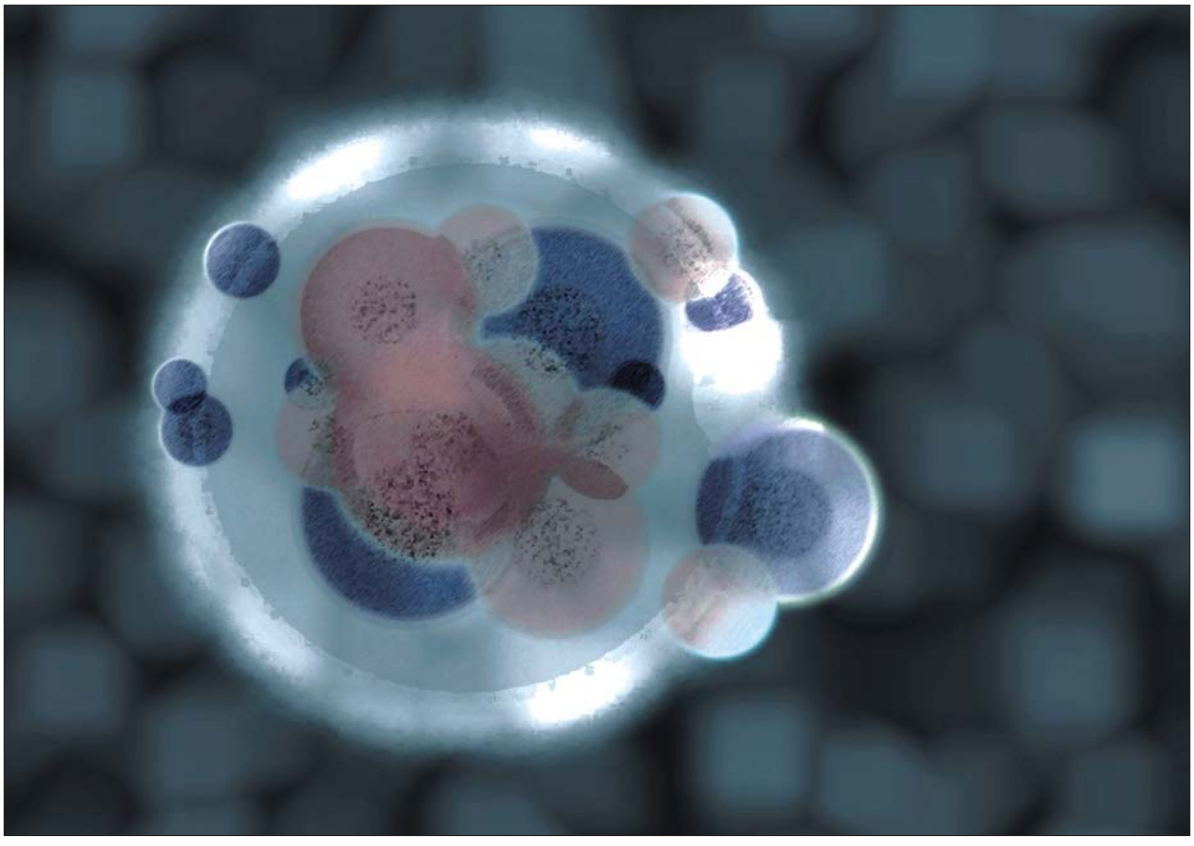

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Leaf Anatomy of *Pistacia* Species (Anacardiaceae)

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Abstract: *Pistacia* is an economically important genus because it contains the pistachio, *P. vera,* which has edible seeds of considerable commercial importance. The evolutionary history of the genus and the taxonomic relationships among the species are equivocal. This study is part of a comprehensive phylogenetic study that has been conducted on this genus in order to refine taxonomic and evolutionary relationships by utilizing different types of data (including morphological, cytological, anatomical and molecular). We aim to investigate the evolutionary and taxonomic relationships among *Pistacia* species. Here we present the first investigation of the leaf structure in all *Pistacia* species. The following species were studied: *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. Leaflets of *P. vera*, which have random orientation, were isobilateral, while leaflets of the other species were dorsiventral and were oriented horizontally.

