SEASONAL VARIATIONS IN MICROMORPHOLOGY, ULTRASTRUCTURE, AND HISTOCHEMISTRY OF MANGO (*MANGIFERA INDICA* L.) LEAVES

MAHARAJ, A.[1] – NAIDOO, Y.[1] – DEWIR, Y. H.[2*] – MUJIB, A.[3]

[1]*School of Life Sciences, University of KwaZulu-Natal, Westville Campus, Private Bag X54001, Durban 4000, South Africa*

[2]*Plant Production Department, College of Food & Agriculture Sciences, King Saud University, Riyadh 11451, Saudi Arabia*

[3]*Cellular Differentiation and Molecular Genetics Section, Department of Botany, Jamia Hamdard, New Delhi 110062, India*

**Corresponding author*
e-mail: ydewir@ksu.edu.sa

(Received 9th Apr 2024; accepted 8th Jul 2024)

Abstract. *Mangifera indica*, a member of the Anacardiaceae family, is an important medicinal plant renowned for its wide range of biological activities. This study aims to examine *M. indica* from South Africa and describe its key micromorphological features related to foliar structures. Methods employed in this study include stereomicroscopy, scanning electron microscopy (SEM), and transmission electron microscopy (TEM) for an in-depth analysis of foliar biology. Additionally, ImageJ software was used to measure the length and diameter values associated with different trichome types on *M. indica* leaves. The morphological evaluation using stereo- and SEM techniques revealed the presence of non-glandular trichomes with cuticular warts as well as glandular peltate trichomes. TEM micrographs confirmed the presence of numerous mitochondria, starch grains, plastoglobuli, and plastids, providing corroborating evidence of cellular organelles within these cells. Histophytochemical analysis of specific leaf sections further validated that alkaloids and phenolics are the major medicinal compounds obtained through extraction methods. The observations indicated no significant difference between summer and winter seasons concerning common morphology-related characteristics across all categories. The overall findings demonstrate that *M. indica's* usefulness for compound extractions remains consistent throughout the year due to its similar attributes between seasons. Consequently, continuous cultivation is established as crucial for developing sustainable approaches toward modern medicine.

Keywords: *Anacardiaceae, leaf anatomy, bioactive compound, SEM, TEM, trichomes*

Introduction

The family Anacardiaceae comprises around 83 genera and 873 species, predominantly distributed in tropical and subtropical regions worldwide (Herrera et al., 2018). This family has been the subject of extensive research, primarily due to its significant economic value in countries that are engaged in the export of fruits like mango, cashew nut, pistachio, ambarella, yellow mombin, and red mombin (Lorenzi et al., 2015; Coelho et al., 2019). *Mangifera indica*, commonly referred to as mango, is a perennial tree reaching heights of 8-18 m, originating from India and Myanmar (Lorenzi et al., 2015). Various parts of the plant, including the roots, bark, leaves, flowers, fruits, and seeds, are commonly utilized for treating various ailments such as diabetes, anemia, diarrhea, hemorrhoids, indigestion, asthma, bronchitis, and influenza (Shah et al., 2010; Santos et al., 2012; Ghuniyal, 2015; Jahurul et al., 2015; Parvez, 2016; Ribeiro et al.,

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2205_43094328

2017; Ediriweera et al., 2017; Lauricella et al., 2017). The present study aims to investigate the seasonal changes of morphological characteristics through macro- and microscopic evaluation and ascertaining the location and presence of metabolites (bioactive compounds) in the leaves of *M. indica*. Several studies focused on the presence of bioactive compounds in *M. indica* suggesting the presence of several metabolites with proven pharmacological activities (Shah et al., 2010; Ghuniyal, 2015; Jahurul et al., 2015; Parvez, 2016; Ribeiro et al., 2017; Ediriweera et al., 2017; Lauricella et al., 2017).

Seasons were introduced to this study due to global climate change (Intergovernmental Panel on Climate Change, 2021). Heat stress not only affects plant morphology and causes leaf etiolation and wilting but also alters the anatomy, physiology, photosynthetic capability, and genetic expression of plants (Chen et al., 2014). Furthermore, heat stress causes changes in the primary and secondary plant metabolism (Macedo, 2012). Among the deleterious effects, the overgeneration and reactions of reactive oxygen species (ROS), are common under heat stress and may damage chloroplasts and cells by attacking membrane lipids, DNA, and proteins (Chen et al., 2014). Conversely, plants have developed different physiological mechanisms at the transcriptomic, proteomic, and metabolomic levels to counteract ROS and adjust to or avoid prevailing oxidative damage (Dobra et al., 2015; Waqas et al., 2016). The factors that lead the photosynthesis under heat stress include the structural and functional disruptions of chloroplasts, degradation, or decreased accumulations of photosynthetic pigments. Therefore, scavenging ROS, maintaining cell membrane stability, and/or enhancing photosynthesis are effective ways to harvest light and sustain normal growth (Dobra et al., 2015; Waqas et al., 2016). The accumulation of osmotic proline, total soluble sugars, and total soluble protein is helpful to protect the structure of enzymes and proteins and maintain cell membrane integrity in the way of low-molecular-weight chaperones (Huve et al., 2006; Hameed et al., 2012; Manaa et al., 2014). In addition, plants have developed complex anti-oxidative defense systems consisting of an enzymatic system and a nonenzymatic system to counteract the injurious effects of ROS (Xu et al., 2006). An overall tendency exists to survive under heat stress by reducing cell size, enlarging the xylem vessel diameter, increasing stomatal density to benefit water transport, and reducing transpiration (Banon et al., 2004; Chen et al., 2012). High-temperature stress strongly influences cell ultrastructure, especially chloroplasts, which are often assessed for evidence of stress (Banon et al., 2004; Chen et al., 2012). Any heat-related damage to thylakoid membranes in chloroplasts is expected to result in chlorophyll loss (Vacha et al., 2007). Earlier research on abiotic stress affecting *M. indica* was studied earlier, which mainly focused on drought and cold stress (Anisko and Lindstrom, 1996; Lipp and Nilsen, 1997; Cordero and Nilsen, 2002). However, fewer studies about heat stress were reported (Ranney et al., 1995; Banon et al., 2004; Chen et al., 2012). Heat stress studies of many other plants have recently focused on physiological effects (Gupta et al., 2013). Little is known about how heat stress affects anatomical structures, such as stoma, mesophyll tissue, and epidermal cells, and ultrastructure, such as chloroplasts (Machado et al., 2002; Medina et al., 2002). In *M. indica* plants, a decrease in root permeability and in-plant hydraulic conductance due to low temperatures have been reported (Syvertsen et al., 1983; Moreshet and Green, 1984). As a consequence of low temperature in plant water relations, the reduction of stomatal conductance of mango plants was noticed during winter (Ribeiro and Machado, 2007). The reduced stomatal aperture may impair

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2205_43094328

leaf photosynthesis by decreasing CO_2 availability to Rubisco (Jones, 1985; Machado et al., 2002; Medina et al., 2002). Cool temperatures also modify the biochemical reactions underlying CO_2 fixation (Allen and Ort, 2001), with mango plants showing the reduction in Rubisco carboxylation and regeneration during winter (Ribeiro et al., 2007).

Specialized hair-like epidermal cells are known as trichomes (Kariyat et al., 2018). Trichomes play a role in a plants defense against biotic threats such as predators acting as both a chemical mediator and physical barrier, furthermore acting as protection mechanisms from abiotic factors such as sunlight by reflecting excess radiation (Valverde et al., 2001; Kariyat et al., 2018). The location of these structures differs with species and can be found on the leaves, stems, roots, and even seed coats (Levin, 1973b; Naidoo et al., 2014). Trichomes are known to be classified as either glandular or non-glandular. This classification depends on their shape and function (Choi and Kim, 2013). The absence of a glandular head in non-glandular trichomes is the most distinctive morphological difference (Werker, 2000). Although trichomes can be used for taxonomic purposes, these can be subdivided further relatively to their morphological characteristics (de Vargas et al., 2018). Non-glandular trichomes are considered to act exclusively as mechanical barriers, compared to glandular trichomes are responsible for the storage and/or exudation of biologically active phytocompounds (Levin, 1973a; Werker, 2000; Naidoo et al., 2011). *Mangifera indica* trichomes have been described as non-glandular (Lizarraga et al., 2017) with seemingly no consensus. Therefore, this study aimed to evaluate summer and winter leaves by viewing the micro-and macro-morphology to ascertain if there are any morphological differences seasonally as well as to determine the trichome type using stereo- and scanning microscopes. Histo-phytochemical analyses were also performed to elucidate the chemical classes of phytocompounds present in *M. indica* leaves for the summer and winter seasons.

Materials and methods

Collection and identification of plant materials

Fresh leaves of *M. indica* were harvested from Durban, KwaZulu-Natal, South Africa (24° 49’05” S 30°56’46” E). The samples collected in summer spanned from December 2019 to March 2020, while those in winter were gathered between June and August 2020. Leaf samples were collected 4 times per each season and three replicates were used for all tests. Authentication of the species was carried out by Professor Y. Naidoo, and a voucher specimen (accession number: NU0092176) was preserved in the Ward Herbarium, School of Life Sciences (Biology), University of KwaZulu-Natal, Durban, South Africa.

