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Stomatal Distribution in *Pistacia* sp. (Anacardiaceae)

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Abstract: The present research aims to study the stomatal distribution in order to utilize this information for providing more insights into the evolutionary history of the stomata in the genus as well as the evolution and the taxonomy of genus itself. This study is the first one to report the stomatal distribution in all *Pistacia* sp. which was carried out between the months of March and July, 2005. The studied species were the following: *Pistacia aethiopica* J. O. Kokwaro, *P. atlantica* Desf., *P. chinensis* Bunge, *P. eurycarpa* Yaltirik, *P. falcata* Becc. ex Martelli, *P. integerrima* Stew. ex Brand., *P. khinjuk* Stocks, *P. lentiscus* L., *P. mexicana* HBK, *P. mutica* Fisch. and Mey., *P. palaestina* Boiss., *P. terebinthus* L., *P. texana* Swingle, *P. vera* L. and *P. weinmannifolia* Poiss. ex Franch. All species had anomocytic stomata. In most species, the stomata density was higher on the abaxial surface than the adaxial. The ratio of abaxial to adaxial stomatal density varied from 0.0 to 1.7. Stomatal distribution may provide insights into how *Pistacia* species evolve in terms of leaf anatomy and respond to different climatic changes. The study indicates that the primitive anatomical condition in the genus is the occurrence of stomata on both adaxial and abaxial surfaces. Stomatal distribution changed (losing stomata on either surface) as the genus moved into regions of higher rainfall.

Key words: *Pistacia*, stomata, abaxial, adaxial

INTRODUCTION

Pistacia L. is a member of the family Anacardiaceae and consists of 11 species according to Zohary's classification^[1], which is under question. Few systematic studies have been published on this important genus, the first complete classification of the genus was published by Zohary^[1]. In his monograph, Zohary divided the genus into four sections: *Lentiscella* Zoh. (containing *P. mexicana* HBK. and *P. texana* Swingle); *Eu Lentiscus* Zoh. (containing *P. lentiscus* L., *P. saportae* Burnat and *P. weinmannifolia* Poisson); *Butmela* Zoh. (containing *P. atlantica* Desf.) and *Eu Terebinthus* Zoh. (containing *P. chinensis* Bunge., *P. khinjuk* Stocks, *P. palaestina* Boiss. *P. terebinthus* L. and *P. vera* L.). *Pistacia vera*, commonly known as Pistachio, has edible seeds and considerable commercial importance. The other species grow in the wild and their seeds are used as rootstock seed sources and sometimes are used for fruit consumption, oil extraction, or soap production.

Pistacia is a xerophytic genus, which is shown by the presence of many adaptations to aridity, such as advanced development of palisade tissue and extensive root growth that allow *Pistacia* sp. like *P. atlantica* and

P. khinjuk to grow in very harsh and dry areas with low rainfall^[2,3]. A single layer of thin walled epidermal cells characterized both leaflet surfaces of all species. The epidermal cells are covered with a relatively thick layer of cutin in *P. lentiscus*, *P. mexicana* and *P. weinmannifolia*, but little or no cutin is observed in other species. All *Pistacia* sp. have no trichomes. Stomata and their guard cells are the major characteristic of epidermal cells. Stomata occur either on one, or the other, or both surfaces of the leaf^[2,4].

Stomata are small pores on the surface of leaves and stems, bounded by a pair of guard cells that control the exchange of gases, most importantly water vapor and CO₂, between the interior of leaf and the atmosphere^[5]. Gas exchange is regulated by controlling the aperture of the stomatal pore and the number of stomata that form on the epidermis. Environmental signals such as light intensity, the concentration of atmospheric carbon dioxide and endogenous plant hormones control stomatal aperture and development^[5]. For example, plants under high wind conditions develop high stomatal density but stomatal aperture is small. Plants subjected to higher pCO₂ concentrations in the atmosphere decrease the number of stomata^[6].

Rapid stomatal responses to environmental change plays a major role in maintaining the water movement from soil to plant. Aasamaa *et al.*^[7] has demonstrated that stomatal size has a key role in this control and for six forest trees there is a clear negative relationship between the length of the stomatal pore and sensitivity to increasing drought. In these species larger stomata were slower to close and demonstrated a greater potential for hydraulic dysfunction under drought. Ferns from deep shade possess large stomata at low densities^[8] and in this natural environment, which may be cool and humid, it is found that truly shade-tolerant species often retain open stomata, even in deep shade, at least for early parts of the day^[9]. The constancy of the open stomata will minimize the impact of what would be slow opening limitations to photosynthesis during short-lived periods of sunlight, which are crucial for enhancing photosynthesis in this light-limited environment.

