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LEAF SURFACE ANATOMY IN SOME WOODY PLANTS FROM NORTHEASTERN MEXICO

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Abstract

Studies on leaf surface anatomy of woody plants and its significance are rare. The present study was undertaken in the Forest Science Faculty Experimental Research Station, UANL, Mexico, with objectives to determine the variability in leaf surface anatomy in the woody plants of the Tamaulipan thornscrub and its utility in taxonomy and possible adaptation to the prevailing semiarid conditions. The results show the presence of large variability in several leaf anatomical traits viz., waxy leaf surface, type of stomata, its size, and distribution. The species have been classified on the basis of various traits which can be used in species delimitation and adaptation to the semiarid condition such as waxy leaf surface, absence sparse stomata on the leaf surface, sunken stomata. The species identified as better adapters to semi-arid environments on the basis of the presence and absence of stomata on both adaxial and abaxial surface viz., *Eysenhardtia texana, Parkinsonia texana, Gymnosperma glutinosum, Celtis laevigata, Condalia hookeri* and *Karwinskia humboldtiana*.

Key words: Leaf surface, Stomata type, Abundance, Waxiness, Woody plants, Northeastern Mexico.

Introduction

Leaves contribute greatly to the productivity of plants in a forest ecosystem through photosynthesis, gas exchange (CO_2, O_2), and transpiration. Stomata plays an important role in these vital functions through stomatal pores. The types and size of stomata vary greatly among species, used in the taxonomic determination of the species along with leaf anatomical features.

Leaves among different plant species vary widely in shape, size, margin, and venation. The epidermis consists of the upper epidermis called adaxial surface and lower epidermis called abaxial surface; it aids in the regulation of gas exchange via stomata. The epidermis is one layer thick, but may possess more layers to prevent transpiration. The epidermis helps in the regulation of gas exchange. It contains stomata, which are openings through which the exchange of gases takes place. Two guard cells surround each stomata regulating its opening and closing. Guard cells are the only epidermal cells to contain chloroplasts.

Leaf anatomical traits play an important role in the taxonomic delimitation of species and its relation to adaptation to environment such as arid or semiarid conditions. The compact or loose palisade layers also vary. The density of trichomes on leaf surface has been related to the tolerance to insects and drought resistance in crops like sorghum, cotton, sunflower, etc. Only few studies have been undertaken on the variability in leaf surface anatomy and its utility in taxonomy and adaption to the environmental stresses.

A study was undertaken on the morphological and anatomical characteristics of cones, leaves and branchlets on 293 trees of *Pseudotsuga* in Mexico obtained from 19 localities of different geographic regions. The results showed significant differences in morpho-anatomical characters sufficient to separate different species (Reyes Hernández *et al*., 2005). Similarly, a study was carried on the variability in twelve species of *Populus* leaf anatomy and morphology in relation to canopy position, biomass production and varietal taxon (Afas *et al*., 2007). They examined canopy position, anatomical characteristics and morphological leaf characteristics such as thickness of epidermis, of palisade and spongy parenchyma layers, density and length of stomata, leaf area, specific leaf area (SLA) and nitrogen concentration for mature leaves from all genotypes at two canopy positions (upper and lower canopy). The spongy parenchyma layer was thicker than the palisade parenchyma layer for all genotypes and irrespective of canopy position, except for genotypes belonging to the *P. deltoides* × *P. nigra* taxon (section *Aigeiros*). Leaves at the upper canopy position had higher stomatal density and thicker anatomical layers than leaves at the lower canopy position. A principal component analysis (PCA) showed that genotypes belonging to the same taxon had similar anatomical characteristics, and genotypes of the same section also showed common leaf characteristics. However, Wolterson (*P. nigra*) differed in anatomical leaf characteristics from other genotypes belonging to the same section (section *Aigeiros*). Hybrids between the two sections (Aigeiros × Tacamahaca) expressed leaf characteristics intermediate between both sections, while their biomass production was low (Afas *et al*., 2007). On the other hand, various anatomical traits are used to determine the adaptation of the species to different habitats. In this regard, a study was carried out on the variability of leaf anatomical characteristics of *Solanum nigrum* from different habitats in Europe and in Yugoslavia revealed that *S. nigrum* lamina has the mesomorphic structure with some xero-heliomorphic adaptations. The differences were observed in terms of stomata number, number of hairs, thickness of lamina, palisade and spongy tissue, as well as the size of mesophyll cells of the species collected from different localities. The highest values for most of the parameters were observed for the plants from cultivated soil. Largest variations of the characters were observed for the leaves from ruderal habitats, under highly variable environments (Krstic *et al*., 2002). A similar study was carried out on the anatomical basis of resistance on plant species of a typical arid Mediterranean ecosystem to confirm the presence of several adaptive properties for the macchia ecosystem. Most of the species examined showed the presence in their internal leaf tissues of ergastic substances, mainly tannins and calcium oxalate, which act with defensive functions, crucial in the adaptive resistance of plants to water stress. Nearly all the species presented adaptations for protection against the photo damage possibly induced from the strong UV-B solar irradiance in the summer. The more significant anatomical features were the trichomes, covering the abaxial surfaces of leaves which maintained water budget of the plants both by influencing the diffusion boundary layer of the leaf surface and by regulating leaf optical parameters and, hence, leaf temperature. In many species, when trichomes or wax layers reduced radiation absorbance, two or three layers of palisade parenchyma were present, presumably to provide a better efficiency in utilizing the photosynthetic light. In almost every plant examined, stomata were sunken or well protected (Rotondi *et al*., 2003). The orchid *Cattleya jenmanii* has ornamental value in Venezuela. A comparative study made on leaf epidermis of *C. jenmanii In vitro* conditions demonstrated that compared to normal ones, the epidermal cells showed larger in size, lower anticlinal cell wall, lower size of stomata for adaptation of orchid, the leaves suffered changes in cells with an increase of mechanical resistance and rigidity (Torres *et al*., 2006).

