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Scanning electron microscopic study of leaf epidermal features of selective members of Cucurbitaceae

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Abstract
Micromorphology of trichomes of selective members of Cucurbitaceae like *Lagenaria vulgaris, Benincasa hispida, Cucurbita maxima, Luffa acutangula and Cucumis sativus* were studied microscopically using scanning electron microscope. The trichomes among the selected members were differ from unicellular to multicellular, conical to elongated, smooth to ridges, with or without flattened disk at base and cystolithic appendages, thin to thick walled, unbranched, glandular or non-glandular. Stomata are minute apertures bounded by two guard cells. They are found mainly in the epidermis of the leaves, they have actinocytic stomata. Large numbers of stomata present in most of the species. This study helps in identifying taxonomically the difference within the selective members with regards to the stomata and trichomes micromorphology.

Keywords: trichomes, stomata, cucurbitaceae, SEM

Introduction
Plant classification has been a subject of discussion among plant taxonomists over the years. Plants are classified and reclassified as soon as new evidences arise and this is going to be a continuous exercise over some year to come. Most plants are classified based on external morphological structure such as flowers and fruits. These structures are not always available on plants because they are seasonal in production. Due to this reason, other means of classification need to be involved; one of which is anatomical studies especially of leaves (Davis and Heywood. 1963) [6]. The epidermis is the outer layer of cells covering the leaf. It has several functions: protecting the plants against water loss by transpiration, regulation of gaseous exchange, secretion of metabolic compounds and absorption of water.

Most leaves show dorsiventral anatomy: the upper (adaxial) and lower (abaxial) surfaces have somewhat different construction and may serve different functions (Hardie, 2009) [11]. It can also be used as taxonomically include several differentiated cell type: epidermal cells, subsidiary cells, guard cells and epidermal hairs (i.e., trichomes). These features have been used previously to resolve some taxonomic problems or to contribute to ever increasing taxonomic data base in some genera and even families of plants (Watson, 1964; Van Wyk *et al*., 1982; Ren *et al.*, 2007; Saheed and Illoh, 2010) [34, 31, 26, 27]. Continuity and/or discontinuity of the characters can be used to show relationships and differences between plant taxa within a genus or between families.

The Cucurbitaceae family ranks among the highest of plant families for number and percentage of species used as human food. The Cucurbitaceae consist of 130 proposed genera with 800 species (Jeffrey, 2005) [14] mainly in regions tropical and subtropical. All species are sensitive to frost. Most of the plants in this family are annual vines, but some are woody lianas, thorny shrubs, or trees. Many species have large, yellow or white solitary flowers. The stems are hairy and pentangular. Tendrils are present at 90° to the leaf petioles at nodes. Leaves are exstipulate, alternate and simple palmately lobed or palmately compound. The flowers are unisexual, with male and female flowers on different plants (dioecious) or on the same plant (monoecious). The female flowers have inferior ovaries. The fruit is often a kind of modified berry called a pepo.

Scanning Electron Microscopy is an ideal technique for examining plant surfaces at high resolution (Pathan *et al.*, 2008) [24]. Micromorphology of vegetative and reproductive plant organs is the object of research to resolve the taxonomic problems of critical species and genera. Trichomes are distributed at the surface of aerial plant parts, having various functions and are extremely variable in their presence across plant, location on plant organs, density, form, etc., a perusal of literature reveals that trichome morphology has not been studied in the systematic of Cucurbitaceae. The present study aims to survey and evaluate the trichomes micromorphology in Cucurbitaceae using SEM to provide new insights into its potential taxonomic value.

Materials and Methods
Five species of Cucurbitaceae *viz., Benincasa hispida, Lagenaria vulgaris, Cucumis sativus, Luffa acutangula, Cucurbita maxima* were scanned using scanning electron microscope (SEM). Leaves were collected from each plant and the specimens were dried in shade. Squares of leaves were excised using a razor blade from the plant, avoiding the midrib areas so as to give a relatively consistent surface. Leaf segments of approximately 20 mm^2 were mounted on aluminum stubs using two-sided adhesive carbon tape. The samples were then coated with very thin layer of gold in a sputtering coater and observed with a scanning electron microscope. (Make: SEM-TESCON, Czechoslovakia).

Electron images were recorded using a digital image processor.

Results and Discussion

The importance of epidermal characters of leaves in angiosperms has been reviewed by several authors (Hagerup, 1953; Stebbins and Jain, 1960; Borril, 1961; Stace, 1984; Nyawuame and Gill, 1990; Edeoga, 1991; Parveen *et al.*, 2000; Edeoga and Ikem, 2001) [9, 30, 4, 29, 21, 7, 23, 8].The trichomes in Cucurbitaceae vary from unicellular to multicellular, short to long, smooth to ridges, with or without flattened disk at base and cystolithic appendages, thin to thick walled, unbranched, glandular or non-glandular. Stomata are minute apertures bounded by two guard cells. They are found mainly in the epidermis of the leaves, they have Actinocytic stoma, in which the guard cells are surrounded by four or more subsidiary cells that elongated radially to the guard cells cyclocytic in which the 4 or more subsidiary cells are arranged in the form of a ring around the stomata.