Key words: *Pistacia*, *vera*, anatomy, taxonomy, isobilateral, dorsiventral

INTRODUCTION

Pistacia L. is a member of the family Anacardiaceae and consists of 11 species according to Zohary's classification (Zohary, 1952). Few systematic studies have been published on this important genus, the first complete classification of which was published by Zohary (1952). 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* Poiss.); *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 are used as rootstock sources and for fruit consumption, oil extraction, or soap production.

Few anatomical studies have been published on *Pistacia* species and those studies have not utilized all species in the genus. Grundwag and Werker (1976) described the wood anatomy of *Pistacia* species in Israel and Palestine (*P. atlantica*, *P. khinjuk, P. lentiscus*, *P. palaestina*, *P.* X *saportae, P. terebinthus* and *P. vera*) and Dong and Bass (1993) performed a similar study in China (*P. chinensis* and *P. weinmannifolia*). Lin *et al.* (1984) characterized leaf morphology, photosynthesis and leaf conductance of nine *Pistacia* species. (*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 (1996) described *Pistacia* species in Jordan (*P. atlantica*, *P. lentiscus* and *P. palaestina)* morphologically and anatomically. Castro-Díez *et al.* (1998) studied leaf morphology, leaf chemical composition and stem xylem characteristics in two *Pistacia* species. (*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. AL-Saghir and Porter (2005) studied leaflet stomatal distribution in the genus.

The evolutionary history of the genus and the taxonomic relationships among the species are not well understood. This study is a part of a comprehensive phylogenetic study that has been conducted on this genus in order to refine taxonomic and evolutionary relationships by utilizing different types of data (including morphological, cytological, anatomical and molecular). We aimed to study the leaf structure in order to utilize this information for providing more insights into the evolutionary history and taxonomy of the genus. This study is the first to investigate internal leaf structure of all *Pistacia* species.

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MATERIALS AND METHODS

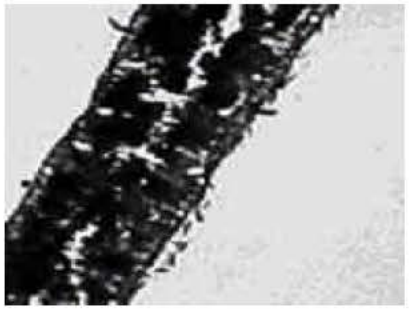
Leaves of *P. atlantica*, *P. khinjuk*, *P. lentiscus* and *P. palaestina* were collected by the senior author during a field trip to Jordan in mid-summer 2004, while leaves of *P. aethiopica*, *P. chinensis*, *P. eurycarpa*, *P. falcata*, *P. integerrima*, *P. mexicana*, *P. mutica*, *P. terebinthus*, *P. texana*, *P. vera* and *P. weinmannifolia* were obtained from herbarium specimens. Herbarium specimens were examined from the Field Museum, Chicago, Illinois, USA (F); Missouri Botanical Garden, St. Louis, Missouri, USA (MO); Royal Botanic Garden, Edinburgh, UK (E); Royal Botanic Gardens, Kew, UK (K); and Virginia Polytechnic Institute and State University, Blacksburg, Virginia, USA (VPI). Portions of leaflet lamina were cut into 4-10 mm^2 sections prior to rehydration. Tissues were rehydrated in a series of 25% alcohol, 10% alcohol and distilled water. The rehydrated sections were stained in saturated aqueous safranin-O. The stained tissues were dehydrated in an alcohol series, 50: 50% alcohol and Xylene and 100% Xylene. The dehydrated tissues were infiltrated in a series of 50: 50% Xylene and paraffin oil and 100% paraffin oil followed by a second series of molten paraplast. The infiltrated tissues were embedded in paraffin in casting boats. Paraffin embedded blocks were sectioned transversely at 2 μm thickness with razor blades on a rotary microtome (MICROM Inc. Walldorf, Germany). Slides were observed and photographed using an Olympus BX50 microscope with a 40x ocular.

RESULTS

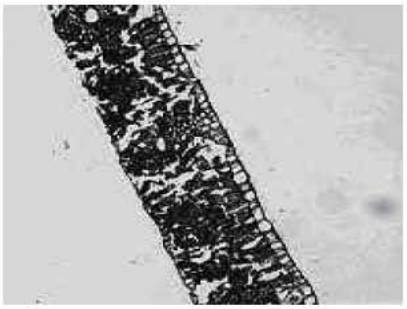
The cross sections of the leaflet lamina show a single layer of thin-walled epidermal cells on both leaflet surfaces of all species covered with a relatively thick layer of cutin in *P. aethiopica*, *P. lentiscus*, *P. mexicana*, *P. taxana* and *P. weinmannifolia* (Fig.1c), but little or no cutin was observed in other species All *Pistacia* species have trichomes on their leaves, which has never been reported for the genus.