An ecophysiological study was undertaken on two urban forestry species (*Azadirachta indica* and *Millettia thonningii*) which included the pattern of transpiration, stomatal movement, relative humidity and few anatomical features. It was assessed that the pattern of transpiration in *M. thonningii* was low in the morning, high in the noon and low in the afternoon. The relative water content was more than 50% in both the species. *Millettia thonningii* escaped drought by shading with leaves but *A. indica* was evergreen. Leaf anatomical study revealed the presence of thick cuticle and high stomatal frequency in *A. indica* and a low stomatal frequency in *M. thonningii* (Dzomeku & Enu-Kwes, 2006). Noman *et al*. (2014) studied the inter-cultivar genetic potential of *Hibiscus* in relation to leaf anatomical characteristics demonstrating significant variability among cultivars anatomical characteristics. Compared to other cultivars, cultivars Lemon shiffon and Wilder's white possessed more epidermal thickness, increased epidermal cell area, high cortical cell area and incremented stomatal density as compared with other cultivars. On the other hand, cultivars Cooperi alba, Mrs. George Davis and Frank green possessed least cortex cell area, lowest xylem region thickness and minimum phloem region thickness respectively. Overall, it was concluded that anatomical genetic potential has endorsed cultivars Lemon chiffon and Wilder's white with enormous capability to grow well under variable environments.

The leaf anatomy of 15 species of vascular plants occurring in coastal zones of the Falcon State (Venezuela) was carried out to evaluate their potential adaptive value of leaf anatomical features to the saline environment. The results revealed that the development of water storing tissue in the mesophyll and/or epidermal cells is the main characteristic associated with the saline habitat in those species. Besides, other characteristics of potential adaptive value were: presence of trichomes, stomata protected by papillae, crystals in mesophyll cells, secretory tissues, and Kranz anatomy (García *et al*., 2008).

A study undertaken on the foliar anatomy of shrubs in the semiarid region in Argentina reveals that some of the characters related to adaptation were stellate trichomes in *Capparicordis tweediana* and *Ruprechtia triflora*, cystolitic trichomes in *Celtis* species, and multicellular peltate scales in *Zanthoxylum coco* (Arambarri *et al*., 2011). Presence of papillose epidermis (e.g., *Schinopsis lorentzii*); ciclocytic stomata in *Bulnesia sarmientoi*, *Maytenus vitis-idaea*, *Moya spinosa* and *Schinopsis* spp.; idioblastic crystalifer epidermal cells (*Scutia buxifolia*); crystaliferous epidermis (*Maytenus vitis-idaea*); multilayered epidermis (*Jodina rhombifolia*); presence of hypodermis in *Castela coccinea*, *Maytenus vitis-idaea*, *Prosopis ruscifolia* and *Ziziphus mistol*; bicollateral vascular bundles in *Lycium cestroides*; presence of crystal sand in *Calycophyllum multiflorum* and *Lycium cestroides*; absolute absence of crystals in the family Capparaceae (Arambarri *et al*., 2011). In another study, Arambarri *et al*. (2012) evaluated leaf anatomical traits that contribute to better understanding of the plant-environment relationships, and to the development of technologies for a sustainable use and conservation of Chacoan forests in Argentina. Various anatomical characters such as the density of epidermal cells, stomata and trichomes, mesophyll types and the type and distribution of vascular and sclerenchymatic tissues were studied. Most trees of Chacoan Oriental possessed distinct hypostomatic leaves with dorsiventral mesophyll and had a high density of epidermal cells (4000-7000/mm^2), intermediate density of stomata (300-500/mm^2) and low density of trichomes (<35/mm^2). Subsequently, a study was undertaken on morpho-anatomical characteristics of *Celtis ehrenbergiana* revealed that its shade leaf structure was bifacial, epidermis was with wavy-sinuous anticlinal cell walls, mesophyll formed by palisade and spongy parenchyma, and angular-lacunar collenchyma. The sun leaf type had a thick leaf-limb which was leathery and dark green and its anatomical structure was equifacial, epidermis with straight anticlinal cell walls, mesophyll formed by homogeneous palisade parenchyma, and angular-massive collenchyma. There was nothing to scarcely stomata on adaxial surface in shade type leaf, however there were numerous in the sun type leaf. *Celtis ehrenbergiana* exhibited phenotypic plasticity indicating that it might be able to survive the climatic changes because of its adaptability and has an advantage over other species (Nughes *et al*., 2013).