Benincasa hispida

The trichomes in *Benincasa hispida* are unicellular, simple, elongated, uniseriate and unbranched, swollen at base, without cystolithic appendages and ended with a pointed tip (fig.1.A) The cell walls are thin and the cell lumen is wide (fig 1.D). The basal cells are bigly dilated and form a whorl of subsidiary cells. Compare to ventral surface more trichomes seen in dorsal surface of leaf (fig.1.B). Actinocytic stomata seen only in ventral surface (fig.1.C).

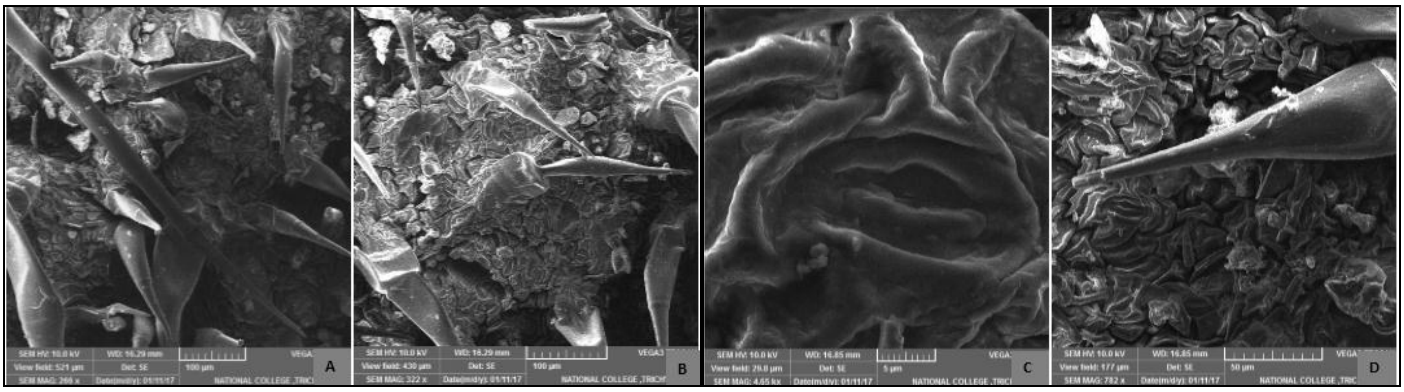

Fig 1: Scanning electron micrographs of leaf surface of *Benincasa hispida*

A-trichomes seen in the dorsal surface of the leaf epidermis (Mag-266x) | B- Basal cell of trichome (Mag-322x) | C- Stoma seen in ventral surface of leaf epidermis (Mag-4.65kx) | D- Trichome in adaxial epidermis (Mag-782x)

Cucumis sativus

In *Cucumis sativus*, the trichomes are distributed all over the leaf surface and comparatively dense at dorsal surface. Some trichomes are smaller in size. The trichomes are 2–3 celled, thick walled, conical, with few cystolithic appendages, swollen at base and ended with pointed tips (fig2.A). The trichomes originate from a circle of four or five epidermal cells (fig.2.B) which encircle the wide foot cell. Stomata are visible in both sides. The stomata are narrowly elliptical (fig.2.C).

