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Comparative Morphological and Anatomical Studies on Cucurbita maxima Duchesne and Lagenaria siceraria (Molina) Standl.

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ABSTRACT

Comparative studies on the morphology and anatomy of Cucurbita maxima Duchesne and Lagenaria siceraria(Molina) Standl. grown in Egypt were carried out. The morphological features of significance included variations in the leaf (color, size, shape, stomatal type and both upper and lower epidermis patterns), pollen grain shape and seed (shape, size, color, hilum and seed coat patterns).

Key words: Anatomy, Cucurbita maxima Duchesne, Lagenaria siceraria (Molina) Standl., Morphology, Scanning Electron Microscope (SEM).

Introduction

The family Cucurbitaceae (cucurbit or gourd family) consists of about 120 genera and over 800 species (Teppner, 2004). The family is widespread in tropical and subtropical regions, with relatively few species occurring in temperate and cool climates (Cronquist, 1981 and Teppner, 2004). The Cucurbitaceae are important as source of food; pumpkin, squash, cucumber, muskmelon and watermelon. A few species are grown as ornamentals. Some are weedy (Jones and Luchsinger, 1987).

Cucurbita maxima Duchesne, pumpkin or winter squash is a member in the gourd family native to South America and cultivated by indigenous people for over 2000 years but now is cultivated worldwide particularly in South America, India and Africa. Winter squash is eaten as vegetable, mashed or in purees, soups or pies. The blossoms are also edible and may be cooked into fritters. Seeds are high in protein and minerals and are eaten raw, toasted or pressed to make oil. Seeds are also used medicinally as a diuretic, antipyretic and anthelmintic (Robinson and Decker-Walters, 1997).

Lagenaria siceraria (Molina) Standl. (Syn. Cucurbita lagenaria L., Cucurbita siceraria Molina, Cucurbita leucantha Duchesne, Lagenaria vulgaris Ser., Lagenaria leucantha Rusby) is also another member in the same family and known as bottle gourd, calabash and white-flowered gourd (Bailey, 1969; Robinson and Decker-Walters, 1997; Teppner, 2004 and Anon., 2013). Its origin is in Africa and it migrated to South America, North America and Asia as a wild or cultivated plant thousands of years ago. Immature fruits are cooked. However, mature fruits used as containers for food and water and to make musical instruments, bird houses, ladles, rattles and decoration. Also, it is prepared as a diuretic, emetic or antipyretic (Robinson and Decker-Walters, 1997).

With increased sophistication of classification systems it has become increasingly important to have more elaborative means for identification. The leaf has not lost its importance as a taxonomic tool but rather has proved to be more useful when a fuller understanding of all its characteristics are known and appreciate.

Pollen grain shapes are described, as well as markings, size, color, stickiness, abundance and other features. It is found that, in general, the shape and size of grains in the various genera exceptions are the thread-shaped pollen grains of some eelgrass and in aspect: round, oval, disc or bean-shaped and sometimes filamentous. The natural color is mostly white, cream, yellow or orange. The texture of the cell wall shows also great variations, from smooth to spiky. (Maxy, 1925 and Thanikaimoni and Van der Ham, 1999). Defining sculpture of those forms that have been described only the basis of observations using TEM and SEM (Basil, 1995). Fossil pollen grains are distinguished primarily by their form and their surface sculpture and Key distinguishes 17 basic pollen classes (Christopher, 1979).

Pollen grains of the neotropical subtribe Cuspariinae (Rutaceae) were examined by LM, SEM and TEM. The pollen morphology of this subtribe is very diverse (Morton and Kallunki, 1993).

The great variations in the morphology and the different ornamentations of the seed coat support the study of taxa delimitation and may solve and facilitate many taxonomic problems. Netolizky (1926) confirmed also that the morphology of seed coat surface should be the principles of natural classification of the flowering
plants. Vaughan (1968) suggested that the structure of the mature seeds, especially the coat structure is considered the more taxonomic useful information.