In the context of the literature survey, it is well documented that leaf anatomical traits play an important role in taxonomy and adaptation of the tree species to environmental stress such as drought, cold and high temperature. The density of trichomes, cuticular thickness, compact palisade, presence of tannins, phenolics, other exudates are related to resistance to water loss. On the basis of these traits, the species can be categorized for their adaptation to different environments of abiotic and biotic stress. There is a great necessity to direct research inputs in this direction.

Materials and Methods

A preliminary study was undertaken on dermal anatomy of few native woody species of northeastern Mexico (Table 1). The study involves 3 aspects: 1) Development of two techniques for leaf impressions; 2) Characterization of leaf surface architecture of 18 woody species on both adaxial and abaxial leaf surface and 3) Characterization of leaf surface of 8 species on the abaxial surface (owing to the fact we could not take good impression for presence of high pubescence) which are discussed in the following section.

Table 1. Scientific name and family of studied species.

Species	Family
Acacia berlandieri (Benth.) Britton & Rose	Fabaceae
Acacia farnesiana (L.) Willd.	Fabaceae
Acacia rigidula (Benth.) Seigler & Ebinger	Fabaceae
Acacia schaffneri (S. Watson) F.J. Herm.	Fabaceae
Amyris madrensis S. Watson.	Rutaceae
Amyris texana (Buckley) P. Wilson	Rutaceae
Azadirachta indica A.Juss.	Meliaceae
Berberis chococo Schlecht.	Berberidaceae
Celtis laevigata C. von Willdenow.	Ulmaceae
Celtis pallida Torrey	Ulmaceae
Condalia hookeri M.C. Johnst.	Rhamnaceae
Diospyros palmeri Eastw.	Ebenaceae
Diospyros texana Scheele.	Ebenaceae
Ehretia anacua (Terán & Berland.) I.M.Johnst.	Boraginaceae
Eysenhardtia texana Scheele	Fabaceae
Eysenhardtia texana Scheele	Fabaceae
Forestiera angustifolia Torr.	Oleaceae
Guaiacum angustifolium Engelm.	Zygophyllaceae
Gymnosperma glutinosum (Spreng.) Less.	Asteraceae
Havardia pallens (Benth.) Britton & Rose	Fabaceae
Karwinskia humboldtiana (Schult.) Zucc.	Rhamnaceae
Leucaena leucocephala (Lam.) de Wit	Fabaceae
Parkinsonia texana (A. Gray) S. Watson	Fabaceae
Prosopis laevigata (Humb. & Bonpl. ex Willd.) M.C.Johnst.	Fabaceae
Quercus virginiana Mill.	Fagaceae
Rhus virens Lindh. ex A. Gray.	Anacardiaceae
Sideroxylon celastrinum (Kunth) T.D.Penn	Sapotaceae
Uncaria guianensis (Aubl.) J.F.Gmel.	Rubiaceae
Zanthoxylum fagara (L.) Sarg.	Rutaceae

Development of techniques: We are presenting here the dermal structure of few plant species that grow and develop in the northeastern region of Mexico. Simple techniques have been developed to study epidermal tissue of leaves (Maiti unpublished).

Technique 1: Small pieces of thermocol are slowly dissolved in small amount of xylene in a petri dish to bring to a consistency of honey. Then, the solution is applied with the help of little finger on both upper and lower surface of leaves of each species in the region in between the midrib and margin. Then, they left on bench for drying. Once dried, a piece of transparent tape is applied and pressed on the region with a finger. Finally the tape is taken out with leaf impression and pressed on a slide in the same direction. Now it is permanent and ready to observe under microscope.

Technique 2: In this technique, a small portion of leaf lamina of each species are put in a mixture of 10% (v/v) chromic acid:10% (v/v) nitric acid in a test tube and the mouth of test tube is plugged with cotton. Thereby, several test tubes containing several species are kept boiling in water bath in a beaker for 15 minutes. Thereafter, leaf lamina is clear. Then a portion of the leaf lamina is kept on a slide and covered with cover slip with few drops of glycerine and stained with a stain and observed under microscope. The anatomical structures observed for the species are presented in the following section.