Stereomicroscopy

For stereomicroscopy analysis, the fresh leaves underwent examination through the Nikon AZ100 stereomicroscope (Nikon Corporation, Yokohama, Japan) outfitted with a Nikon Fiber Illuminator. Images were captured using the NIS-Elements Software (NIS-elements D 3.00), with a focus on the surface details of the adaxial and abaxial sides across the emergent, young, and mature developmental stages. All stages were collected at the same sampling time.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2205_43094328

Scanning electron microscopy (SEM)

To investigate the morphology and distribution of trichomes on the leaf surfaces, a scanning electron microscope was employed. Detailed examination of the micromorphology of chemically-fixed samples from both leaf surfaces at each developmental stage was conducted. The process commenced with the primary fixation of 5 mm^2 fresh leaf sections in 2.5% glutaraldehyde for 18-24 h. Subsequently, the specimens underwent a triple rinsing process lasting 5 min each, utilizing a 0.1 M sodium phosphate buffer at a pH of 7.2, followed by a post-fixation step in 0.5% osmium tetroxide for a duration of 3 h at a temperature of 24°C. The specimens underwent another triple washing step lasting 5 min each, employing the sodium phosphate buffer, and were dehydrated through exposure to escalating concentrations of ethanol (30%, 50%, 75%, 100%) in two sessions, each lasting 5 min, succeeded by exposure to 100% ethanol in two sessions, each lasting 10 min. Subsequently, the dehydrated specimens were subjected to critical point drying utilizing the Quorum K850 Critical Point Dryer (Quorum Technologies Ltd., Laughton, East Sussex, UK) equipped with a vertical chamber. Following this, the specimens were affixed onto small aluminum stubs using double-sided adhesive carbon tape and coated with a layer of gold using the Quorum 150 RES (Quorum Technologies Ltd.), an integrated system for carbon and sputter coating. The specimens were observed and imaged utilizing the LEO 1450 SEM at a working distance (WD) of 12-15 mm, with images captured using the SmartSEM image software (Zeiss, Jena, Germany).

Freeze drying

A separate collection of fresh leaves representing three developmental stages (emergent, young, and mature) of *M. indica* were flash-frozen in liquid nitrogen (-196°C) and subsequently cryopreserved in an Edwards Modulyo freeze dryer (Edwards High Vacuum International Ltd., UK) at temperatures ranging from -40 to -60°C in a vacuum of 10^{-1} Torr for a duration of 72 h. The samples were fixed onto aluminum stubs using carbon conductive tape, underwent dual gold sputter coating utilizing a Polaron SC500 Sputter Coater (Quorum Technologies Ltd., UK) under a vacuum of 0.1 Torr. The sputter coating duration and thickness were standardized by the Polaron SC500 Sputter Coater equipment. The prepared leaf sections were examined using a Zeiss Ultra-Plus FEG-Scanning electron microscope operated at 20 kV.

Morphometric analysis of trichomes

Ten selected micrographs of trichomes acquired through scanning electron microscopy (SEM) were subjected to analysis utilizing the ImageJ software (Schindelin et al., 2015). The diameter (μm) of the trichome head, as well as the length and width of the stalk for each type, were measured.

Transmission electron microscopy (TEM)

The ultrastructure of leaf tissue was observed, analyzed, and captured using TEM. Leaf segments from various growth phases (±2 mm^2) were excised and primarily fixed in a 2.5% glutaraldehyde solution for a duration of 24 h. These segments were triple-washed in a 0.1 M phosphate buffer (pH 7.2) and subsequently post-fixed in 0.5%

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2205_43094328

osmium tetroxide for 3 h. Following this, the samples underwent triple rinsing for 5 min each using the phosphate buffer. Subsequently, the specimens were dehydrated through a series of acetone solutions of increasing concentration (30%, 50%, 75%) for 5 min each, culminating in two immersions in 100% acetone lasting 10 min each. Post-dehydration, the samples were immersed in propylene oxide, a clearing agent, for 15 min, and gradually infiltrated using escalating concentrations of Spurr's low-viscosity epoxy resin in propylene oxide solution (25%, 50%, 75%, and 100%) (Spurr, 1969). The specimens were embedded in equal ratios of Spurr's resin and acetone for 4 h, followed by pure resin for 24 h at 70°C (Spurr, 1969). Subsequent to this, they were placed into silicon molds and polymerized for 8 h at 70°C. Glass blades were fashioned using the LKB Knifemaker 7801A (Elekta, Stockholm, Sweden) and were utilized to section the resin blocks. Ultrathin resin-embedded slices were produced employing the Reichert Jung ultra-microtome (Leica, Wetzlar, Germany). These sections were initially examined to identify areas of interest, stained with 1% Toluidine Blue, mounted on slides, and observed using the Nikon Eclipse 80i light microscope (Nikon Corporation) equipped with a Nikon DS-Fi1 camera and the NIS-Elements imaging software suite. Subsequently, the ultrathin sections were sliced at 90–110 nm using the Reichert Jung ultra-microtome and positioned on copper grids. These sections were stained with 2.5% uranyl acetate for 10 min at 23°C, rinsed with distilled water, and then subjected to a 10-min lead citrate staining. The copper grids were washed with distilled water, visualized, and captured using the JEOL 1010 TEM (JEOL, Tokyo, Japan) equipped with the iTEM software.

Histochemistry

Hand-cut sections of fresh *M. indica* leaves were histochemically dyed as delineated below. The dyed sections were observed and photographed utilizing the Nikon Eclipse 80i compound light microscope, along with the Nikon DS-Fi1 compound microscope (Nikon).

(a) Alkaloids

Sections for Ditmars and Wagners staining reagents were treated separately for a duration of 10 min each. Following staining, the sections were rinsed with distilled water, mounted, and observed. The presence of a deep brown-orange hue was considered indicative of a positive reaction (Furr and Mahlberg, 1981).

(b) Cellulose

For the fast-green staining procedure, sections were immersed in the stain for a period of 1 min and then thoroughly rinsed with distilled water. The appearance of a vivid green color was interpreted as a positive reaction, particularly with regards to cell walls (Tos et al., 1980).

(c) Lipid, lignin, cutin and suberin

To apply the Sudan black B stain, sections were subjected to staining for 30 min, followed by rinsing with 70% ethanol and distilled water. Subsequently, the sections were affixed to a slide using glycerol. The presence of a blue-black tint on tissues served as an indication of a positive reaction (Pearse, 1985; Demarco, 2017).

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2205_43094328

To apply the Nile blue stain, sections were treated with 1% Nile blue solution at 60°C for 5 min, then immersed in 1% acetic acid for an additional minute. Post rinsing with distilled water, the sections were mounted for observation. Acidic lipids exhibited a blue staining pattern (Cain, 1947; Demarco, 2017).

(d) Monochromatic staining

In the Toluidine Blue staining process, sections were exposed to the stain for 1 min before being rinsed with distilled water and mounted for examination. The manifestation of a bright pink-purple hue signified positive identification of carboxylated polysaccharides and polyphenols, which stained in shades of blue to green. Phosphate groups associated with macromolecules displayed a purple to blue staining pattern (O'Brien et al., 1964; Sridharan and Shankar, 2012).

(e) Mucilages and polysaccharides

Sections were immersed in a 0.1% ruthenium red solution for 5 min, followed by two washes in distilled water. They were mounted in glycerol, and observations were made. A color spectrum ranging from pink to red indicated a positive reaction (Gregory and Baas, 1989; Demarco, 2017).

(f) Phenolic compounds

For the application of ferric trichloride stain, sections were exposed to a 10% solution of the reagent, with the addition of aqueous sodium carbonate for 15 min at room temperature. The development of deep black deposits was considered a positive indication of the presence of phenolic compounds (Johansen, 1940).

(g) Total proteins

Sections underwent staining with 0.25% Coomassie Blue for 15 min, followed by differentiation in 7% acetic acid. Subsequent rinsing in distilled water and mounting in glycerol revealed a blue staining of tissues, confirming the presence of total proteins (Fisher, 1968).

Results and discussion

Stereomicroscopy

Stereomicroscopy images showing a mature leaf that is dark green (*Fig. 1A*), the adaxial surfaces of an emergent leaf (reddish in color) (*Fig. 1B*), and a young leaf (green-yellow color) (*Fig. 1C*). The presence of non-glandular trichomes was evident on the adaxial and abaxial surfaces, with a denser cover on the abaxial leaf surface (*Fig. 1B, C*). In addition, there are considerably fewer trichomes on mature leaves in comparison to the emergent and young leaves. According to Werker (2000), this is due to the surface area increasing in mature leaves, which disperse the peltate glands.

Scanning electron microscopy

Trichomes are highly diverse structures that are in contact with the external environment whose function is in response to different biotic and abiotic stimuli

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2205_43094328

(Tooker et al., 2010; Li et al., 2018). Trichomes, therefore, serve as the first line of defense against predators and with some producing bioactive compounds that may attract and guide pollinators (Wagner, 1991; Hegebarth et al., 2016). The trichomes of *M. indica* densely cover the leaf surface (*Figs. 2A* and *3A*), occurring more frequently on the adaxial surfaces of the emergent and young leaves (*Fig. 1B* and *C*).

