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Micromorphological Characters of the Vegetative and Floral Organs of Some *Cleome* Species from Nigeria

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Abstract: Investigations into the micromorphological characters of the vegetative and floral organs were carried out in three species of *Cleome* common in eastern Nigeria. These *Cleome* species are *C. gynandra*, *C. rutidosperma* and *C. viscosa*. The results revealed the undulate nature of leaf epidermal cell walls of *C. gynandra* and *C. rutidosperma*, in contrast to that of *C. viscosa*, which were straight. The result of the stomata frequency was also significant where the highest was observed on the lower epidermis (23.81%) and upper epidermis (5.88%) of *C. viscosa*. Glandular, club shaped trichomes were observed in the leaf epidermis of *C. viscosa*. The palisade parenchyma of *C. rutidosperma* was single layered while that of *C. viscosa* and *C. gynandra* ranged btw 1-3 layers. Aggregate and rectangular shaped calcium oxalate crystals occurred in the stem and root of *C. viscosa*, while the aggregate type occurred in the other two species. The pollen grains of the three species were circular in outline tricolpate with a porus aperture type. The result of this investigation on the anatomy, histochemistry and palynology of these three *Cleome* species could be exploited for taxonomic and breeding purposes.

Key words: Anatomy · *Cleome* · Epidermis · Pollen · Taxonomy · Vegetative

INTRODUCTION

The genus *Cleome* belongs to the family Cleomaceae (syn-Capparaceae). *Cleome* comprises 150-200 species about 50 of these occurring in Africa [1]. *C. gynandra* occurs throughout the tropics and sub-tropics. In Africa it is mainly found near human settlements. *C. rutidosperma* is a pantropical weed of coastal regions occurring as a weed of rice fields in Nigeria. *C. viscosa* is also a pantropical weed common in cultivated fields, waste areas and settlements throughout West Africa [2]. *C. gynandra* is an erect annual herb up to 150cm tall and strongly branched. *C. rutidosperma* is an erect annual herb up to 50cm tall and branched from the base with pubescent stem. *C. viscosa* is up to 1m tall with yellowish, glandular hairs. The leaves of these *Cleome* species are alternate and palmately compound.

The essential oil extracted from the seeds of *C. gynandra* is occasionally used as an insecticide, especially against ticks in Livestock [3]. In many communities, women use *C. gynandra* before and after giving birth. It is believed to restore blood supply. It is also used for stomach ailment and to treat headaches [4]. There are also reports of anti-HIV and anti-bacteria activities of *C. gynandra* [5]. *C. rutidosperma* has similar medicinal uses as *C. gynandra*. In Nigeria, it is used to treat convulsions and in Ghana for irritated skin [1].

In Africa and Asia, the leaves and seeds of *C. viscosa* are used as a rubefacient and to treat infections, fever, rheumatism and headache. A decoction is used as an expectorant and digestive stimulant. The seeds are carminative [6]. The young shoots and leaves of these three species of *Cleome* are eaten as a cooked vegetable or added to soup in most part of Africa and Asia [5, 6]. These plants are highly appreciated vegetables in many African communities with good potential for further development. According to Jansen [1], these *Cleome* species commonly known as spider plants need more research in its medicinal, nutritional as well as the taxonomy of the complex. Hence this work on the anatomy of the three *Cleome* species will go far in further contributing to the taxonomy of these important crops. Also the investigations into the pollen structure are of much importance to plant breeders since information on the breeding of *Cleome* species is scarce.

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MATERIALS AND METHODS

This study was carried out between April and July 2007 at Michael Okpara University of Agriculture, Umudike Nigeria. Fresh parts of plant [inflorescence, leaves, stems and roots) were collected from plants growing in natural conditions in different locations of southeastern Nigeria. These specimens were fixed in FAA (1:1:18) glacial acetic acid: 40 % formaldehyde: 10% ethanol (v/v) for 48-72 hours. The fixed materials were later sectioned using a Reichert rotary microtome at 26nm following a slightly modified method of Cutler [7]. Anatomical staining was done by staining with few drops of alcian blue for 5 mins and counter staining with safranin solution for 2 mins. Sectioned specimens were mounted on slides using Canada balsam.

For leaf epidermal peels leaf samples were boiled in concentrated HNO3 for a minute, the samples were then carefully washed in water and the upper and lower epidermis teased from the mesophyll using fine forceps and dissecting needles. The peelings of the leaves were then mounted in glycerine after staining with safranin for 2mins

Calcium oxalate crystals in the different parts of the plants were histochemically localized following the method of Silver and Price [8]. The types of crystals of calcium oxalate localized in different tissues were microscopically examined and their morphology and location noted. For palynological studies, pollens were collected from fresh and matured flowers of the three Cleome species. Pollen samples for light microscopy were acetolyzed following the method of Erdtman [9] with slight modifications. Unstained acetolyzed pollen grains were embedded in aqueous glycerine. Observations were made on the slides using a light microscope.