Small stomata can open and close more rapidly and their general association with high densities provides the capacity for rapid increases in the stomatal conductance of a leaf, maximizing CO₂ diffusion into the leaf during favourable conditions for photosynthesis^[6]. The effect of growth at elevated concentrations of CO₂ on stomatal density and stomatal index (the fraction of epidermal cells that are stomata) is one of the most intensively studied environmental controls on stomatal development. The reduction in stomatal density with CO₂ enrichment leads generally to a decrease in maximum stomatal conductance but an increase in the maximum rate of photosynthesis, at the elevated CO₂ concentration^[10].

The occurrence of stomata on both adaxial and abaxial surfaces of leaves of some species and not others not readily attributed to any particular selection pressure under which the species may have originated. We suggest that this difference could be related to the ecological plasticity of *Pistacia* sp. to a wide range of environmental conditions. During the field trip in Jordan, plants of *P. atlantica* were found at 200 m below sea level, but the same species was also found at 1200 m above sea level. In addition, during this field trip *P. atlantica* was found in cold regions with high rainfall and in arid regions with low rainfall. This indicates the high level of plasticity of *Pistacia* sp. to live under radically different environmental conditions.

Few anatomical studies have been published on this genus. Grundwag and Werker^[11] described the wood anatomy of *Pistacia* sp. in Israel and Palestine (*P. atlantica*, *P. khinjuk*, *P. lentiscus*, *P. palaestina*, *P. X saportae*, *P. terebinthus* and *P. vera*) and Dong and Bass^[12] performed a similar study in China (*P. chinensis* and *P. weinmannifolia*). Lin *et al.*^[2] characterized leaf

morphology, photosynthesis and leaf conductance of nine *Pistacia* sp. (*P. atlantica*, *P. chinensis*, *P. integerrima*, *P. khinjuk*, *P. lentiscus*, *P. mexicana*, *P. mutica*, *P. terebinthus*, *P. texana*, *P. vera* and *P. weinmannifolia*). EL-Oqlah^[4] described *Pistacia* sp. in Jordan (*P. atlantica*, *P. lentiscus* and *P. palaestina*) morphologically and anatomically. Castro-Díez *et al.*^[13] studied leaf morphology, leaf chemical composition and stem xylem characteristics in two *Pistacia* (*P. lentiscus* and *P. terebinthus*) along a climatic gradient in a study area located in the NE quadrant of the Iberian Peninsula, which extended 350 km from the Atlantic coast to the middle Ebro Basin.

Pistacia is an economically important genus because it has the pistachio crop, *P. vera*, which has edible seeds of considerable commercial importance. The evolutionary history of the genus (including the context of the evolution of the stomata) and the taxonomic relationship among the species are controversial and not well understood. This study is a part of a comprehensive phylogenetic study that has been conducted on this genus to refine taxonomic and evolutionary relationship utilizing different types of data (including morphology, cytology, anatomy and molecular) for a doctoral thesis. It aims to study the stomatal distribution in order to utilize this information for providing more insights into the evolutionary history of the stomata in the genus as well as the evolution and the taxonomy of genus itself. This study is the first one to report the stomatal distribution in all *Pistacia* sp.

MATERIALS AND METHODS

This study was carried out between the months of March and July 2005. Leaves of *P. atlantica*, *P. khinjuk*, *P. lentiscus*, and *P. palaestina* were collected by the senior author during a field trip to Jordan in mid-summer 2004, while leaves of *P. aethiopica*, *P. chinensis*, *P. eurycarpa*, *P. falcata*, *P. integerrima*, *P. mexicana*, *P. mutica*, *P. terebinthus*, *P. texana*, *P. vera* and *P. weinmannifolia* were obtained from herbarium specimens. Herbarium specimens were examined from the following herbaria: Field Museum, Chicago, Illinois, USA (F); Missouri Botanical Garden, St. Louis, Missouri, USA (MO); Royal Botanic Garden, Edinburgh, UK (E); Royal Botanical Gardens, Kew, UK (K) and Virginia Polytechnic Institute and State University, Blacksburg, Virginia, USA (VPI).