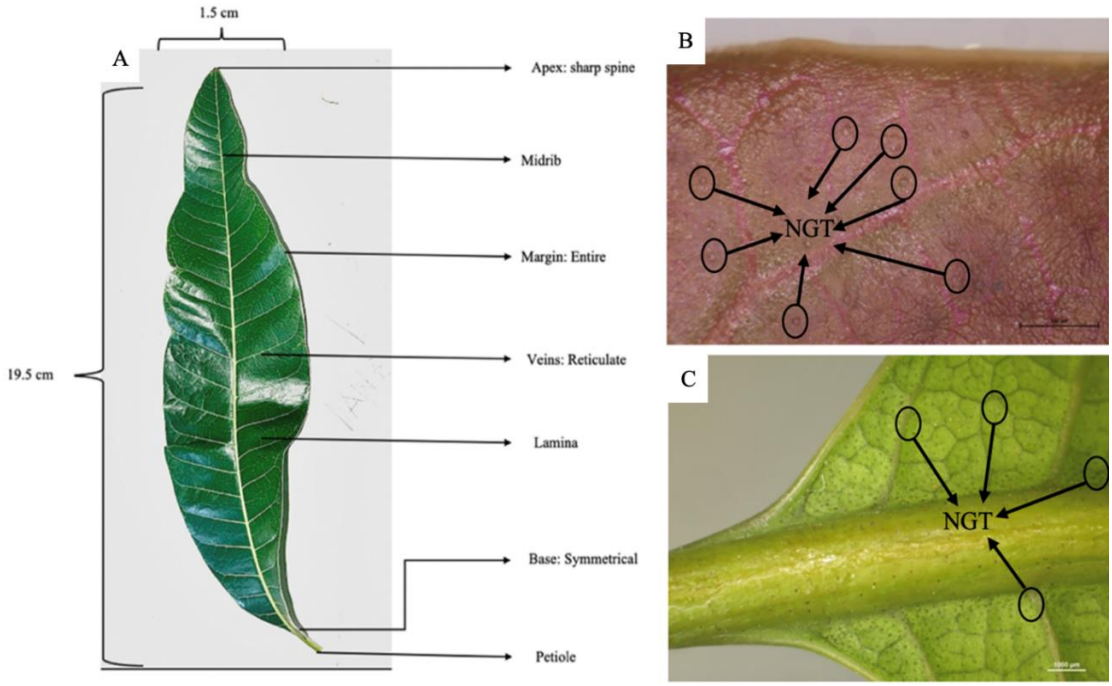

Figure 1. *Stereomicroscopic micrographs showing characteristics of M. indica leaf. (A) Adaxial mature entire leaf. (B) Adaxial emergent leaf showing non- glandular peltate glands. (C) Abaxial young leaf showing non- glandular peltate glands. Abbreviations: NGT = non-glandular trichome*

Two types of trichomes were observed on the leaves of *M. indica,* and it appears to be peltate glandular trichomes and non-glandular trichomes with cuticular warts. The non-glandular trichome is uniseriate and multicellular with a tapering end as shown in *Figures 2E, F* and *3E, F* (Metcalfe and Chalk, 1957; Norfaizal and Latiff, 2013; Ganogpichayagrai et al., 2016). The non-glandular trichome lengths are inconsistent, ranging between 70-200 μm. The peltate gland trichome is multicellular, consisting of 2 rows of 8 oblong cells, each with a size ranging from 32-48 μm. Non-glandular trichomes contribute to the enhancement of plant defense mechanisms by mitigating the impact of UV radiation through surface reflectance. Moreover, they play a crucial role in enhancing drought tolerance by lowering leaf temperatures and preventing the onset of photo-inhibition stress (Levin, 1973; Wagner, 1991; Werker, 2000). Szyndler et al. (2013) demonstrated that trichomes might also limit the movement of herbivores, such as insects, thereby restricting plant tissue damage. Kariyat et al. (2017) proved that non-glandular trichomes deter insects by causing post-ingestive gut damage since some trichomes are reinforced by silica, which damage the peritrophic matrix (PM), a protective sheath that lines the guts of most insects and which serves to prevent mechanical damage to the gut epithelium, inhibit pathogen invasion and assist in digestion and nutrient absorption. In general, trichomes provide mechanical and chemical barriers against herbivores (Terra,

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2205_43094328

2001). The functional properties of glandular trichomes' secretory metabolites have led to commercial applications in the cosmetic, food, and pharmaceutical industries, e.g. The glandular trichomes that secrete essential oils, which give those leaves their distinctive fragrance. Natural essential oils have great commercial value. Many species of *Mangifera* are aromatic and are used as spices, herbs, medicines, and a source of fragrance (Valkama et al., 2003; Balcke et al., 2017). Trichomes are highly diverse, and thus, their morphological traits have been key characteristics in plant taxonomic studies (Ko et al.,2007; Huang et al., 2008; Luo et al., 2010). Non-glandular trichomes are metabolically active during the earliest stages of development (Levin, 1973; Mayekiso et al., 2008) and are thought to play a minor role throughout the remaining lifespan of the plant (Levin, 1973; Mayekiso et al., 2008). Santos et al. (2016) showed that while the traditional roles of non-glandular trichomes were to protect plant materials from predators, UV radiation, and abiotic factors, they also have the potential to produce, store, and liberate bioactive substances (Levin, 1973; Mayekiso et al., 2008).

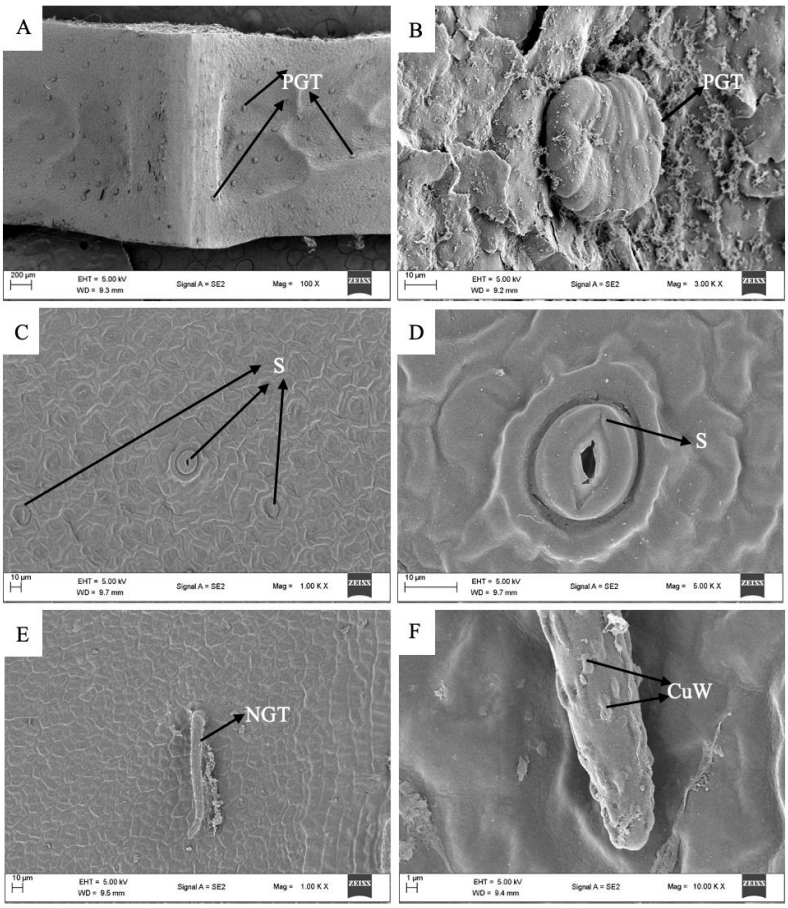

Figure 2. *Scanning electron micrographs of M. indica leaf for Summer. (A) Abaxial surface peltate gland trichome. (B) Adaxial surface peltate gland trichome consisting of 2 rows of 8 oblong cells each. (C and D) Abaxial surface showing anomocytic stomata. (E and F) Adaxial surface of non-glandular trichome with cuticular warts. Abbreviations: PGT = Peltate gland trichome; S = Stomata; NCT = non-glandular trichome; CuW = Cuticular warts*

Previous studies have emphasized the identification and characteristics of trichomes, including their presence, size, color, distribution pattern, and type, as valuable features for

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2205_43094328

the classification of plants (Cooper, 1932; Navarro and Oualidi, 2000; Shaheen et al., 2009). The observation of peltate trichomes in our study (*Figs. 2B* and *3B*) confirm a prior research by Metcalfe and Chalk (1957) but contrasts with the findings of Norfaizal and Latiff (2013), who noted the presence of trichomes in *M. indica* epidermis. The characteristics of sunken peltate trichomes are deemed significant and potentially linked to ecological adaptations (Johnson, 1975; Bibi et al., 2014). The influence of water scarcity on plant anatomy modifications, such as increased cuticular thickness, higher trichome density (*Figs. 2A* and *3A*), and the development of cuticular warts on trichomes, has been highlighted by Ganong (1895), Werker (2000), and Bibi et al. (2014). This relationship implies a potential connection between water availability and the presence or absence of glandular trichomes (*Figs. 2B* and *3B*) as well as non-glandular trichomes with cuticular warts (*Figs. 2E, F* and *3E, F*). This may vary based on the plant's environment (Ganong, 1895; Werker, 2000; Bibi et al., 2014). This study does not agree with that as the summer and winter leaves resembled similar-to-identical morphological characteristics on all fronts. The reduced sample size utilized during one-year time span in this study may have hindered the ability to observe significant differences. It is possible that the plant's resilience to various climatic conditions is a result of adaptation to climate change. Additional investigation is imperative to validate the aforementioned assertion.