Characterization of leaf surface anatomy: Using technique 2, we took impression of leaf surface (both upper and lower surface) of few species to study the anatomical variability of the species.

Quantification of stomata on leaf surface: Measurement of stomata is difficult to assess owing to the fact that some species show absence of stomata and possess sunken stomata. We took data on stomatal counts and size of only eight species owing to the absence and or to the presence of sunken stomata. Stomata were counted per mm^2 of leaf surface. With the help of an ocular micrometer we measured the length and breadth of 50 stomata of each species and counted the number of stomata per unit area of leaf surface.

Results

The species show a large variability in stomatal presence and abundance. There exists a large variability in the type and size of stomata, epidermal cell structure which can be utilized in taxonomic delimitation of species and adaptation to abiotic and biotic stress (Figs. 1 and 2). Therefore, there is a great necessity to study leaf surface structure of different species which may help in the taxonomic delimitation and adaptation of the species to xeric environments. Each species has specific epidermal and stomatal characteristics to distinguish from each other. Large variation exists in size and form, intensity of epidermal cells, types, size and intensity of stomata both on the adaxial and abaxial surface, the presence and absence of glands and exudes. Most of the species in Tamaulipan thornscrub possess few special characteristics such as waxy leaf surface and the presence of sunken stomata. Most of the species possess waxy leaf surface. Some species with a waxy surface are *Amyris texana, Celtis pallida, Guaiacum angustifolium,* and *Acacia berlandieri*. Many species possessed sunken stomata viz., *Amyris texana, Celtis pallida, Guaiacum angustifolium, Forestiera angustifolia, Eysenhardtia texana, Parkinsonia texana, Gymnosperma glutinosum, Prosopis laevigata, Condalia hookeri, Diospyros palmeri, Diospyros texana, Karwinskia humboldtiana, Zanthoxylum fagara, Sideroxylon celastrinum, Leucaena leucocephala* and *Ehretia anacua*.

