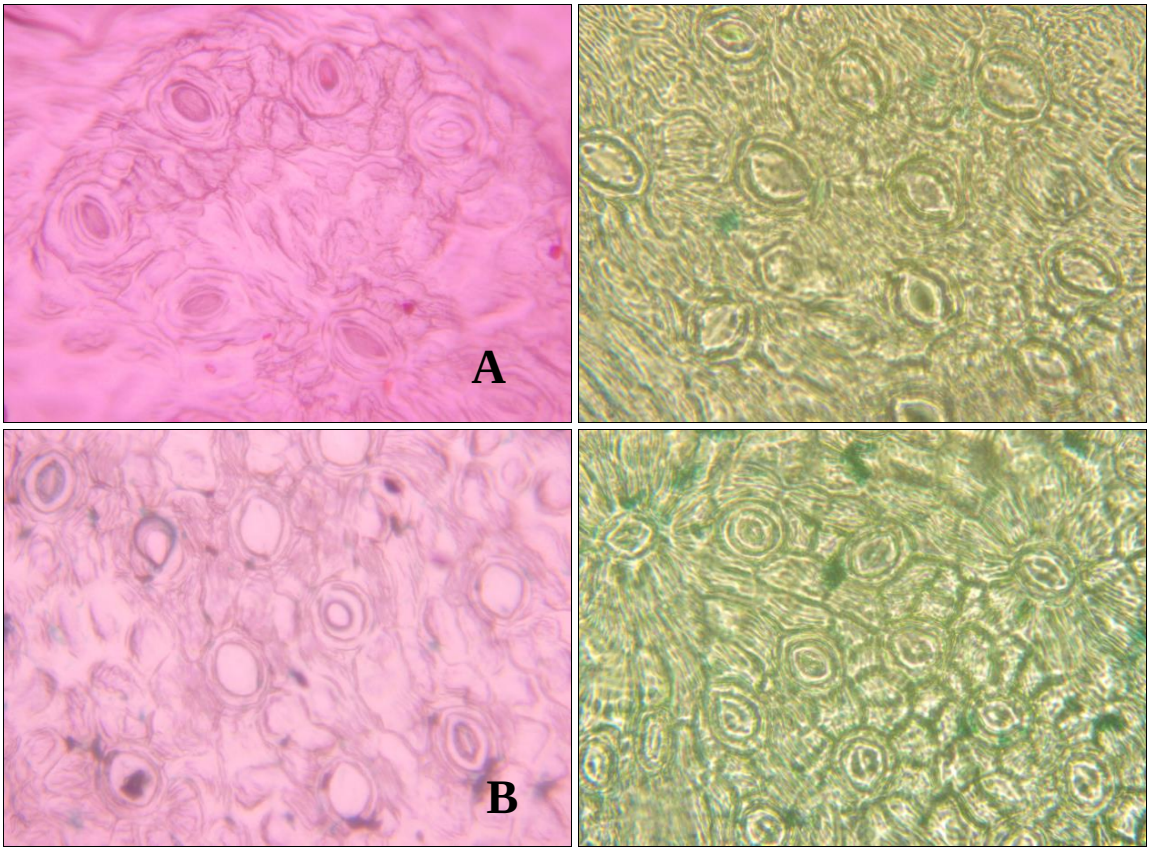
This method is used to measure stomata using a ×40 light microscope. Measurements of stomatal size (width and length) were taken on the abaxial face of P. lentiscus leaves. In contrast, stomatal densities were assessed. An optical microscope with a computer-connected digital camera was used to take these measurements as well as the software (ToupView) for the micromorphological study of leaf surfaces.

Fig 1: Stomata slide processing tools

Results

This study showed that the height factor directly affected the anatomical traits of stomata of leaflets *Pistacia lentiscus* L. such as stomata length, width, size, and density. The first terrace's Karsa area is 90 m above sea level, and had the largest size of stomata in *P. lentiscus* among the study areas, as it contains stomata found at the level of the surface of the epidermis. They have an oval shape. The second terrace's Akfanta area is 282 m above sea level and stomata of *P. lentiscus* are also found at the surface level of the epidermis, and also have an oval shape. Situated on the third terrace, Balagra area is 623 m above sea level, stomata of *P. lentiscus* are also located at the surface level of the epidermis, and have an oval shape, Ras al-Hilal area is located on the third terrace, *P. lentiscus* stomata are also found at the surface level of the epidermis and have an oval shape. Results included stomata's length and width decreased with elevation, hence decreasing their size. On the other hand, as height increases, so does the stomata's density.