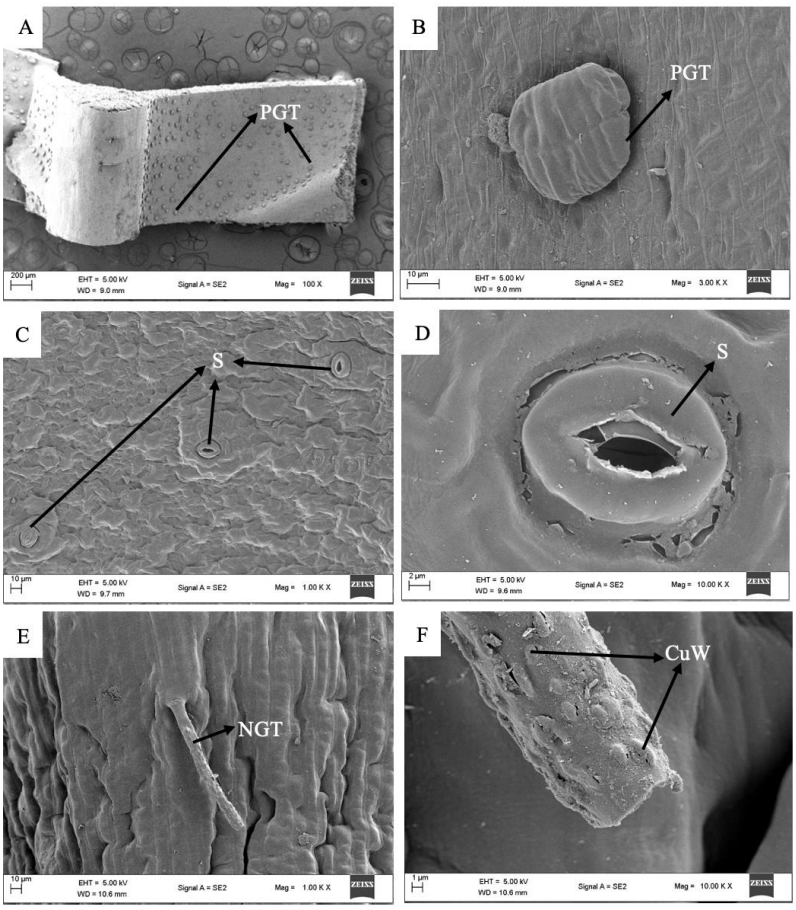

Figure 3. *Scanning electron micrographs of M. indica leaf for Winter. A) Abaxial surface peltate gland trichome. (B) Adaxial surface peltate gland trichome consisting of 2 rows of 8 oblong cells each. (C and D) Abaxial surface showing anomocytic stomata. (E and F) Adaxial surface of non-glandular trichome with cuticular warts. Abbreviations: PGT = Peltate gland trichome; S = Stomata; NCT: non-glandular trichome; CuW = Cuticular warts*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2205_43094328

Transmission electron microscopy

Similar anatomical characteristics among *Mangifera* species are helpful for the division of genera in Anacardiaceae (Norfaizal and Latiff, 2013). The typical anatomical characteristics of all *Mangifera* species are as follows: (i) the typical cyclocytic and anomocytic stomata with a limited number of subsidiary cells in adaxial and abaxial surfaces, respectively; (ii) the amphistomatic leaves; (iii) the jigsaw shape with deeply undulate cell wall in adaxial epidermal cell; (iv) the presence of sunken peltate trichomes on lamina and midrib; (v) the presence of bundle sheath extension to both surfaces; (vi) the presence of fiber at the apex of leaf margin, midrib and petiole; (vii) the presence of resin ducts; and (viii) the presence of mucilaginous cells in the epidermis and midrib (Mckay et al., 2003; Sharma et al., 2012; Norfaizal and Latiff, 2013; Ferrenberg, 2014; Cahyanto et al., 2017). The deeply undulate cell walls are of jigsaw shape. The irregular shape with slightly undulate and straight cell walls is found on adaxial and abaxial surfaces, respectively (*Figs. 4A, B, C* and *5A, B, D, E*). Although this finding corresponded partially to Norfaizal and Latiff (2013) investigations, the oval and round shapes of epidermal cells in Ganogpichayagrai et al. (2016) disagree with the epidermal features of this study. According to Sharma et al. (2012) and Cahyanto et al. (2017), *Mangifera indica* leaves have a 1-layered epidermis, parenchymatous cortex, closed vascular system, and resin ducts that corresponded with this study. As the function of the resin canal, the complex mixtures of substances in the resin duct resist the herbivorous pest, bacterial invasion, and wounding (Mckay et al., 2003; Ferrenberg, 2014). Noticeably, starch grains (*Figs. 4* and *5*) in parenchyma cells of *M. indica* leaf sections have not been reported in previous studies but have been reported in the present study for both the summer and winter seasons. Starch grains are used to store energy/food for the plant (Lacchia and Carmello-Guerreiro, 2009). These findings may indicate why the summer and winter seasons show very little to no visible seasonal morphological differences. Further studies should be conducted to quantify the phytochemicals present in the seasonal leaves, indicating any variation between the seasons. The pattern of stomatal apparatus varies in each plant group, so these characteristics enhance the species identification (Van Cotthem, 1970). *Mangifera indica* generally has anomocytic stomata (Metcalfe and Chalk, 1957; Norfaizal and Latiff, 2013; Ganogpichayagrai et al., 2016), which correspond with this study; however, work done by Cahyanto et al. (2017) noticed the presence of actinocytic stomata. The results from this study disagree with the results found by Cahyanto et al. (2017). Moreover, the anomocytic stomata are different from the anisocytic stomata of *Mangifera odorata, M. pentandra,* and *M. quadrifida* in Norfaizal and Latiff (2013).

Light microscopy and histochemical analysis

In the transverse section of the leaf, the epidermal cells exhibit slightly sinuous and thick walls on both the upper and lower surfaces during the seasons of summer and winter (*Fig. 2* and *3*). The leaf blade demonstrates hypostomatic characteristics, with anomocytic stomata (*Figs. 2D* and *3D*). As outlined by Metcalfe and Chalk (1950), the Anacardiaceae family is recognized by either hypostomatic or amphistomatic leaf blades. Furthermore, multicellular glandular trichomes were identified on both surfaces of the leaf (*Figs. 1, 2* and *3*). In a study concerning the leaf of *M. indica*, Rocha et al. (2015) acknowledged the existence of trichomes. Additionally, non-glandular trichomes were observed in the leaf of *M. laurina* by the authors. The

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2205_43094328

occurrence of non-glandular trichomes has also been documented in the leaf of *M. altissima* (Vasconcelos and Randau, 2016), the same type of trichome found in *M. indica* (*Fig. 2E, F* and *3E, F*). The midrib, when viewed in the transverse section, appears biconvex (*Fig. 1*). A similar midrib shape was noted by Rocha et al. (2015). The epidermis is single-layered, encompassed by a substantial cuticle (*Fig. 6*).

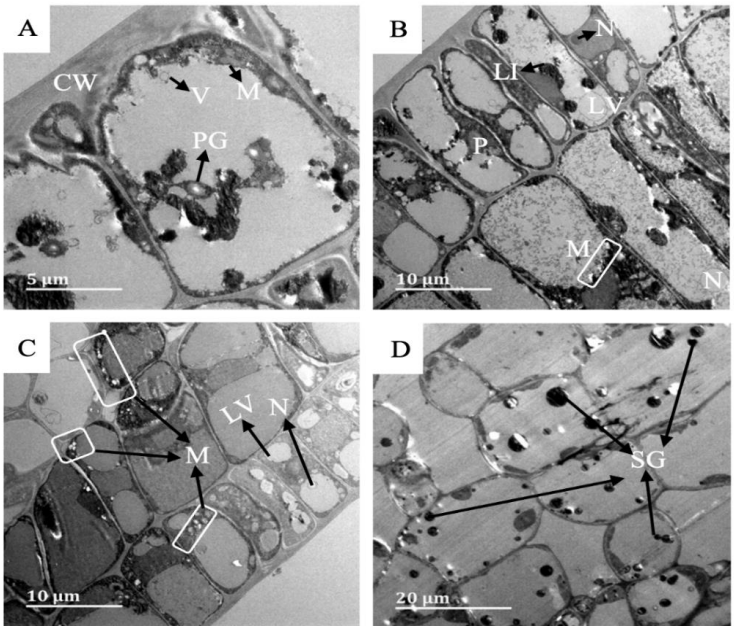

Figure 4. *Transmission electron micrographs of M. indica leaf for Summer. A) Upper epidermis. (B) Upper epidermis and palisade cells. (C) Lower epidermis and spongy mesophyll. (D) spongy mesophyll. Abbreviations: CW = Cell wall; LV = Large vacuole; PG = Plastoglobuli; M = Mitochondria; SG = Starch grain; V = Vesicle; LI = Lipid inclusion*

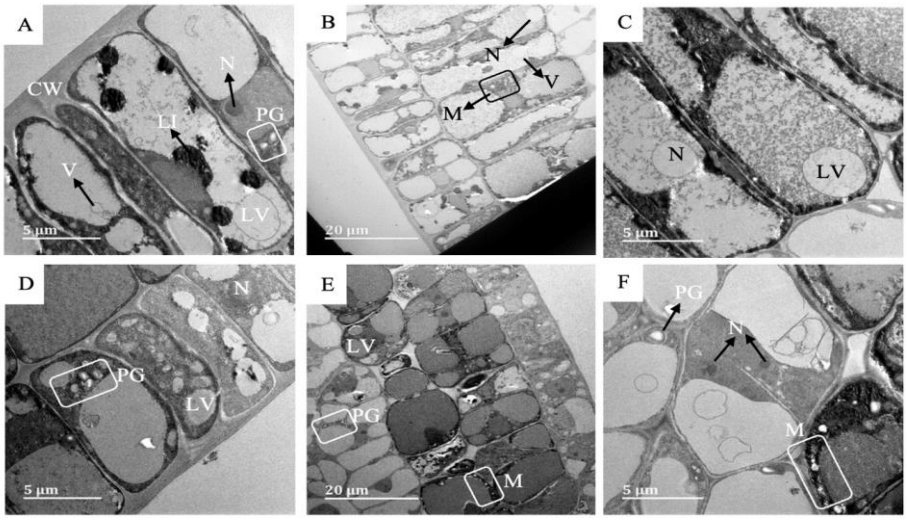

Figure 5. *Transmission electron micrographs of M. indica leaf for Winter. A) Upper epidermis. (B) Upper epidermis and palisade cells. (C) Palisade cells. (D) Lower epidermis. (E) Lower epidermis and spongy mesophyll. (F) Spongy mesophyll. Abbreviations: CW = Cell wall; LV = Large vacuole; PG = Plastoglobuli; M = Mitochondria; SG = Starch grain; V = Vesicle; LI = Lipid inclusion*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2205_43094328