Surface sculpturing may aid in solving problems of identity or relationship concerning taxa at various levels (Werker, 1997). The SEM examinations of seed surface features could be applied in taxonomy and there are many characters (e.g. seed coat) could be used to characterize groups of related species, genera or taxonomic categories up to the sub-family level. Some characters of the micro-morphology and orientation of epicuticular wax crystalloid are surprisingly high systematic significance (Barthlott, 1981). More detailed structural information are found about seeds obtained by using the Scanning Electron Microscope. However, Yeh and Kakuma (1987) suggested that seed characters; color, coat patterns, shape, size, outer arils and hilum lead to better criteria for species identification and support the taxonomical positions of taxa.

The aim of this study is to distinguish the taxonomic relationship between two species of Cucurbitaceae grown in Egypt; namely, Cucurbita maxima Duchesne and Lagenaria siceraria (Molina) Standl. Morphological descriptions and Scanning Electron Microscope (SEM) survey on the leaf, pollen grain and seed surface as well as leaf anatomy of the studied species were investigated.

Materials and Methods

In this study, two species of Cucurbitaceae; namely, Cucurbita maxima Duchesne and Lagenaria siceraria (Molina) Standl. were studied. These species were planted in Balteem, Kafer El-Sheikh at the winter growing season of 2010/2011. Specimens of investigated plants were subjected to identification. Authentication was carried out by means of comparison at the Herbarium, Flora & Phyto-Taxonomy Researches, Horticultural Research Institute, ARC, Dokki, Giza, Egypt (CAIM), where plant herbarium specimens were deposited. In addition, description of studied species mentioned by (Bailey,1969; Claugher,1990; Hesse et al., 2009 and Simpson, 2010) were consulted. Scientific names of studied species were revised through the Catalogue of Life Annual Checklist (Anon., 2013).

The present investigation aimed to represent the taxonomical relationships among the studied species. Taxonomical evidences and characters which will explore this relationships were gathered from the following different sources during this study; morphological, anatomical descriptions and Scanning Electron Microscope (SEM) on the surfaces of leaves, pollen grains and seeds of the two selected species.

Ultra structural features of leaf, pollen grain and seed surfaces of each species were carried out using Scanning Electron Microscope (SEM). The specimens were mounted on the copper specimen holder stub and coated with a thin layer of gold palladium using Edwards sputter coater unit S 150 B. The specimens were examined in different positions using different magnifications by JEOL- JSM T 100 Model Scanning Electron Microscope, at the Research Park of Faculty of Agriculture (FARP), Cairo University, Giza, Egypt.

For anatomical studies, specimens were taken from well developed leaves at different parts of the main stem. Specimens were killed and fixed at least for 48 hrs. in F.A.A. (10 ml formalin, 5 ml glacial acetic acid and 85 ml ethyl alcohol 70%). The tested materials were washed in 50% ethyl alcohol, dehydrated in a normal butyl alcohol series, embedded in paraffin wax of melting point 56°C, sectioned to a thickness of 20 microns, double stained with crystal violet-erythrosin, cleared in xylene and mounted in Canada balsam (Nassar and El-Sahhar, 1998). Slides were analyzed microscopically and photomicrographed.

Results and Discussion

The species under consideration were studied and the results were gathered according to the following aspects:

Macromorphological and Scanning Electron Microscope (SEM) descriptions of leaf (upper and lower surfaces), pollen grain and seed surface for each species as well as leaf anatomy.

Cucurbita maxima Duchesne:

1. Leaf:

a. Macromorphological and Scanning Electron Microscope (SEM) descriptions of leaf:

Leaves are simple, petiolate, alternate, dark green in color at their upper surface, 4.5–8 cm in length, 5–12.5 cm wide, nearly reniform, lobes distinguished, denticulate margined, apex mucronulate, base reniform, venation palmate and yellow, covered with coarse hairs on both surfaces, petiole 2.5–4 cm and hollow (Fig. 1).
Fig. 1: Photographs of leaf of *Cucurbita maxima* Duchesne.
A-Upper surface.
B- Lower surface.