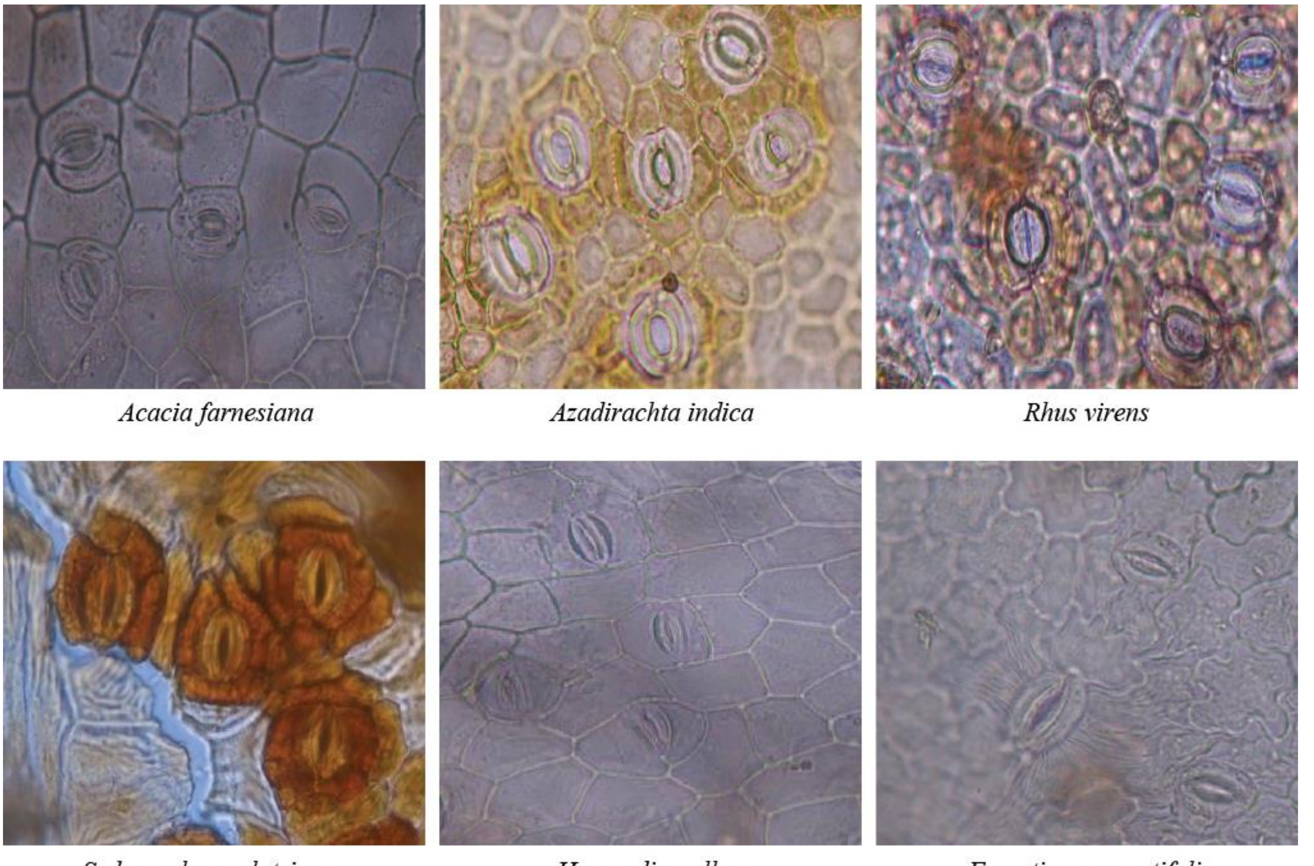

Fig. 1. Stomatal photographs (40X) of leaf impressions of some studied species treated with chromic acid and nitric acid.

Stomatal density, size and epidermal characteristics: Owing to the absence of stomata on leaf surface and sunken stomata it was possible to count stomata and size of stomata of only few species is mentioned herein. *Celtis pallida* presented 51.5 stomata per mm^2 on the adaxial surface but possessed 191.4 per mm^2 on the abaxial surface. *Condalia hookeri* had 145.1 per mm^2, the length and breadth of stomata were 20.6 µm and 11.3 µm, respectively. The type of stomata anomocytic. Stomata are absent on the upper surface. Leaf surface waxy, glands present on the abaxial surface. For species *Diospyros palmeri*, the length and breadth of stomata were 29.4 µm and 19.4 µm, respectively. Stomata anomocytic, few stomata present on the adaxial surface, but very few on the abaxial surface, glands present on the abaxial surface. With respect to *Diospyros texana*, had on the abaxial surface 134.3 stomata per mm^2, length and breadth of stomata were 28.4 µm and 17.1 µm, respectively. Stomata anomocytic, very few stomata on the adaxial surface, stomata are medium sunken. *Helietta parvifolia*, had stomata 74.9 per mm^2 on the abaxial surface, length and breadth are 36.7 µm and 18.3 µm, respectively. Stomata anisocytic, leaf surface waxy, but higher number of stomata present on the abaxial surface. *Karwinskia humboldtiana* had 517.0 stomata per mm^2, length and breadth of stomata were 17.2 µm and 10.1 µm, respectively. Stomata anomocytic, very few stomata present or absent on the adaxial surface. In *Sideroxylon celastrinum*, stomata length and breadth were 22.3 µm and 9.3 µm, respectively. Density of stomata was 155.2 per mm^2. Very few stomata were present or absent on the adaxial surface. Stomata anisocytic. In *Zanthoxylum fagara* stomata length and breadth were 21.95 µm and 10.88 µm, respectively, stomata anomocytic, leaf surface waxy, few stomata present on the adaxial surface, some were present on the abaxial surface.

Discussion

The present study is mainly concentrated on stomatal architecture, its distribution and abundance. The species can broadly be classified on the basis of the types of stomata (old system): 1) Anomocytic: *Acacia berlandieri, Acacia farnesiana, Acacia schaffneri, Gymnosperma glutinosum, Celtis pallida, Celtis laevigata, Condalia hookeri, Diospyros palmeri, Leucaena leucocephala, Amyris madrensis, Berberis chococo, Havardia pallens, Forestiera angustifolia, Uncaria guianensis,* and *Parkinsonia texana*. 2) Anisocytic: *Prosopis laevigata, Sideroxylon celastrinum*. 3) Paracytic: *Amyris texana, Eysenhardtia texana, Zanthoxylum fagara, Azadirachta indica*.

In the present study, most of the species possessed very few or absence of stomata on the adaxial surface. Species with negligible or absence of stomata on adaxial leaf surface (10): *Forestiera angustifolia, Parkinsonia texana, Gymnosperma glutinosum, Celtis laevigata, Condalia hookeri, Diospyros palmeri, Diospyros texana, Helietta parvifolia, Karwinskia humboldtiana, Zanthoxylum fagara.* Species with very few stomata on the adaxial leaf surface (4): *Amyris texana, Celtis pallida, Eysenhardtia texana,* *Sideroxylon celstrianum.* Species containing many stomata on the adaxial leaf surface (5): *Guaiacum angustifolium, Acacia berlandieri, Uncaria guianensis, Acacia schaffneri, Prosopis laevigata.* Similarly we can group the species with presence or absence of stomata on the lower surface. Species with negligible or absence of stomata on abaxial leaf surface leaf surface (1): *Gymnosperma glutinosum*. Species with moderate or very few stomata on the abaxial leaf surface (12): *Eysenhardtia texana, Parkinsonia texana, Forestiera angustifolia, Condalia hookeri, Diospyros palmeri, Karwinskia humboldtiana, Zanthoxylum fagara, Leucaena leucocephala, Ehretia anacua, Amyris madrensis, Diospyros texana, Quercus virginiana*. Species containing many stomata on the abaxial leaf surface (9): *Amyris texana, Guaiacum angustifolium, Acacia berlandieri, Uncaria guianensis, Acacia schaffneri, Prosopis laevigata, Celtis pallida, Sideroxylon celastrinum, Berberis chococo.*