The transverse section across the midrib during both summer and winter displays a dorsiventral structure with upper palisade and lower spongy parenchyma cells (*Figs. 4* and *5*) in each period. For both summer and winter seasons, the midrib presents a centrally positioned vascular bundle (*Fig. 4* and *5*). A thorough transverse section reveals upper and lower single-layered epidermal cells that are compactly arranged in a barrel shape, along with a cuticle. Some of the epidermal cells appear to be interrupted by stomatal openings (*Fig. 6A*). Mesophyll cells consisting of an upper compactly arranged group of cells without any air spaces. There are 1-2 layers of the elongated palisade and lower 5-6 layers of an oval to rounded shaped with intercellular spaces (*Fig. 4* and *5*). Characteristics from the midrib transverse sections in this study are in acceptance with a previous study that coincides with this study's findings (Norfaizal and Latiff, 2013). The transverse section indicates that there are no distinct anatomical differences between the summer and winter leaves, as depicted in *Figure 6*.

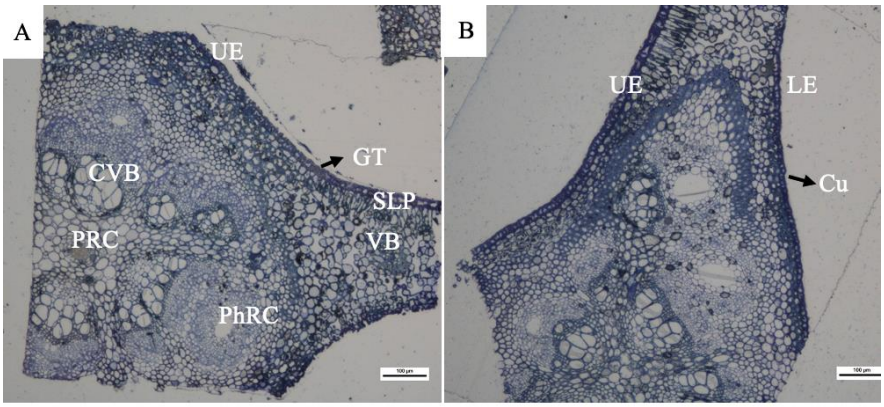

Figure 6. *Transverse section micrographs of M. indica L. leaves (Left: A- Summer) (Right: B-Winter). (A-B) Toluidine blue stain. Abbreviations: CVB = Central Vascular bundle; PRC = Pith resin canal; VB = Vascular bundle; SLP = Singly layer palisade; Cu = Cuticle; LE = Lower epidermis; UE = Upper epidermis; GT = Glandular trichome; PhRC = Phloem resin canal; S = Stomata*

The vascular bundle has a biconvex shape and is collateral, surrounded by sclerenchyma (*Fig. 6*), corroborating with some studies that indicated the presence of a thick cuticle, uniseriate epidermis and the vascular bundle having a biconvex shape (Santhan, 2014; Rocha et al., 2015). The presence of these secretory structures such as laticifers and idioblasts is one of the family Anacardiaceae (Metcalfe and Chalk, 1950). In vegetative organs, secretory structures are found mainly in the phloem and pith (Lacchia and Carmello-Guerreiro, 2009); however, in this study, secretory structures were not indicated as no laticifers and idioblasts were found. This observation may be due to some geographical conditions. However, further studies should be conducted with an increased sample size and have samples from different locations; this may improve may reveal the presence of these secretory structures (laticifers and idioblasts). The mesophyll presents an organization of the dorsiventral type, with one-two layer of palisade parenchyma and around six-eight layers of spongy parenchyma (*Figs. 4* and *6A, B*). Santhan (2014) observed two-three layers of palisade parenchyma in the *M. indica* species. Lignin was observed in xylem sclerenchyma (*Fig. 7D* and *8D*). The tests for alkaloids, proteins, suberin, lipids, resin acids, mucilage and gums, and

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2205_43094328

phenolics were all positive for the summer and winter seasons as depicted in *Figures 7* and *8*, which agrees with literature (Okwu and Ezenagu, 2008; Helen et al., 2013; Somkuwar and Kamble, 2013; Nwankwo and Osaro-Mathew, 2014; Dhital, 2017; Diso et al., 2017; Divyalashmi and Sharmili, 2017). The summer and winter leaves did not show any variation, as the histochemical tests revealed positive results for both seasons. This suggests that *M. indica* L. can be used throughout the year for qualitative metabolites extraction, however further studies are necessary.

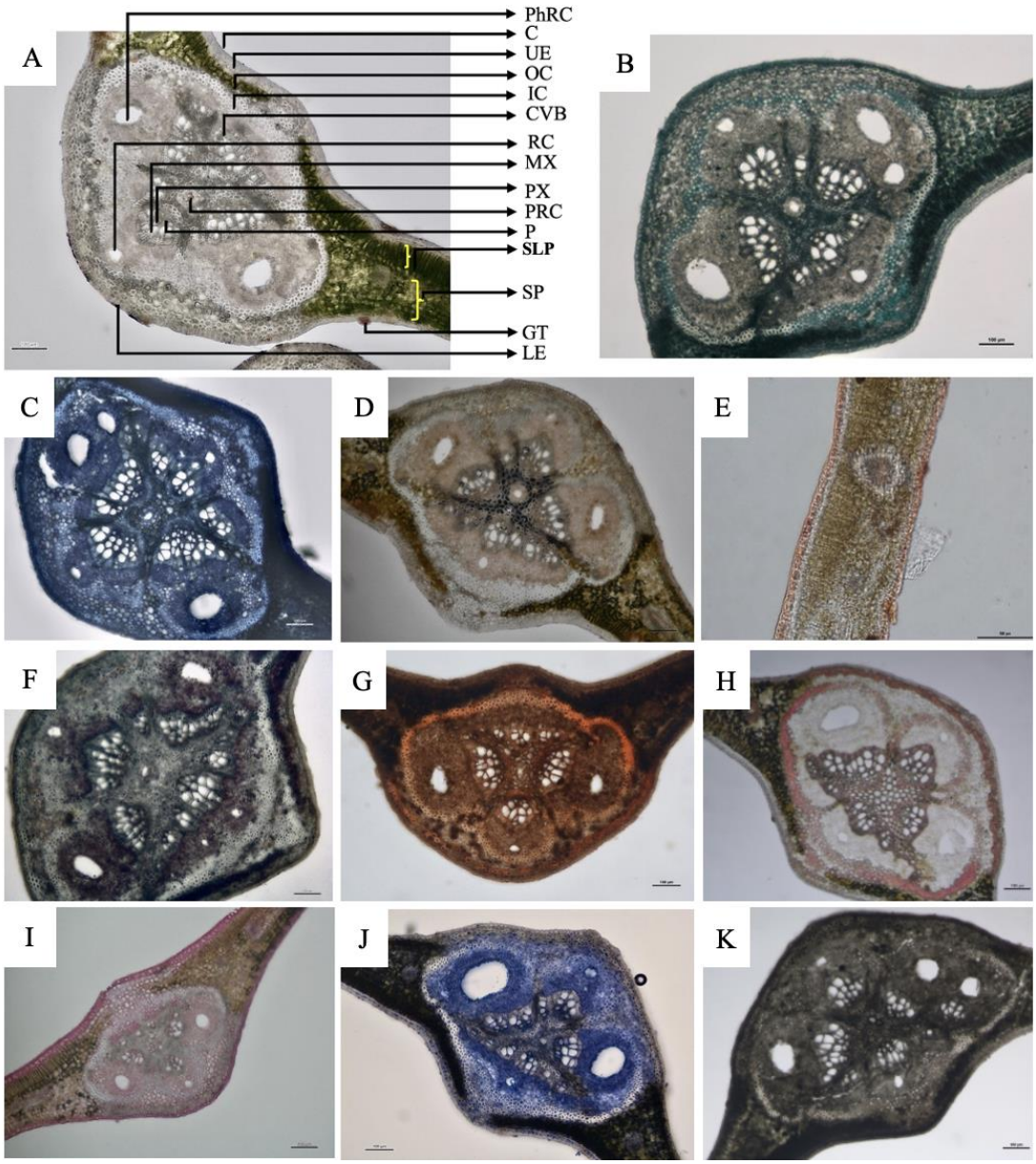

Figure 7. *Cross-sectional histochemical staining micrographs of M. indica leaf for Summer. A) Control. (B) Sudan Black. (C-Toluidine blue. (D) Wagners reagent. (E) Sudan III and IV. (F) Bromophenol blue. (G) NADI. (H) Phloroglucinol. (I) Ruthenium Red. (J) Coomassie blue. (K) Ferric trichloride. Abbreviations: PhRC = Phloem resin canal; C = Cortex; UE = Upper epidermis; OC = Outer cortex; IC = Inner cortex; CVB = Central vascular bundle; RC = Resin canal; MX = Meta xylem; PX = Phloem xylem; PRC: = Pith resin canal; P = Phloem; SLP = Single layer palisade; SP = Spongy parenchyma; GT = Glandular trichome; LE = Lower epidermis*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2205_43094328