Results showed that elevation had an impact on *P. lentiscus* leaves stomata characteristics, including stomata length, width, size and density While stomata length and width did not change significantly at lower elevations, they both did at higher elevations, except for stomata density, which increased with elevation. The highest recorded measure of length and width, the largest size and the lowest density of *P. lentiscus* leaves stomata during this study was in Karsa region. The lowest recorded measure of length and width, the lowest size and the highest density of *P. lentiscus* leaves stomata was in Ras al Hilal region.



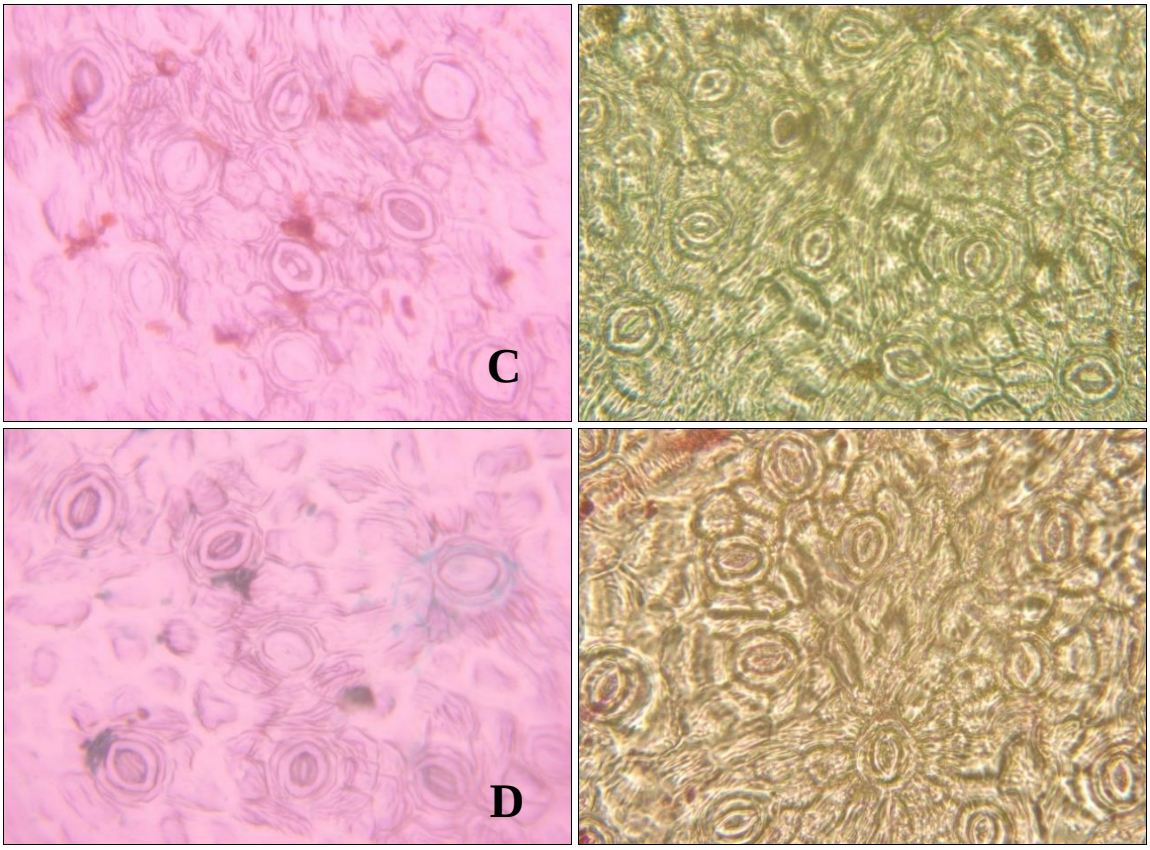


Fig 3: Light microscopic photographs of stomata of *Pistacia lentiscus* L. grown in (A) Karsa region (B) Akfanta region (C) Balagra region (D) Ras al-Hilal region

Table 1: Anatomical traits stomata of a leaflet of compound leaves of *Pistacia lentiscus* L.