Scanning electron micrograph of the upper surface in pumpkin leaf blade shows paracytic stomata with raised level and reticulate-foveate sculpture pattern, whereas the lower surface shows paracytic and anomocytic stomata with raised level and reticulate sculpture pattern. Trichomes of non-glandular and glebulate ornamentation are present on both surfaces (Fig. 2).

Fig. 2: Scanning electron micrographs of leaf blade of *Cucurbita maxima* Duchesne
A: Surface of upper epidermis
B: Surface of lower epidermis
C:Trichomes.
b. Structure of the leaf:

1. The leaf blade:

Transverse sections through the mature foliage leaves of *Cucurbita maxima* Duchesne plant is shown in Figure (3). It is clear that leaf is dorsiventral; i.e., the palisade tissue is located on the adaxial side of the blade and the spongy tissue on the abaxial one. There are two epidermal layers on adaxial and abaxial surfaces of the leaf. Each is uniseriate, composed of a row of compactly barrel cells and covered with a thin cuticle layer. Trichomes of nonglandular and glandular hairs are present on both surfaces (Fig. 4). The nonglandular hairs are multicellular uniseriate (2-5 cells) and most of them with compound foot. Some trichomes are nearly conical in shape and some others seem to be sharp like spine. The glandular hairs composed of stalk (1-3 cells) and spherical head (3-6 cells). These types of trichomes were mentioned by Metcalfe and Chalk (1979) for *Cucurbita*. At the midrib region, both upper and lower epidermis are convex. The palisade tissue consists of two layers of cells which elongated perpendicularly to the surface of the blade being characterized by an abundance of chloroplasts. The palisade tissue occupies about half of the whole thickness of the mesophyll. The spongy tissue is composed of three layers of chlorenchymatous loosely arranged cells with many intercellular spaces. There is a mass of collenchymatous cells below the adaxial and abaxial epidermis at the midrib region. Three bicollateral vascular bundles are arranged perpendicularly at the midrib region; the large one at the center of the midvein and the other smaller two being on the same side and each one above the other. The xylem of the main bundle consists of about 11 to 13 parallel rows, each with 3 to 4 vessels.

![Fig. 3: Transverse section through the leaf blade of *Cucurbita maxima* Duchesne. (X 100)](image)

![Fig. 4: Non-glandular multicellular hairs (A and B) and glandular hairs (C) developing from the epidermis of the leaf blade of *Cucurbita maxima* Duchesne. (X 360)](image)
The petiole:

The petiole of *Cucurbita maxima* Duchesne leaf as appeared in the transverse section shown in Figure (5) is oval in shape, wavy in outline, having distinct ridges and furrows and becoming hollow; i.e., a large hollow cavity is present in the central region and represents about 60% of the whole section. It is bounded by an uniseriate epidermis of nearly square-shaped cells and covered with a thin layer of cuticle. Trichomes are present and similar to those found on the leaf blade. The ribs, underlying the epidermis, consist mainly of collenchyma. The rest of the ground tissue is composed of nearly thin-walled large parenchyma cells, except at the distance between the ribs where there are 3 to 4 layers of chlorenchyma cells directly beneath the epidermis. The vascular cylinder is composed of 16 bicolateral vascular bundles arranged in a complete circle and being separated from one another by wide spaces of ground tissue. Worthy to state that vascular bundles are lying on the radii which pass through the petiole ridges; i.e., number of both the ridges and the vascular bundles are similar.

![Fig. 5: Transverse section through the leaf petiole of *Cucurbita maxima* Duchesne.](image1)

(A) Whole section. (X 40)

(B) Magnified portion of A. (X 100)

Pollen grain:

Pollen grains free, spheroidal, pantoporate, spiny. The zone of germination pores raised. Aperture one and poroid. Tectum aculeate (Fig. 6).

![Fig. 6: A scanning electron micrograph of pollen grains of *Cucurbita maxima* Duchesne.](image2)

Seed:

Seeds numerous, 1.7–2 x 0.7-1.1cm in dimensions, ovate, with an angular and raised side, smooth, glabrous and shiny surfaced, yellow, fine waved. Hilum conspicuous, terminal, and white. Base rounded (Fig. 7).
Fig. 7: A photograph showing mature seeds of *Cucurbita maxima* Duchesne.