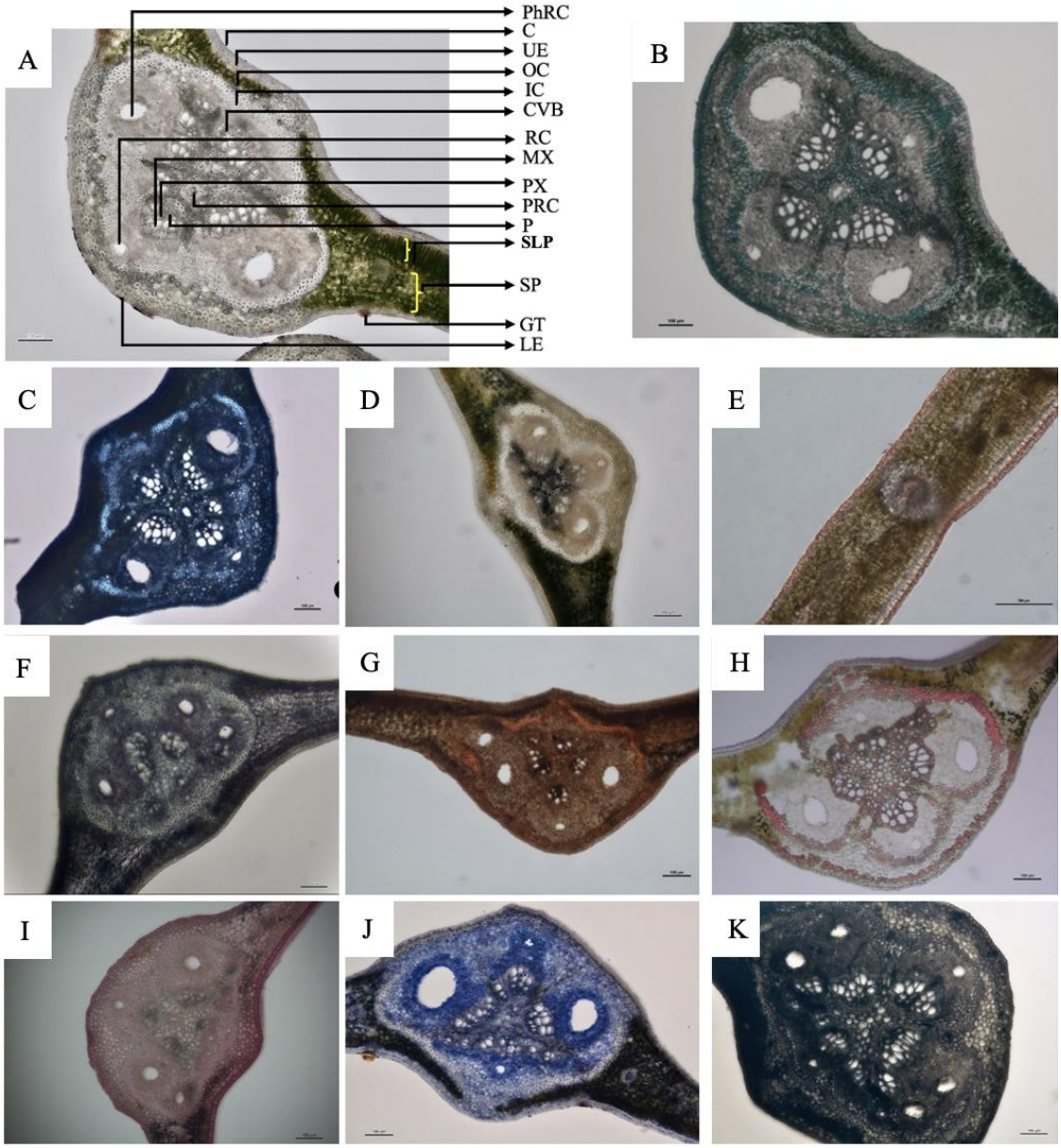

Figure 8. *Cross-sectional histochemical staining micrographs of M. indica leaf for Winter. A) control. (B) Sudan Black. (C) Toluidine blue. (D) Wagners reagent. (E) Sudan III and IV. (F) Bromophenol blue. (G) NADI. (H) Phloroglucinol. (I) Ruthenium red. (J) Coomassie blue. (K) Ferric trichloride. Abbreviations: PhRC = Phloem resin canal; C = Cortex; UE = Upper epidermis; OC = Outer cortex; IC = Inner cortex; CVB = Central vascular bundle; RC = Resin canal; MX = Meta xylem; PX = Phloem xylem; PRC: = Pith resin canal; P = Phloem; SLP = Single layer palisade; SP = Spongy parenchyma; GT = Glandular trichome; LE = Lower epidermis*

Conclusions

The integration of stereo- and scanning electron microscopy enabled detailed identification of the leaf morphology of *M. indica*. Previously, our understanding of *M. indica* leaf morphology using microscopy techniques has been limited. The identified leaf structures were compared with existing data on the species, significantly enhancing

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2205_43094328

our knowledge of its leaf anatomy. This study unveiled new anatomical features crucial for identifying *M. indica*, including the presence of glandular and non-glandular trichomes on the leaf surface. Histochemical tests pinpointed the sites of metabolite accumulation and synthesis, contributing to the pharmaco-botanical standardization of this species. These metabolites, such as alkaloids and phenols, are known for their potent medicinal properties. The present study delineates several anatomical features: (i) the shape of epidermal cells; (ii) the outline of the leaf margin midrib; (iii) the characteristics of epidermal shape; (iv) the hypodermis layer; v) the number of palisade cell layers; (vi) the presence of peltate glandular and non-glandular trichomes with cuticular warts; (vii) the distribution of peltate trichomes; (viii) and the inclusion of various organelles (e.g., vacuoles, starch grains). Further research focusing on the internal features of cells and organelles, particularly the trichomes, as well as the micromorphology of the seeds and roots of *M. indica*, is warranted for a more comprehensive understanding.

Acknowledgements. Funding by the National Research Foundation (NRF) is greatly appreciated. The authors acknowledge Researchers Supporting Project number (RSP-2024R375), King Saud University, Riyadh, Saudi Arabia.

REFERENCES

[1] Allen, D. J., Ort, D. R. (2001): Impacts of chilling temperatures on photosynthesis in warm-climate plants. – Trends Plant Science 6: 36-42.

[2] Anisko, T., Lindstrom, O. M. (1996): Cold hardiness and water relations parameters in *Rhododendron* cv Catawbiense Boursault subjected to drought episodes. – Physiologia Plantarum 98: 147-155.

[3] Balcke, G. U., Bennewitz, S., Bergau, N., Athmer, B., Henning, A., Majovsky, P., Jim´enez- Go´mez, J. M., Hoehenwarter, W., Tissier, A. (2017): Multi-omics of tomato glandular trichomes reveals distinct features of central carbon metabolism supporting high productivity of specialized metabolites. – Plant Cell 29: 960-983.

[4] Banon, S., Fernandez, J. A., Franco, J. A., Torrecillas, A., Alarcón, J. J., Sanchez-Blanco, M. J. (2004): Effects of water stress and night temperature preconditioning on water relations and morphological and anatomical changes of *Mangifera indica* plants. – Scientia Horticulturae 101: 333-342.

[5] Bibi, H., Afzal, M., Muhammad, A., Kamal, M., Ullah, I., Khan, W. (2014): Morphological and Anatomical studies on selected dicot xerophytes of district Karak, Pakistan. – American-Eurasian Journal of Agricultural & Environmental Sciences 14: 1201-1212.

[6] Cahyanto, T., Sopian, A., Efendi, M., Kinasih, I. (2017): The Diversity of *Mangifera indica* cultivars in Sabang West Java based on morphological and anatomical characteristics. – Biosaintifika: Journal of Biology & Biology Education 9: 156-167.

[7] Cain, A. J. (1947): The use of Nile Blue in the examination of lipoids. – Quarterly Journal of Microscopical Science 88: 383-392.

[8] Chen, W. R., Zheng, J. S., Li, Y. Q., Guo, W. D. (2012): Effects of high temperature on photosynthesis, chlorophyll fluorescence, chloroplast ultrastructure, and antioxidant activities in fingered citron. – Russian Journal of Plant Physiology 59: 732-740.