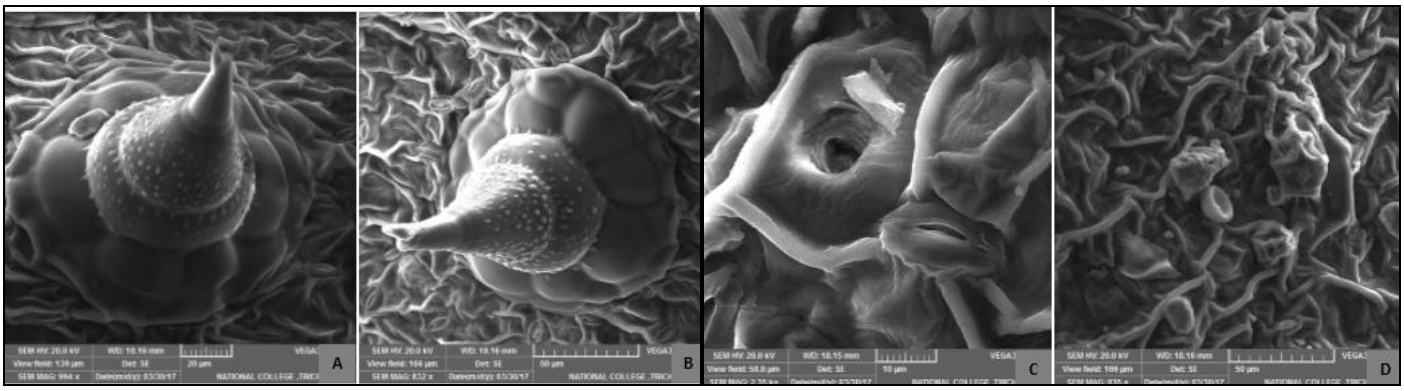

Fig 2: Scanning electron micrographs of leaf surface of *Cucumis sativus*

A – Trichomes in the dorsal surface of the leaf epidermal surface (Mag. –994x) | B – basal cell of trichome (Mag. – 832x) | C – Ventral view, stomata and trichome scar (Mag.– 2.35kx) | D – Ventral view of epidermis (Mag.–835x)

Lagenaria vulgaris

The trichomes in *Lagenaria vulgaris* are covering type without any secretory structure, elongated, thin walled, with ridges, usually ended with acute apex and without cystolithic appendages. They are multicellular, uniseriate and unbranched

structure. They are broad at the base and gradually tappering with pointed ends (fig 3.B). Numerous actinocytic stomata were seen in both sides. The cells surrounding the stomatal pores are uniformly arranged in the form of ring (fig.3.A).

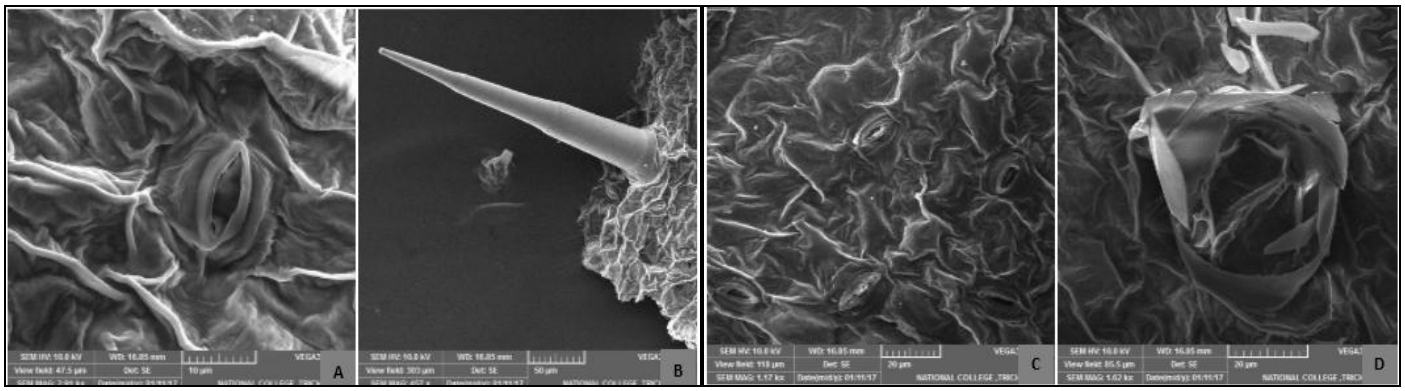

Fig 3: Scanning electron micrographs of leaf surface of *Lagenaria vulgaris*

A - Stoma seen in the dorsal surface of the leaf epidermis (Mag-2.91kx) | B - Trichome in dorsal surface of leaf epidermis (Mag-457x) | C - Stomata seen in ventral surface of leaf epidermis (Mag-1.17kx) | D - Scar of trichome (Mag 1.62 kx).

Luffa acutangula

The trichomes in *L. acutangula* are rudimentarily developed, with 3–4 rudimentary developed flattened basal cells. The apical cells are small and suddenly pointed. Basal cells are with cystolithic appendages. The trichomes are also seen isolated from the leaf. The isolated trichome has have bulged basal cell with which the trichomes are attached epidermal layer of the leaf (fig 4.A). The trichomes may be straight or curved (fig4.A, D). Stomata are commonly seen in adaxial surface of the leaf epidermis (fig.4.C).