On the basis of the presence and abundance of stomata on both adaxial and abaxial surface we can further classify species into three categories viz., Species with stomata absent, rare or very few on both adaxial and abaxial surface: *Eysenhardtia texana, Parkinsonia texana, Gymnosperma glutinosum, Celtis laevigata, Condalia hookeri, Karwinskia humboldtiana*. It is expected that these species may be tolerant to drought and are expected to exhibit high stomatal control to prevent loss of water by transpiration. Species with stomata absent or few on the adaxial surface but low on lower surface: *Amyris texana, Celtis pallida, Guaiacum angustifolium, Forestiera angustifolia, Diospyros palmeri, Diospyros texana, Helietta parvifolia.* It is expected that these species will be moderately tolerant to drought. Species with many stomata on adaxial and abaxial surface are: *Acacia rigidula, Acacia berlandieri, Acacia schaffneri, Eysenhardtia texana.* The species having many stomata are expected to be susceptible to drought, but these species might have deep root system for adaptation to drought situations.

It is observed that there exist variation in stomata density and size of stomata among eight species studied which influence water balance through control of transpiration. In the context of the results of the present study, it is observed that there exists a large variability in the type and size of stomata, epidermal cell and stomatal architecture structure which can be utilized in taxonomic delimitation of species and adaptation to abiotic and biotic stress. Therefore, there is a great necessity to study leaf surface structure of different species which may help in the taxonomic delimitation and adaptation of the species to xeric environments. Various authors (Rossatto & Kolb, 2010; Noman *et al*., 2014; Cotta Coutinho *et al*., 2016; Oggero *et al*., 2016) have used leaf anatomy in the taxonomic classification and possible adaptations to environmental stress as discussed below.

The species studied in the Tamaulipan thornscrub are adapted in the semiarid environments in northeastern Mexico, although varying in its adaptability depending on the species (Stienen *et al*., 1989; González Rodríguez *et al*., 2011). Some of the species possess special traits related to adaption in the environments such as waxy leaf surface, sunken stomata, the absence of stomata on the adaxial and or abaxial surface, thereby reducing loss in transpiration mentioned before. The presence of waxy leaf surface in most of the species such as *Amyris texana, Celtis pallida, Guaiacum angustifolium, Senegalia berlandieri, Karwinskia humboldtiana* could help in the reflectance of solar radiation, thereby keeping leaf temperature cooler. In addition, the presence of sunken stomata in many of the species *viz. Amyris texana, Celtis pallida, Guaiacum angustifolium, Forestiera angustifolia, Eysenhardtia texana, Parkinsonia texana, Gymnospermum glutinosum, Prosopis laevigata, Condalia hookeri, Diospyros palmeri, Karwinskia humboldtiana, Leucaena leucocephala, Forestiera angustifolia, Ehretia anacua* helps to maintain microclimate in the stomatal cavity, thereby reduce transpiration in the semiarid environment. This hypothesis needs to be confirmed in future studies. In this respect, the variability in epicuticular wax has been observed in 37 woody species in the Tamaulipan thornscrub (Maiti unpublished). The results of the present study with respect to the importance of stomata and epidermal cell characteristics coincide with the findings of other researchers, Souza *et al*. (2004) in the taxonomic delimitation of species in Piperaceae, and Chernetskyy (2012) in the genus *Kalanchoë*. Few anatomical characters such as stellate trichomes, cystolitic trichomes, papilate epidermis, cystolitic trichomes (Arambarri *et al*., 2011) are utilized to distinguish different species. The variability in the anatomical characteristics have been used in the adaptation of the species in stress environment (xeric) in *Solanum nigrum* in Europe and Yugoslovakia (Krstic *et al*., 2002), in the case of species in arid Mediterranean ecosystem (Rotondi *et al*., 2003), in orchids (Torres *et al*., 2006), and in the adaptation of *Azadirachta indica* and *Millettia thonningii* (Dzomeku & Enu-Kwesi, 2006). The stomatal frequency in *A. indica* was very high, while it was low in *Milletia thonningii*, thickness of epidermis, of palisade and spongy parenchyma layers, density and length of stomata (Afas *et al*., 2007. Similarly density of epidermal cells, density of stomata in the adaptation of species in Chacoan forests in Argentina (Arambarri *et al*., 2012) and the use of few morpho-anatomical characteristics in the case of *Celtis ehrenbergiana* (Nughes *et al*., 2013). The present study mention the presence of special adaptive leaf anatomical traits which need to be confirmed in future studies.