[9] Chen, W. L., Yang, W. J., Lo, H. F., Yeh, D. M. (2014): Physiology, anatomy, and cell membrane thermostability selection of leafy radish (*Raphanus sativus* var. *Oleiformis* Pers.) with different tolerance under heat stress. – Scientia Horticulturae 179: 367-375.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2205_43094328

[10] Choi, J. S., Kim, E. S. (2013): Structural features of glandular and non-glandular trichomes in three species of *Mentha*. – Applied Microscopy 43: 47-53.
[11] Coelho, B. E. S., Nascimento, M. M., Carvalho, I. R. B., Sousa, K. D. S. M., Machado, N. S., Neto, A. F. (2019): Solar drying curve and physical-chemical evaluation of "Espada" mango. – Journal of Environmental Analysis and Progress 4: 187-194.
[12] Cooper, D. C. (1932): The Development of the peltate hairs of *Mangifera indica*. – American Journal of Botany 9: 423-428.
[13] Cordero, R. A., Nilsen, E. T. (2002): Effects of summer drought and winter freezing on stem hydraulic conductivity of *Mangifera species* from contrasting climates. – Tree Physiology 22: 919-928.
[14] Van Cotthem, W. R. J. (1970): A classification of stomatal types. – Botanical Journal of the Linnean Society 63: 235-246.
[15] Demarco, D. (2017): Histochemical Analysis of Plant Secretory Structures. – In: Pellicciari, C., Biggiogera, M. (eds.) Histochemistry of Single Molecules. Humana Press, New York, pp. 313-330.
[16] Dhital, K. S. (2017): Phytochemical screening and antioxidant activities of *Mangifera indica* leaves grown in temperate region of Nepal. – Journal of Pharmacognosy and Phytochemistry 6: 205-209.
[17] Diso, S., Ali, M., Mukhtar, S., Garba, M. (2017): Antibacterial activity and phytochemical screening of *Mangifera indica* (Mango) stem and leaf extracts on clinical isolates of methicillin resistant *Staphylococcus aureus*. – Journal of Advances in Medical and Pharmaceutical Sciences 13: 1-6.
[18] Divyalashmi, L., Sharmili, A. (2017): Phytochemical analysis and antibacterial activity of *Mangifera indica* L and *Piper betle*. – International Journal of Pharma and Bio Sciences 8: 84-91.
[19] Dobra, J., Cerny, M., Storchova, H., Dobrev, P., Skalak, J., Jedelsky, P. L., Luksanova, H., Gaudinova, A., Pesek, B., Malbeck, J., Vanek, T., Brzobohaty, B., Vankova, R. (2015): The impact of heat stress targeting on the hormonal and transcriptomic response in *Arabidopsis*. – Plant Science 231: 52-61.
[20] Ediriweera, M. K., Tennekoon, K. H., Samarakoon, S. R. (2017): A review on ethnopharmacological applications, pharmacological activities, and bioactive compounds of *Mangifera indica* (mango). – Evidence Based Complementary and Alternative Medicine 1: 6949835.
[21] Ferrenberg, S., Kane, J. M., Milton, J. B. (2014): Resin duct characteristics associated with tree resistance to bark beetles across Lodgepole and limber pines. – Oecologia 174: 1283-1292.
[22] Fisher, D. B. (1968): Protein staining of ribboned epon sections for light microscopy. – Histochemistry and Cell Biology 16: 92-96.
[23] Furr, M., Mahlberg, P. G. (1981): Histochemical analyses of laticifers and glandular trichomes in *Mangifera indica*. – Journal of Natural Products 44: 153-159.
[24] Ganogpichayagrai, A., Rungsihirunrat, K., Palanuvej, C. and Ruangrungsi, N. (2016): Characterization of *Mangifera indica* cultivars in Thailand based on macroscopic, microscopic, and genetic Characters. – Journal of Advanced Pharmaceutical Technology & Research 7: 127-133.
[25] Ganong, W. F. (1895): Present Problems in the Anatomy, Morphology, and Biology of the Cactaceae. – Botanical Gazette 20: 129-138.
[26] Ghuniyal, J. (2015): Ethanomedical, chemical, pharmacological, toxicological properties of *Mangifera indica*: a review. – International Journal of Pharma Research & Review 4: 51-64.
[27] Gregory, M., Baas, P. (1989): A survey of mucilage cells in vegetative organs of the dicotyledons. – Israel Journal of Botany 38: 125-174.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2205_43094328

[28] Gupta, N. K., Agarwal, S., Agarwal, V. P., Nathawat, N. S., Gupta, S., Singh, G. (2013): Effect of short-term heat stress on growth, physiology and antioxidative defence system in wheat seedlings. – Acta Physiologiae Plantarum 35: 1837-1842.
[29] Hameed, A., Goher, M., Iqbal, N. (2012): Heat stress-induced cell death, changes in antioxidants, lipid peroxidation and protease activity in wheat leaves. – Journal of Plant Growth Regulation 31: 283-291.
[30] Hegebarth, D., Buschhaus, C., Wu, M., Bird, D., Jetter, R. (2016): The composition of surface wax on trichomes of *Arabidopsis thaliana* differs from wax on other epidermal cells. – Plant Journal 88: 762-774.
[31] Helen, P. A. M., Aswathy, M. R., Deepthi, K. G., Mol, R. R., Joseph, J. J., Sree, S. J. (2013): Phytochemical analysis and anticancer activity of leaf extract of *Mangifera indica* (Kottukonamvarika). – International Journal of Pharmaceutical Sciences and Research 4: 823-828.
[32] Herrera, F., Mitchell, J. D., Pell, S. K., Collinson, M. E., Daly, D. C., Manchester, S. R. (2018): Fruit morphology and anatomy of the Spondioid Anacardiaceae. – The Botanical Review 84: 315-393.
[33] Huang, S. S., Kirchoff, B. K., Liao, J. P., (2008): The capitate and peltate glandular trichomes of *Lavandula pinnata* L. (Lamiaceae): Histochemistry, ultrastructure, and secretion. – Journal of Torrey Botanical Society 135: 155-167.
[34] Huve, K., Bichele, I., Tobias, M., Niinemets, U. (2006): Heat sensitivity of photosynthetic electron transport varies during the day due to changes in sugars and osmotic potential. – Plant, Cell and Environment 29: 212-228.
[35] Jahurul, M. H. A., Zaidul, I. S., Ghafoor, K., Al- Juhaimi, F. Y., Nyam, K. L., Norulaini, N. A., Sahena, F., Mohd Omar, A. K. (2015): Mango (*Mangifera indica* L.) by-products and their valuable components: a review. – Food Chemistry 183: 173-180.
[36] Johansen, D. A. (1940): Plant Microtechnique. – McGraw-Hill Book Co. Inc, New York.
[37] Johnson, H. B. (1975): Plant pubescence: an ecological perspective. – The New York Botanical Garden 41: 233-258.
[38] Jones, H. G. (1985): Partitioning stomatal and non-stomatal limitations to photosynthesis. – Plant, Cell and Environment 8: 95-104.
[39] Kariyat, R. R., Hardison, S. B., Ryan, A. B., Stephenson, A. G., De Moraes, C. M., Mescher, M. C. (2018): Leaf trichomes affect caterpillar feeding in an instar-specific manner. – Communicative & Integrative Biology 11: 1-6.
[40] Ko, K. N., Lee, K. W., Lee, S. E., Kim, E. S. (2007): Development and ultrastructure of glandular trichomes in *Pelargonium fragrans* 'Mabel Grey'. – Journal of Plant Biology 50: 362-368.
[41] Lacchia, A. P. S., Carmello-Guerreiro, S. M. (2009): Ultrastructural aspects of secretory channels in vegetative and reproductive organs of Anacardiaceae. – Acta Botanica Brasilica 23: 376-378.
[42] Lauricella, M., Emanuele, S., Calvaruso, G., Giuliano, M., D'Anneo, A. (2017): Multifaceted health benefits of *Mangifera indica* L. (mango): The inestimable value of orchards recently planted in Sicilian rural areas. – Nutrients 9: E525.
[43] Levin, D. (1973a): The role of trichomes in plant defense. – The Quarterly Review of Biology 43: 3-15.
[44] Levin, D. A. (1973b): The role of trichomes in plant defense. – The Quarterly Review of Biology 48: 3-15.
[45] Li, S., Tosens, T., Harley, P. C., Harley, P. C., Jiang, Y., Kanagendran, A., Grosberg, M., Jaamets, K., Niinemets, Ü. (2018): Glandular trichomes as a barrier against atmospheric oxidative stress: relationships with ozone uptake, leaf damage, and emission of LOX products across a diverse set of species. – Plant, Cell and Environment 41: 1263-1277.
[46] Lipp, C. C., Nilsen, E. T. (1997): The impact of subcanopy light environment on the hydraulic vulnerability of *Mangifera indica* to freeze-thaw cycles and drought. – Plant, Cell and Environment 20: 1264-1272.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2205_43094328