RESULTS AND DISCUSSION

The three species of *Cleome* investigated had the amphistomatic type of stomata though the stomatal frequency were much higher on the lower epidermis compared to the upper epidemis in the three species. The stomatal frequencies of the lower epidermis of *C. viscosa, C. gynandra* and *C. rutidosperma* were 23.31%, 16.67% and 17.85% respectively. The upper epidermis of *C. vicosa* also had the highest stomatal frequency of 5.88% compared to 2.78% of that of *C. rutidosperma,* while the lowest 2.32% was observed in *C. gynandra*.. Ahmad [10]), Olowokudejo and Pereira-Sheteolu [11] found stomatal frequency value very reliable in distinguishing between the leaves of co-generic species.

In *C. gynandra* and *C. rutidosperma*, the anticlinal cell walls were undulate in nature and the epidermal cells were irregularly shaped. The anticlinal cell walls of *C. viscosa* were straight and the epidermal cells were isodiametric in shape, mostly triangular and pentagonal. The straight anticlinal cell wall of *C. viscosa* separates it from the other two species. Edeoga and Jimoh [12] have earlier used the nature of epidermal cell walls in the intra-specific separation in the family Lamiaceae.

In the leaves of *C. viscosa* multicellular trichomes were observed, these trichomes were large and long with a club shaped head and glandular basal end. These glandular hairs have earlier been described by Jansen [1]. This feature separates it from other *Cleome* species. Reports on trichomes of dicots have been documented by Metcalfe and Chalk [13]. The epidermis of the leaves of all the three *Cleome* species studied was one layer thick on both the upper and lower surfaces. The mesophyll was differentiated into palisade parenchyma and spongy parenchyma. The palisade parenchyma of *C. rutidosperma* was single layered while that of *C. viscosa* and *C. gynandra* ranged between 1-3 layers. Though the species have basically the same leaf anatomy variation of the palisade parenchyma is a means of separation.

The transverse section of the stem and the three C*leome* species studied were externally bounded by the epidermis, which was one layer thick. A cylindrical region of cortex lies below the epidermis. The parenchyma cells of the *Cleome* species investigated are thin walled and mostly hexagonal. The endodermis is present and one layered. It delimits the cortex from the vascular bundles in matured stems. The vascular bundles are oval in transverse section, concentric with phloem in the centre and surrounded by xylem. There was presence of multicellular hairs on the stem of all the species. These hairs were however more prominent on *C. rutidosperma*.

The roots of all the *Cleome* species investigated are composed of an outer piliferous layer that is one layer thick. The cortex is composed of parenchyma that cells are mostly oval shaped and contain intercellular air spaces. The vascular bundles lie at the center. The protoxylems are smaller and lie in the outer layer while the metaxylems are larger and lie towards the center. In matured roots of the three species, the pith is well developed, thick walled and lignified. The information from the vegetative anatomy of these medicinally important plants has provided clues for clearer understanding and identification of these *Cleome* species. This has been done in other medicinal plants [14, 15].

Table 1: Some micromorphological characters of the three *Cleome* species studied *mean ± SD

Characteristics		*Cleome viscosa*	*Cleome gynandra*	*Cleomerutidosperma*
Shape of Epidermal Cell wall	Upper epidermis	Isodiametric	irregular	Irregular
	Lower epidermis	Isodiametric	irregular	Irregular
Nature of anticlinal cell walls		Straight	undulate	undulate
Type of stomata		Diacytic	Diacytic	diacytic
*Stomatal frequency				
	Upper epidermis	5.88± 0.98	2.32± 1.25	2.78± 0.88
	Lower epidermis	23.31± 3.28	16.67± 2.54	17.85± 1.76
Trichome type (Leaf lamina)		Multicellular and glandular type	Absent	Absent
Type of calcium oxalate crystals		Aggregate and rectangular	Aggregate	Aggregate
Nature of pollen grains		Tricolpate and oval	Tricolpate and oval	Tricolpate and oval
No. of palisade parenchyma layer (leaf lamina)		1-3	1	1-3

An important observation in the stem and root anatomy of these taxa studied is the presence of two types of calcium oxalate crystals, namely aggregate and rectangular crystals. The aggregate and rectangular shaped crystals occurred only in the stem of *C. viscosa*, while the aggregate type occurred in the root and stem of *C viscosa, C. gynandra* and *C. rutidosperma*. The crystals mostly occurred in the parenchyma cells. The localization of these crystals within parenchyma cells suggests that these substances could be involved in the synthesis of carbohydrate [16, 17]. Edeoga and Osuagwu [18] had earlier used calcium oxalate crystals in taxonomic delimitation of some legumes.

The pollen of the *Cleome* species was circular or oval in outline. The pollen grains were all tricolpate with a porus aperture type. The pollen characters observed could be exploited for systematic and breeding purpose. Nyananyo [19], Edeoga *et. al.* [20], Mbagwu and Edeoga [21] has used pollen morphology to produce a more acceptable classification of the species in the plants they studied. Similarly, the pollen aperture indicated that the Cleome species are advanced groups of dicotyledons. They are also entomophilous and could be pollinated by insects and other animals. The advantage of this remains area of further investigation by plant breeders.

The general description of the micromorphological characters of the vegetative and floral organs of these *Cleome* species from from Nigeria will help in identifying them correctly to avoid substitution and adulteration of these vital leafy vegetables and medicinally important plants. From this study it is likely that the nature of the pollen grain could lead to the improvement of breeding of these plants, which before now breeding information has been scarce [1, 5].

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