The epidermal cells of the seed coat are glebulate. The anticlinal walls are raised and usually 4-5 gonal, straight with irregular channels. The outer periclinal walls are convex with delicate furrows. Hilum conspicuous, terminal, broad and rounded (Fig. 8).

![Seed surface sculpture patterns (A) and hilum shape (B) of *Cucurbita maxima* Duchesne as detected by SEM.](image)

Fig. 8: Seed surface sculpture patterns (A) and hilum shape (B) of *Cucurbita maxima* Duchesne as detected by SEM.

*Lagenaria siceraria* (Molina) Standl:

1. Leaf:

   a. *Macromorphological and Scanning Electron Microscope (SEM) descriptions of leaf:*

   Leaves are simple, petiolate, alternate, green in color, 4–9 cm in length, 6–14 cm wide, reniform, lobes obscured, ciliate margined, apex mucronate, base reniform, venation palmate and green, covered with fine hairs on both surfaces, petiole 2.7–6 cm (Fig. 9).
Fig. 9: Photographs of leaf of *Lagenaria siceraria* (Molina) Standl.
A: Upper surface
B: Lower surface

Figure (10) illustrates scanning electron micrographs of upper and lower surfaces in bottle gourd leaf blade. The upper surface has paracytic stomata with superficial and semiraised level, while the lower surface has paracytic stomata with raised level. Rugose sculpture pattern on both epidermis. Trichomes; non-glandular and smooth ornamentation.

Fig. 10: Scanning electron micrographs of leaf blade of *Lagenaria siceraria* (Molina) Standl.
A- Surface of upper epidermis
B- Surface of lower epidermis
C- Trichomes
b. Structure of the leaf:

The leaf blade:

Transverse section through the mature foliage leaf of *Lagenaria siceraria* (Molina) Standl. plant is shown in Figure (11). Compact arrangement of epidermal cells, presence of cuticle layer and trichomes are the main features of the bottle gourd leaf epidermis. Trichomes of nonglandular and glandular hairs are present on both surfaces (Fig. 12). The nonglandular hairs are multicellular uniseriate composed of 3 to 7 cells of which the terminal cell tapers and some of them with compound foot. This finding is in agreement with that given by Badmanaban et al. (2009) who recorded that numerous uniseriate covering trichomes (1 to 4 cells) with pointed edge occur throughout the upper and lower epidermis of *Lagenaria siceraria*. The glandular hairs composed of stalk (1-4 cells) and spherical head (3-6 cells). This result is in harmony with the finding of Metcalfe and Chalk (1979) who mentioned that glandular hairs with uniseriate stalk of variable length and spherical or disk-shaped heads are present in species of *Lagenaria*. The mesophyll is differentiated into palisade and spongy cells. This finding is in agreement with those given by Metcalfe and Chalk (1979) and Badmanaban et al. (2009). Leaf of *Lagenaria* is dorsiventral. The palisade tissue consists of two layers elongated perpendicularly to the surface of the blade being characterized by an abundance of chloroplasts. The palisade tissue occupies about half of the whole thickness of the mesophyll. The spongy tissue occurs towards the lower epidermis and consists of four layers of chlorenchymatous cells with wide intercellular spaces. At the midrib region, both upper and lower epidermis are convex and three bicollateral vascular bundles are arranged perpendicularly and embedded in ground tissue of parenchyma cells with a mass of collenchyma cells underlying the two epidermis. The main bundle lies in the lower portion of midrib region and its xylem consists of about 11 parallel rows, each with 3 vessels. This result is in agreement with that given by Metcalfe and Chalk (1979). The three vascular bundles of the midribs are arranged perpendicularly. There are collenchymatous cells below the upper epidermis and a few layers of the same above the lower epidermis at the midrib of *Lagenaria siceraria*, being in agreement with Badmanaban et al. (2009).