[47] Lizarraga, E. F., Mercado, M. I., Galvez, C. E. D. V., Ruiz, A. I., Ponessa, G. I., Catalan, C. A. N. (2017): Morpho anatomical characterization and essential oils of *Tagetes terniflora* and *Tagetes minuta* (Asteraceae) growing in Tucuman (Argentina). – Boletín de la Sociedad Argentina de Botánica 52: 55-68.
[48] Lorenzi, H., Lacerda, M. T. C., Bacher, L. B. (2015): Fruits in Brazil: Native and Exotic (in Natural Consumption). – Plantarum Institute of Flora Studies, São Paulo.
[49] Luo, S. H., Luo, Q., Niu, X. M., Xie, M. J., Zhao, X., Schneider, B., Gershenzon, J., Li, S. H. (2010): Glandular trichomes of Leucosceptrum Canum Harbor Defensive sesterterpenoids. – Angewandte Chemie, 122: 4573-4577.
[50] Macedo, A. (2012): Abiotic Stress Responses in Plants: Metabolism to Productivity. – In: Ahmad, P., Prasad, M. N. V. (eds.) Abiotic Stress Responses in Plants. Springer, New York, pp. 41-61.
[51] Machado, E. C., Medina, C. L., Gomes, M. M. A., Habermann, G. (2002): Seasonal variation of photosynthetic rates, stomatal conductance and leaf water potential in 'Valencia' orange trees. – Scientia Agricola 59: 53-58.
[52] Manaa, A., Gharbi, E., Mimouni, H., Wasti, S., Aschi-Smiti, S., Lutts, S., Ben Ahmed, H. (2014): Simultaneous application of salicylic acid and calcium improves salt tolerance in two contrasting tomato (*Solanum lycopersicum*) cultivars. – South African Journal of Botany 95: 32-39.
[53] Mayekiso, B., Magwa, M. L., Coopoosamy, R. (2008): The morphology and ultrastructure of glandular and non-glandular trichomes of *Pteroniaincana* (Asteraceae). – African Journal of Plant Science 2: 52-60.
[54] Mckay, S. A. B., Hunter, W. L., Godard, K., Wang, S. X., Martin, D. M., Bohlmann, J. Plant, A. L. (2003): Insect attack and wounding including traumatic resin duct development and gene expression of (-)-pinene synthase in Sitka spruce. – Plant Physiology 133: 368-378.
[55] Medina, C. L., Souza, R. P., Machado, E. C., Ribeiro, R. V., Silva, J. A. B. (2002): Photosynthetic response of citrus grown under reflective aluminized polypropylene shading nets. – Scientia Horticulturae 96: 115-125.
[56] Metcalfe, C. R., Chalk, K. L. (1950): Anatomy of the Dicotyledons: Leaves, Stem, and Wood in Relation to Taxonomy with Notes on Economic Uses. – Clarendon, Oxford.
[57] Metcalfe, C. R., Chalk, L. (1957): Anatomy of the Dicotyledons. – Oxford University Press, London.
[58] Moreshet, S., Green, G. C. (1984): Seasonal trends in hydraulic conductance of field-grown 'Valencia' orange trees. – Scientia Horticulturae 23: 169-180.
[59] Naidoo, Y., Heneidak, S., Bhatt, A., Kasim, N., Naidoo, G. (2014): Morphology, histochemistry, and ultrastructure of foliar mucilage-producing trichomes of *Harpagophytum procumbens* (Pedaliaceae). – Turkish Journal of Botany 38: 60-67.
[60] Navarro, T., El Oualidi, J. (2000): Trichome morphology in *Teucrium* L. (Labiatae). a Taxonomic Review. – Annals of the Botanical Garden of Madrid 57: 277-297.
[61] Norfaizal, M., Latiff, A. (2013): Leaf anatomical characteristics of *Bouea*, *Mangifera* and Spondias (Anacardiaceae) in Malaysia. – Journal of Life Sciences 8: 758-767.
[62] Nwankwo, I. U., Osaro-Mathew, R. C. (2014): Assessment of the phytochemical components of *Mangifera indica* (leaf) and *Musa paradisiaca* (roots) extracts and their antibacterial activity against some common pathogenic bacteria. – Journal of Pharmacy and Biological Sciences 9: 8-11.
[63] O' Brien, T. P., Feder, N., McCully, M. E. (1964): Polychromatic staining of plant cell walls by toluidine blue O. – Protoplasma 59: 368-373.
[64] Okwu, D. E., Ezenagu, V. I. T. U. S. (2008): Evaluation of the phytochemical composition of mango (*Mangifera indica* Linn) stem bark and leaves. – International Journal of Chemical Sciences 6: 705-716.
[65] Parvez, G. M. M. (2016): Pharmacological activities of mango (*Mangifera indica*): a review. – Journal of Pharmacognosy and Phytochemistry 5: 1-7.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2205_43094328

[66] Pearse, A. G. E. (1985): Histochemistry: Theoretical and Applied. Fourth Ed. – Churchill Livingstone, Edinburgh.
[67] Ranney, T. G., Blazich, F. A., Warren, S. L. (1995): Heat tolerance of selected specie and populations of *Rhododendron*. – Journal of the American Society for Horticultural Science 120: 423-428.
[68] Ribeiro, R. V., Machado, E. C. (2007): Some aspects of citrus eco- physiology in subtropical climates: re-visiting photosynthesis under natural conditions. – Brazilian Journal of Plant Physiology 19: 393- 411.
[69] Ribeiro, R. V., Bieski, I. G. C., Balogun, S. O., Martins, D. T. O. (2017): Ethnobotanical study of medicinal plants used by Ribeirinhos in the North Araguaia microregion, Mato Grosso, Brazil. – Journal of Ethnopharmacology 205: 69-102.
[70] Rocha, L. A., Rocha, A. M., Pacheco, A. C. L., Abreu, M. C. (2015): Foliar morphoanatomical differences of four species of the Anacardiaceae family. – Research Notebook, Biology Series 27: 35-48.
[71] Santhan, P. (2014): Leaf structural characteristics of important medicinal plants. – International Journal of Research in Ayurveda and Pharmacy 5: 673-679.
[72] Santos, M. M., Nunes, M. G. S., Martins, R. D. (2012): Empirical use of medicinal plants for the treatment of diabetes. – Brazilian Journal of Medicinal Plants 14(2): 327-334.
[73] Santos, T., Luiz, R. d., Silva, d. M., Stefany, C. R., Tatiane, M. (2016): Non-glandular trichomes in Lamiaceae and Verbenaceae species: morphological and histochemical features indicate more than physical protection. – New Zealand Journal of Botany 54: 446-457.
[74] Schindelin, J., Rueden, C. T., Hiner, M. C., and Eliceiri, K. W. (2015): The ImageJ ecosystem: an open platform for biomedical image analysis. – Molecular Reproduction and Development 82: 518-529.
[75] Shah, K. A., Patel, M. B., Patel, R. J., Parmar, P. K. (2010): *Mangifera indica* (Mango). – Pharmacognosy Review 4: 42-48.
[76] Shaheen, N., Ajab, M., Yasmin, G., Hayat, M. Q. (2009): Diversity of Foliar Trichomes and their Systematics Relevance in the Genus Hibiscus (Malvaceae). – International Journal of Agriculture & Biology 11: 279-284.
[77] Sharma, B. G., Albert, S., Dhaduk, H. K. (2012): Petiolar anatomy as an aid to the identification of *Mangifera indica* L. varieties. – Notes to Biological Science 4: 44-47.
[78] Somkuwar, D. O., Kamble, V. A. (2013): Phytochemical screening of ethanolic extracts of stem, leaves, flower, and seed kernel of *Mangifera indica* L. – International Journal of Pharmacology and Biological Sciences 4: 383-389.
[79] Spurr, A. R. (1969): A low-viscosity epoxy resin embedding medium for electron microscopy. – Journal of Ultrastructure Research 26: 31-43.
[80] Sridharan, G., Shankar, A. A. (2012): Toluidine blue: a review of its chemistry and clinical utility. – Journal of Oral and Maxillofacial Pathology 16: 251-255.
[81] Syvertsen, J. P., Zablotowicz, R. M., Smith Jr., M. L. (1983): Soil temperature and flooding effects on two species of citrus. 1. Plant growth and hydraulic conductivity. – Plant Soil 72: 3-12.
[82] Szyndler, M. W., Haynes, K. F., Potter, M. F., Corn, R. M., Loudon, C. (2013): Entrapment of bed bugs by leaf trichomes inspires microfabrication of biomimetic surfaces. – Journal of the Royal Society Interface 10: 174.
[83] Terra, W. R. (2001): The origin and functions of the insect peritrophic membrane and peritrophicgel. – Archives of Insect Biochemistry and Physiology 47: 47-61.
[84] Tooker, J., Peiffer, M., Luthe, D. S., Felton, G. W. (2010): Trichomes as sensors: detecting activity on the leaf surface. – Plant Signal Behaviour 5: 73-75.
[85] Tos, J., van der Ploeg, M., Mitchell, J. P., Cohn, N. S. (1980): Protein staining methods in quantitative cytochemistry. – Journal of Microscopy 119: 295-311.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2205_43094328

[86] Vacha, F., Adamec, F., Valenta, J., Vacha, M. (2007): Spatial location of photosystem pigment–protein complexes in thylakoid membranes of chloroplasts of Pisum sativum studied by chlorophyll fluorescence. – Journal of Luminescence 122: 301-303.
[87] Valkama, E., Salminen, J. P., Koricheva, J., Pihlaja, K. (2003): Comparative analysis of leaf trichome structure and composition of epicuticular flavonoids in Finnish birch species. – Annals of Botany 91: 643-655.
[88] Valverde, P., Fornoni, J., Núnez-Farfan, J. (2001): Defensive role of leaf trichomes in resistance to herbivorous insects in *Datura stramonium*. – Journal of Evolutionary Biology 14: 424-432.
[89] Vasconcelos, A. L., Vasconcelos, A. L., Randau, K. P. (2016): Pharmacognostic characterization of *Spondias mombin* L. (Anacardiaceae). – Pharmacognosy Journal 8: 513-519.
[90] Wagner, G. J. (1991): Secreting glandular trichomes: more than just hairs. – Plant Physiology 96: 675-679.
[91] Waqas, M., Shahzad, R., Khan, A. L., Asaf, S., Kim, Y. H., Kang, S. M., Bilal, S., Hamayun, M., Lee, I. J. (2016): Salvaging effect of triacontanol on plant growth, thermotolerance, macro-nutrient content, amino acid concentration and modulation of defense hormonal levels under heat stress. – Plant Physiology and Biochemistry 99: 118-125.
[92] Werker, E. (2000): Trichome diversity and development. – Advancement in Botany 31: 1-35.
[93] Xu, S., Li, J. L., Zhang, X. Q., Wei, H., Cui, L. J. (2006): Effects of heat acclimation pre-treatment on changes of membrane lipid peroxidation, antioxidant metabolites, and ultrastructure of chloroplasts in two cool-season turfgrass species under heat stress. – Environmental and Experimental Botany 56: 274-285.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2205_43094328