Stomata were counted from ten samples of each sp. included in this study, two from each specimen. For studying stomatal distribution, epidermal replicas of leaflets were made by coating the adaxial and abaxial

surfaces with clear fingernail polish. The dried films were then peeled and mounted on slides. Replicas were observed using a BX61 Olympus microscope under 40 x magnification. Stomatal density was determined for each surface by randomly counting them for mm² (measured by micrometer) in 10 different fields in five different plants then the average was calculated.

There was no special procedure performed with the samples. No treatment of the herbarium specimens or specific fingernail polish at specific concentration was used.

RESULTS

All species had anomocytic stomata (Fig. 1 and 2). Guard cells were not situated at the same level as adjacent epidermal cells and there were no subsidiary cells surrounding the guard cells. Our results disagree with the results of the study made by Lin *et al.*^[2], who reported that all *Pistacia* sp. (*P. atlantica*, *P. chinensis*, *P. integerrima*, *P. khinjuk*, *P. lentiscus*, *P. mexicana*, *P. mutica*, *P. terebinthus*, *P. texana*, *P. vera* and *P. weinmannifolia*) had actinocytic stomata. Stomatal density on both surfaces as well as the size of the stomata varies from species to species. (Table 1). However, in most species, stomatal density was higher on the abaxial surface than on the adaxial. The ratio of abaxial to adaxial stomatal density varied from 0.0 to 1.7. In *P. atlantica*, *P. eurycarpa*, *P. mutica* and *P. vera*, the stomata were observed to have low ratios on adaxial and abaxial

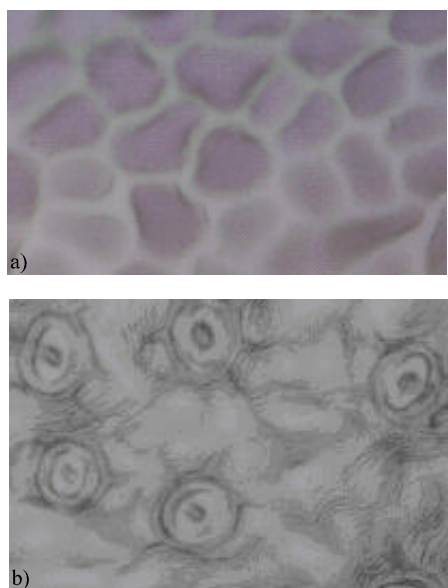


Fig. 1. Paradermal sections of a *P. lentiscus* leaflet
a) adaxial surface, b) abaxial surface

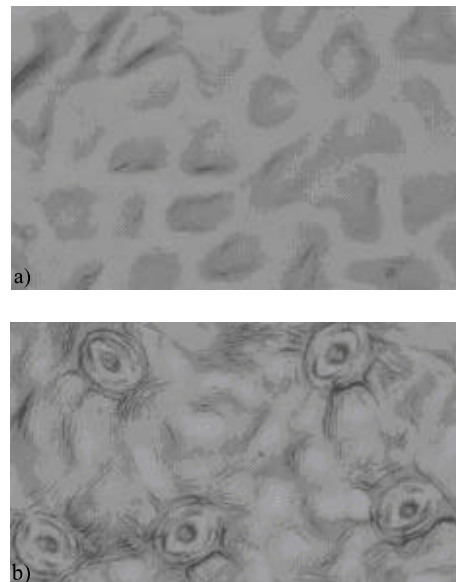


Fig. 2. Paradermal sections of a *P. terebinthus* leaflet
a) adaxial surface, b) abaxial surface

Table 1: Stomatal density (per mm²) on the adaxial and abaxial leaf surfaces of *Pistacia* sp.

	Adaxial	Abaxial	Total	Ratio Ad Ab ⁻¹
<i>P. aethiopica</i>	0.0	292.5	292.5	0.0
<i>P. atlantica</i>	242.5	150.0	392.5	1.6
<i>P. chinensis</i>	467.5	280.0	27.9	1.7
<i>P. eurycarpa</i>	235.0	137.0	372.0	1.7
<i>P. falcata</i>	242.5	0.0	242.5	---
<i>P. integerrima</i>	0.0	545.0	545.0	0.0
<i>P. khinjuk</i>	337.5	0.0	337.5	---
<i>P. lentiscus</i>	0.0	312.53	12.5	0.0
<i>P. mexicana</i>	0.0	225.0	225.0	0.0
<i>P. mutica</i>	210.0	165.0	350.0	1.3
<i>P. palaestina</i>	0.0	255.0	255.0	0.0
<i>P. terebinthus</i>	0.0	285.0	285.0	0.0
<i>P. texana</i>	0.0	225.0	225.0	0.0
<i>P. vera</i>	130.0	162.5	292.5	0.8
<i>P. weinmannifolia</i>	0.0	365.0	365.0	0.0