![Fig. 11: Transverse section through the leaf blade of Lagenaria siceraria (Molina) Standl. (X 100)](image)

![Main bundle](image)

![Midrib region](image)

![Fig. 12: Non-glandular multicellular hairs (A and B) and glandular hairs (C) developing from the epidermis of the leaf blade of Lagenaria siceraria (Molina) Standl. (X 360)](image)
The petiole:

The transverse section shown in Figure (13) proves that the petiole surface of *Lagenaria siceraria* (Molina) Standl. plant is ribbed. The epidermis consists of an uniseriate layer of rectangular cells covered with a thin cuticle layer. The nonglandular and glandular hairs are present in the epidermis. The ridges underlying the epidermis consist of collenchyma. The cortex in the grooves between ridges consists of chlorenchyma. The inner layers of the cortex comprised thin walled compact parenchyma cells. The vascular bundles are arranged in a ring and separated from one another by the ground tissue. The vascular cylinder consists of 11 bicolateral bundles. The pith occupies a large portion of the section and consists of parenchyma cells. The pith is connected with the cortex through medullary rays 5-8 rows wide.

![Fig. 13: Transverse section through the leaf petiole of *Lagenaria siceraria* (Molina) Standl.](image)

- **Fig. 13:** Transverse section through the leaf petiole of *Lagenaria siceraria* (Molina) Standl.
  - **A:** Whole section. *(X 40)*
  - **B:** Magnified portion of A. *(X 100)*

Pollen grain:

Pollen grains free, spheroidal, tricolporate. The zone of germination pores raised. Tectum glebulate (Fig. 14).

![Fig. 14: A scanning electron micrograph of pollen grains of *Lagenaria siceraria* (Molina) Standl.](image)

Seed:

Seeds numerous, 1.8–2.5 x 0.9-1.2 cm in dimensions, narrowly ovate, with a rounded side, smooth, glabrous and dull surfaced, yellow to dark brown, with two sharply dark furrows. The hilum is conspicuous, terminal and brown. Base straight (Fig. 15).
Surface scan of the seed coat by means of SEM shows that the hilum is conspicuous, terminal, pike and rounded. The seed coat epidermal cells are irregular and reticulate. The anticlinal walls are raised and usually 4-6 gonal. The outer periclinal walls are concave (Fig 16).

For sake of ease, various differential characters of *Cucurbita maxima* Duchesne and *Lagenaria siceraria* (Molina) Standl., previously given are summarized in Table (1) in order to compare and accurately identify each of the two studied species. Moreover, a botanical key is constructed as shown in what follows for the same reason.

**Botanical key:**

Leaf margin denticulate, apex mucronulate, dark green in color, venation yellow in color, hairs coarse, upper epidermis with stomata raised, reticulate-foveate sculpture, lower epidermis with raised paracytic and anomocytic stomata, reticulate sculpture, trichomes ornamentation glebulate, petiole hollow

Seed ovate with angular raised side, 1.7-2 X 0.7-1.1 cm., sculpture glebulate, hilum broad and white

Pollen grain aperture one and poroid, tectum aculeate.

*Cucurbita maxima* Duchesne

Leaf margin ciliate, apex mucronate, green in color, venation green in color, hairs fine, upper epidermis with stomata superficial and semiraised, roguse sculpture, lower epidermis with raised paracytic stomata, roguse sculpture, trichomes ornamentation smooth, petiole solid

Seed narrowly ovate with rounded side, 1.8-2.5 X 0.9-1.2 cm., sculpture reticulate, hilum pike and brown

Pollen grain aperture more than one and tricolporate, tectum glebulate.

*Lagenaria siceraria* (Molina) Standl
Table 1: Diagnostic characters of *Cucurbita maxima* Duchesne and *Lagenaria siceraria* (Molina) Standl.

<table>
<thead>
<tr>
<th>Characters</th>
<th><em>Cucurbita maxima</em> Duchesne</th>
<th><em>Lagenaria siceraria</em> (Molina) Standl.</th>
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</thead>
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<td></td>
<td></td>
</tr>
<tr>
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<td>Reniform</td>
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<td>- Margin</td>
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<td>- Apex</td>
<td>Micronulate</td>
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<td>- Color</td>
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<td>- Venation</td>
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<tr>
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<td>Fine hairs</td>
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References