The leaflets of *P. vera* differ from those of other species (Fig.1a). The leaflets are randomly oriented and their adaxial and abaxial surfaces are similar. They are isobilateral and the adaxial palisade cells are shorter than those of other species. The abaxial palisade consists of two layers of cells that appear slightly longer than the spongy mesophyll of other species. These cells are less densely packed than those of the adaxial layer of the same leaves.

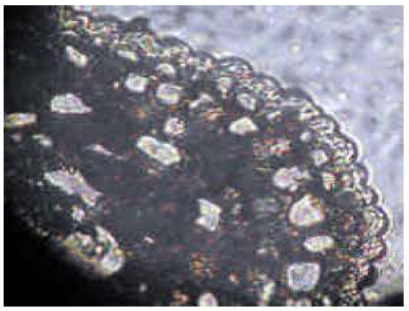
Leaflets of the other species are dorsiventral in appearance (Fig.1b),. Their adaxial palisade consists of one layer that makes up most of the lamina thickness compared to that of *P. vera*. Many cells in the spongy tissue were palisade-like in appearance (i.e., long, narrow and oriented perpendicular to the abaxial surface). However, they were interspersed with spongy mesophyll cells and not arranged in a dense palisade- like tissue. The abaxial spongy mesophyll consists of several layers and the cells are very similar in appearance and density among all species except *P. vera*.



(a)



(b)



(c)

Fig. 1: Cross sections of a: *P. Vera* leaflet, b: *P. Atlantica* leaflet and c: P. Mexicana leaflet

DISCUSSION

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. These adaptive traits allow species like

P. atlantica and *P. khinjuk* to grow in very harsh and dry areas with low rainfall (Spiegel-Roy *et al*., 1977; Lin *et al*., 1984).

The current study indicates, excluding *P. vera*, that the leaflet internal anatomy of *Pistacia* species are homogenous. Our results agree with Lin *et al*. (1984). The similarity in leaf anatomy supports and the presence of trichomes on all species supports a close taxonomic relationship among the different species.

Our other morphological data (not shown here) indicate that *P. vera is* the most primitive species in the genus. We suggest that the isobilateral appearance of both leaflet surfaces is the primitive state of this character and the dorsiventral appearance is the advanced one in *Pistacia* species. There was no reversal to the ancestral state in this genus. Moreover, our other morphological results show that the genus can be divided into two sections, *Lentiscus* and *Terebinthus* (Parfitt and Badenes, 1997). 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 section *Lentiscus* contains the evergreen species (*P. aethiopica, P. lentiscus*, *P. mexicana P. texana* and *P. weinmannifolia*). The present study indicates a close relationship between the two sections and supports the monophyly of the genus (AL-Saghir and Porter, 2005).

This study provides more data that can be used in combination with morphological and molecular data to refine the taxonomic relationships among the different *Pistacia* species. Anatomical differences may be used as key taxonomic traits to distinguish between the highly similar species of *Pistacia* (EL-Oqlah, 1996). For example, we found that there are anatomical differences between *P. khinjuk and P. vera* in terms of their internal leaflet anatomy. The leaflets of *P. vera* are randomly oriented and isobilateral. The adaxial palisade cells are shorter than those of other species. The abaxial palisade consists of two layers of cells that appear slightly longer than those of other species. In contrast, the *P. khinjuk* leaflet is dorsiventral. The adaxial palisade is one-layered and makes up most of the lamina thickness compared to a much smaller portion in *P. vera.* Many cells in the spongy tissue were palisade-like in appearance. These differences were found even though the morphological traits of these two species are highly similar. Thus, the anatomical differences can be used as a key taxonomic trait to distinguish between the two species.

We have identified isobilateral leaves as an additional ancestral trait for *Pistacia*. Moreover, our anatomical evidence supports the monophyletic nature of *Pistacia* because all species have trichomes and all species except *P. vera* have dorsiventral leaves.

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