*Ad = Adaxial, Ab = Abaxial

surfaces with relatively large stomata. In these species, stomatal density on the adaxial surface was slightly higher than on the abaxial surface. In *P. chinensis*, stomata were observed on both adaxial and abaxial surfaces; stomatal density was notably higher on the adaxial surface with small stomata. Stomata were observed only on the adaxial surface in *P. falcata* and *P. khinjuk* with large stomata. Stomata were observed only on the abaxial surface in *P. aethiopica*, *P. Integerrima*, *P. lentiscus*, *P. mexicana*, *P. texana*, *P. palaestina*, *P. terebinthus* and *P. weinmannifolia* and they have large stomata.

DISCUSSION

Stomatal control of water loss allows plants to occupy habitats with fluctuating environmental conditions. Stomata first appeared in terrestrial land plants over 400 million years ago (Myr)^[14] and since then have changed markedly in size and density on plant surfaces.

Loss of stomata may have occurred in *Pistacia* species as an adaptation to changes in climate, from relatively even distribution of rainfall and temperature to seasonal variation with higher rainfall and warmer temperatures. This is supported by the presumably more advanced *Pistacia* sp. having smaller elongated leaflets with pointed shoot apices, which are more efficient for water removal from the leaf surface. This would be a useful adaptation as the genus moved into regions of higher rainfall^[15]. We already elaborated on this relationship between the stomata and the climatic factors in the introduction.

Our morphological results (not shown here) indicate that *P. vera* is the most primitive species in the genus. So we suggest that the occurrence of stomata on both surfaces of leaf is the primitive state of this character and the occurrence on either surface is the advanced one in *Pistacia* sp. The most common character trend in the genus was the occurrence of stomata only in abaxial surface in a low density and large size. There was a reversal of the character to the ancestral state in *P. atlantica*, *P. mutica* and *P. eurycarpa*. Moreover, morphological results show that the genus can be divided into sections, *Lentiscus* and *Terebinthus*. Section *Terebinthus* contains the deciduous species (*P. atlantica*, *P. chinensis*, *P. eurycarpa*, *P. falcata*, *P. integerrima*, *P. khinjuk*, *P. mutica*, *P. palaestina*, *P. terebinthus* and *P. vera*) and the other group section *Lentiscus* contains the evergreen species (*P. aethiopica*, *P. lentiscus*, *P. mexicana*, *P. texana* and *P. weinmannifolia*). We noticed that stomata were observed only in abaxial surface in section *Lentiscus* and in two deciduous species (*P. palaestina* and *P. terebinthus*) in section *Terebinthus*. However, in the section *Terebinthus* there was a difference and no consistency in terms of occurrence of stomata. Moreover, this is consistent with our morphological results in that section *Lentiscus* was more homogeneous than *Terebinthus*. We suggest that the two deciduous species share the same character with ever green because they inhabit the same environmental conditions (*P. lentiscus*, *P. palaestina* and *P. terebinthus* are the major elements of Mediterranean basin).

This study provides more data (stomatal distribution) that can be used in combination with morphological and molecular data to refine the taxonomic relationships

among the different *Pistacia* sp. and map these differences into the phylogenetic tree of the genus. They may be used as key taxonomic traits to distinguish between the highly similar species. of *Pistacia*^[4]. For example, we found that there are anatomical differences between *P. khinjuk* and *P. vera* in terms of the occurrence of stomata (Table 1). In *P. khinjuk*, stomata were found only on the adaxial surface, while in *P. vera* they were found in both adaxial and abaxial surfaces. These differences occurred even though these two sp. are highly similar morphologically, so stomatal distribution can be used as a key taxonomic trait to distinguish between the two species.

Based on the correlation between the stomata and the wide range of the environmental conditions in which *Pistacia* sp. can grow, we suggest that the *Pistacia* sp. mainly inhabit humid and cool areas and are subjected to high wind conditions.

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