Characters	Karsa	Akfanta	Balagra	Ras Al Hilal
Stomata length (µm)	30±5.00 23±2.00	30±3.61 22±2.00	28±2.00 20±3.61	26±1.000 19±2.65
Stomata width (µm)	25±2.52 20±2.00	22±2.00 18±1.000	21±1.000 17±1.000	20±1.000 16±1.000
Stomata size (µm²)	750±187 460±86.0	660±170.1 396±58.0	588±70.0 340±82.7	520±46.0 304±59.8
Stomata density (mm²)	281 ±16.52	325 ±5	303 ±12	346 ±19

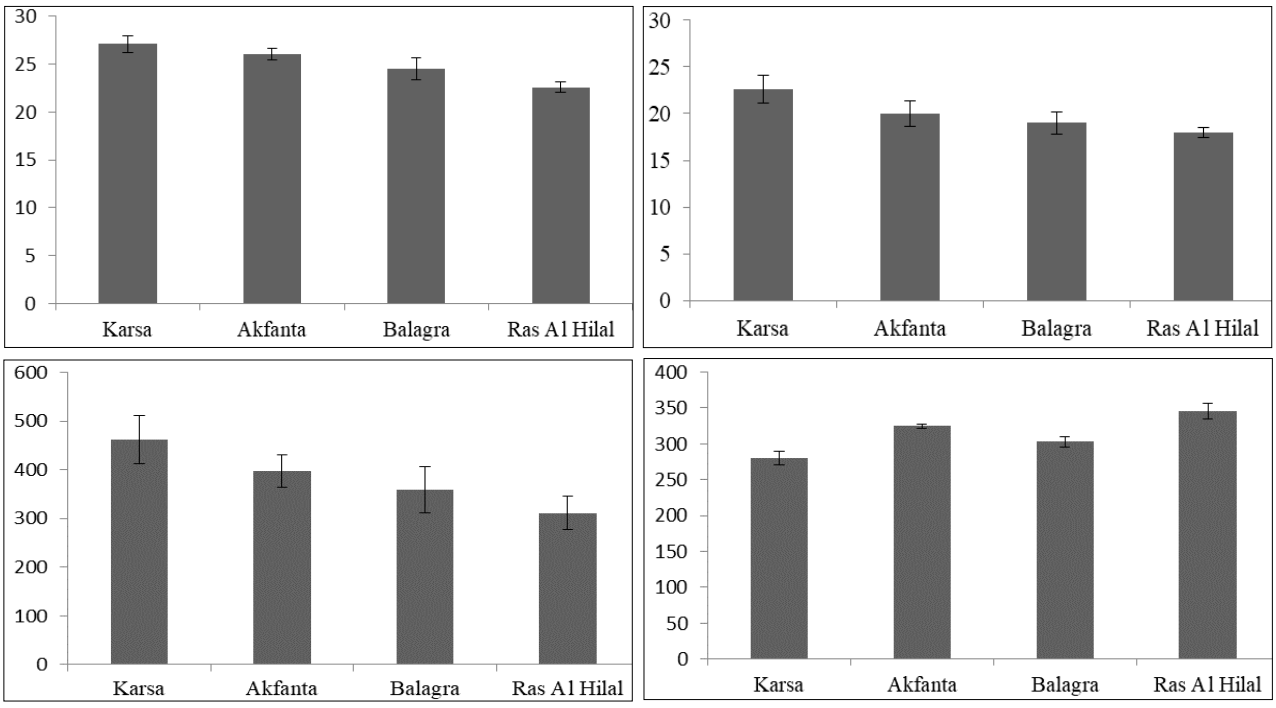


Fig 4: Length, width, size and density of stomata *P. lentiscus* in study regions

Statistical analysis: ANOVA was used to verify the validity and variability of the data, and Minitab version 17 software was used to analyze the numerical values obtained from various parameters measured in order to compute averages and standard deviations that made it easier to compare the impact of altitudinal gradients on *P. lentiscus* leaves stomata characteristics. The alpha level was set at 0.05 ($\alpha = 0.05$), and the means of each treatment were compared to each other using Tukey's test.

Discussion

Studies on the relationship between stomatal density and elevation revealed that leaves at 1,480 m have more stomata on both surfaces than leaves at the two altitudinal extremes, 1,760 m and 950 m (Kofidis & Bosabalidis 2008) [10]. This is in agreement with the current research.

The leaf impression technique was applied to view stomata. Replicas were examined under an optical microscope. Pictures were digitally recorded for each slide at magnifications ×40 and used for stomata measurements. Pistacia species can be reliably identified by leaves stomata. Plants exposed to strong winds develop a high stomatal density but a small stomatal aperture (AL-Saghir & Duncan 2005) [1].

Increased environmental humidity does not always translate into increased stomata number. According to AL-Saghir (2005) [1], the presence of stomata exclusively on the lower surface (hyposto-mati) was the most widespread feature in the genus Pistachio. They identified *P. lentiscus* as hypostomotic and suggested that this variation may be related to the ability of pistachio to adapt to a variety of environmental conditions. The climatic conditions varied at the different sites from which samples were collected (elevation). This illustrates the extraordinary adaptability of this species, allowing it to thrive across a wide range of geographic regions. Describes round and oval lenticular stomata (Doghbage *et al.,* 2023) [6]. This is in agreement with the current research.

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