Conclusions

The techniques developed for observation of leaf epidermal traits may be effectively used in mass scale screening of the forest tress for desirable traits. There exists large variability in various leaf anatomical traits such as stomata, type, its presence, absence, way nature among woody plant species in northeastern Mexico. These variabilities can be effectively used in the taxonomic delimitation and adaption of the species in the environmental stress. Among which waxy leaf surface, absence of stomata, sunken stomata could predict the capacity of the species for adaptation to environmental stresses. These hypotheses may be confirmed in future studies.

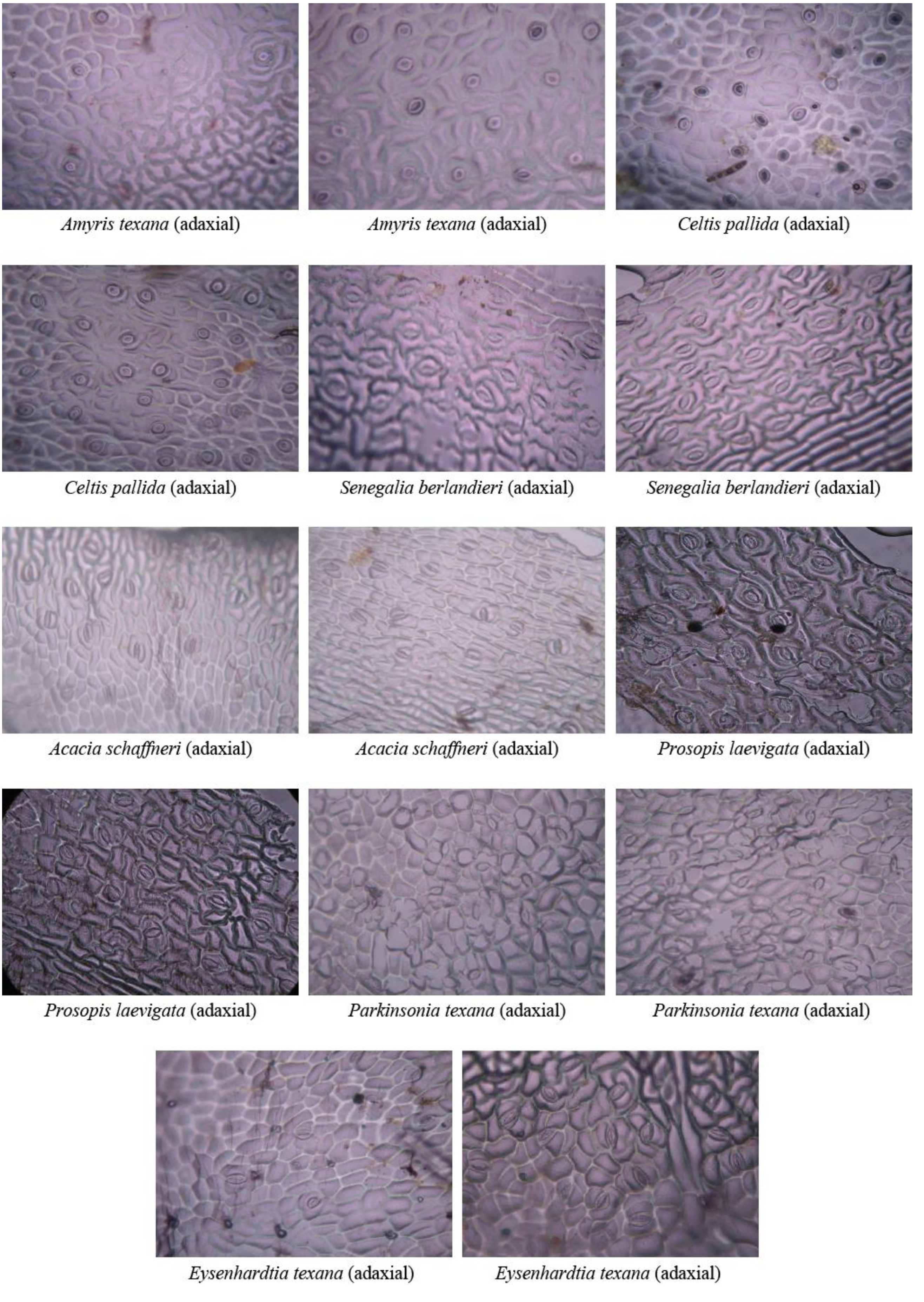

Fig. 2. Stomatal photographs (40X) of leaf impressions of some studied species treated with xylene.