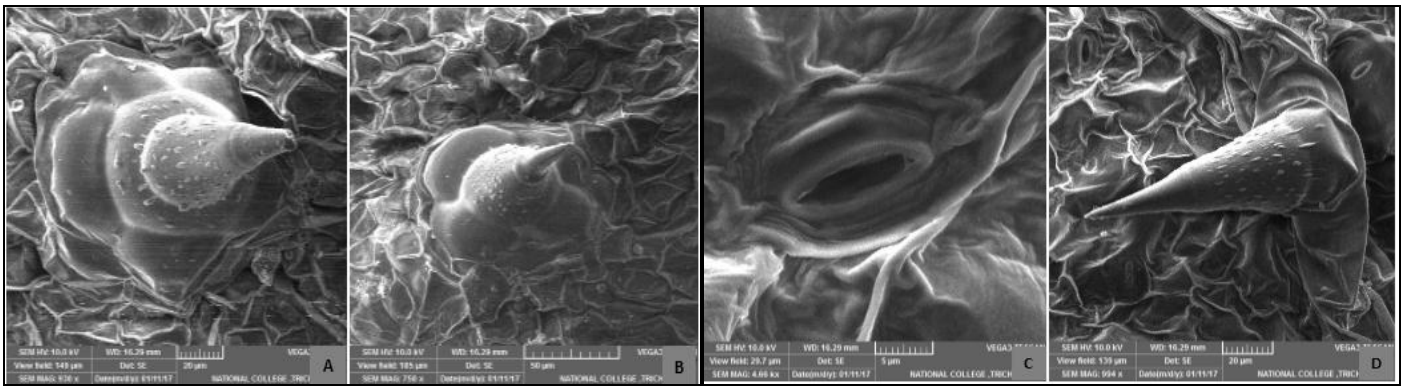

Fig 4: Scanning electron micrographs of leaf surface of *Luffa acutangula*

A- Trichome has have bulged basal cell seen in the dorsal surface of the leaf epidermal surface (Mag-630x) | B- Trichome with cystolithic appendages (Mag-750x) | C- Actinocytic stoma in ventral surface of leaf epidermis (Mag-4.66kx) | D- Non glandular trichome (Mag-994x)

Cucurbita maxima

In *Cucurbita maxima*, Thick non glandular covering types of epidermal trichomes are commonly seen on the both sides of leaf. The trichomes are elongated, uniseriate and unbranched. The trichomes are attached on the epidermal cells. Stomata are common; they are widely elliptic or circular in outline. The stomatal pore was narrow and straight.

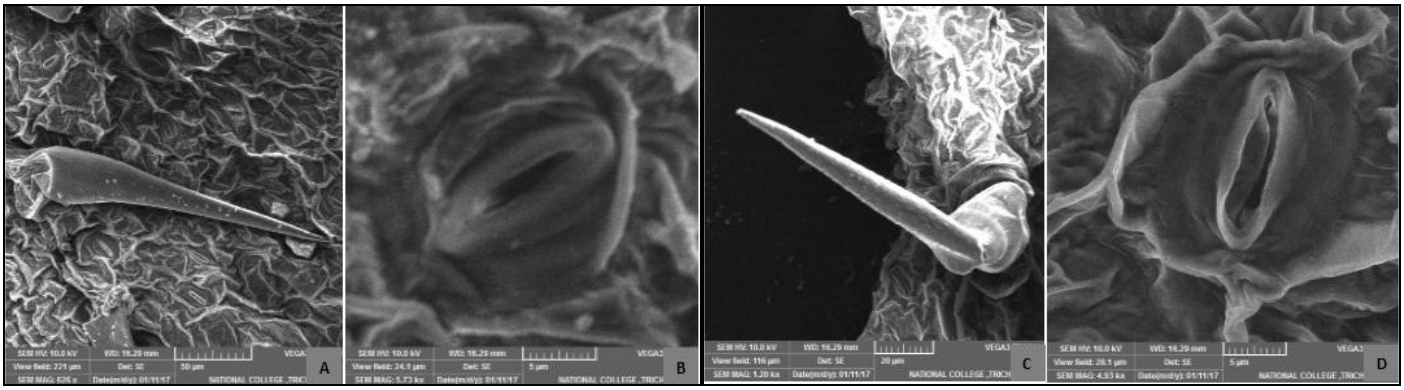

Fig 5: Scanning electron micrographs of leaf surface of *Cucurbita maxima*

A-Trichome seen in the dorsal surface of the leaf epidermal surface (Mag-626x) | **B**-Stoma in abaxial surface of leaf epidermis (Mag- 5.73kx) | C- Ventral surface view of trichome (Mag- 1.20kx) | **D**- Stoma in adaxial surface of leaf epidermis (Mag-4.93kx)

Conclusions

The present scanning electron microscopic study of the leaf epidermal features of all five species of Cucurbitaceae viz., *Benincasa hispida, Lagenaria vulgaris, Cucumis sativus, Luffa acutangula, Cucurbita maxima* provided enough scanned characters which could be implored to support external morphological characters used to classify within the family. The study outcome revealed that the epidermal features such as stomata and trichomes of the selected members can be used as a tool for diagnostic and taxonomic studies, in herbal medicine and forensic science for the identification and authentication of plant specimens.

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