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References

Afas, N.A.L., N. Marron and R. Ceulemans. 2007. Variability in *Populus* leaf anatomy and morphology in relation to canopy position, biomass production, and varietal taxon. *Ann. For. Sci*., 64: 521-532.

Arambarri, A., C. Monti, N. Bayón, M. Hernández, M.C. Novoa and M. Colares. 2012. Ecoanatomía foliar de arbustos y árboles del Distrito Chaqueño Oriental de la Argentina. *Bonplandia*, 21: 5-26.

Arambarri, A.M., M.C. Novoa, N.D. Bayón, M.P. Hernández, M.N. Colares and C. Monti. 2011. Anatomía foliar de arbustos y árboles medicinales de la región chaqueña semiárida de la Argentina. *Dominguezia*, 27: 5-24.

Chernetskyy, M.A. 2012. The role of morpho-anatomical traits of the leaves in the taxonomy of Kalanchoideae Berg. Subfamily (Crassulaceae DC.). *Modern Phytomorphology*, 1: 15-18.

Cotta Coutinho, I.A., J.G. Rando, A. de S., Conceição and R.M.S.A. Meira. 2016. A study of the morphoanatomical characters of the leaves of *Chamaecrista* (L.) Moench sect. *Apoucouita* (Leguminosae-Caesalpinioideae). *Acta Bot. Bras*., 30: 205-221.

Dzomeku, B.M. and L. Enu-Kwesi. 2006. Ecophysiological study on two urban forestry species (*Azadirachta indica* and *Millettia thonningii*) in Ghana. *Res. J. Bot*., 1: 134-138.

García, M., D. Jáuregui and E. Medina. 2008. Adaptaciones anatómicas foliares en especies de Angiospermas que crecen en la zona costera del Estado de Falcón (Venezuela). *Acta Botánica Venezuelica*, 31: 291-306.

González Rodríguez, H., I. Cantú Silva, R.G. Ramírez Lozano, M.V. Gómez Meza, M. Pando Moreno and J.M. López Hernández. 2011. Potencial hídrico xilemático en cuatro especies arbustivas nativas del noreste de México. *Revista Chapingo Serie Ciencias Forestales y del Ambiente Edición Especial*, 17: 97-109.

Krstic, L.N., L.S. Merkulov and P.P. Boza. 2002. The variability of leaf anatomical characteristics of *Solanum nigrum* L. (Solanales, Solanaceae) from different habitats. *Proceedings for Natural Sciences, Matica Srpska Novi Sad*, 102: 59-70.

Noman, A., Q. Ali, M. Hameed, T. Mehmood and T. Iftikhar. 2014. Comparison of leaf anatomical characteristics of *Hibiscus rosa-sinesis* grown in Faislabad region. *Pak. J. Bot*., 46: 199-206.

Nughes, L., M. Colares, M. Hernández and A. Arambarri. 2013. Morfo-anatomía de las hojas de *Celtis ehrenbergiana* (Celtidaceae) desarrolladas bajo condiciones naturales de sol y sombra. *Bonplandia*, 22: 159-170.

Oggero, A.J., M.D. Arana and H.E. Reinoso. 2016. Comparative morphology and anatomy of the leaf and stem of species of *Zanthoxylum* (Rutaceae) from central Argentina. *Polibotánica*, 42: 121-136.

Reyes Hernández, V.J., J.J. Vargas Hernández, J. López Upton and H. Vaquera Huerta. 2005. Variación morfológica y anatómica en poblaciones mexicanas de *Pseudotsuga* (Pinaceae). *Acta Bot. Mex*., 70: 47-67.

Rossatto, D.R. and R.M. Kolb. 2010. *Gochnatia polymorpha* (Less.) Cabrera (Asteraceae) changes in leaf structure due to differences in light and edaphic conditions. *Acta Bot. Bras*., 24: 605-612.

Rotondi, A., F. Rossi, C. Asunis and C. Cesaraccio. 2003. Leaf xeromorphic adaptations of some plants of a coastal Mediterranean macchia ecosystem. *J. Mediterr. Ecol*., 4: 25-35.

Souza, L.A., I.S. Moscheta and J.H.G. Oliveira. 2004. Comparative morphology and anatomy of the leaf and stem of *Peperomia dahlstedtii* C.DC., *Ottonia martiana* MIQ and *Piper diospyrifolium* Kunth (Piperaceae). *Gayana Botánica*, 61: 6-17.

Stienen, H., M.P. Smits, N. Reid, J. Landa and J.H.A. Boerboom. 1989. Ecophysiology of 8 woody multipurpose species from semiarid northeastern México. *Ann. Sci. For*., 46: 454-458.

Torres, J., L. Laskowski and M.E. Sanabria. 2006. Efecto del ambiente de desarrollo sobre la anatomía de la epidermis foliar de *Cattleya jenmanii* Rolfe. *Bioagro*, 18: 93-99.

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