

Full Length Research Paper

The value of morpho-anatomical features in the systematics of *Cucurbita* L. (Cucurbitaceae) species in Nigeria

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Accepted 13 September, 2004

Comparative studies on the morphology and anatomy of the three species of *Cucurbita* L. (*C. moschata*, *C. maxima* and *C. pepo*) in Nigeria were carried out. The morphological features of significance include variations in the number of tendrils, fruit size, shape, nature of fruit stalk, leaf shape and flower colour. Seed-coat anatomy revealed four distinct zones, which varied in thickness and tissue layers. Similarities were observed in the distribution, differentiation and number of layers of cells and tissues in the leaf, petiole and stem sections. There were, however, variations in number of bicollateral vascular bundles in their petiole. While the number is 10 in *C. moschata*, 14 in *C. pepo*, it is 16 in *C. maxima*. Parietal placentation and 15 anther-lobes are reported for these species for the first time. The usefulness of these parameters in the taxonomic delimitation of these species is discussed.

Key words: *Cucurbita*, morpho-anatomy, placentation, seed-coat, taxonomy, vascular bundles.

INTRODUCTION

Brief morphological description of the genus *Cucurbita* L. and its component species have been attempted by Hutchinson and Dalziel (1954) and Purseglove (1968). The genus is represented in Nigeria by three species, which are mostly cultivated. These are *Cucurbita moschata* (Duch.ex Lam) Duch.ex Poir., *Cucurbita maxima* Duch.ex Lam. and *Cucurbita pepo* L.

Cucurbita are rarely found growing in the wild in Nigeria. The 'musk', *C. moschata*, is cultivated in northern Nigeria for the fruits. In southern Nigeria, in a largely unimproved form 'ugboguru', it is cultivated for both the leaves and fruits (Okoli, 1984; Ndukwa and Okoli, 1992). The other two species *C. pepo* and *C. maxima* are cultivated in the northern parts of Nigeria. These species

constitute an important part of the diet of the area. They are cooked and the pulp eaten alone, eaten with yam/potato or used in making palatable stew. Their pulp may be eaten sometimes without cooking. The seeds are frequently fried and eaten by children. Though the economic potentials of these species are immense, information on their morphology and anatomy is either scanty or completely lacking. Except the reports of Agbagwa and Ndukwa (2001, 2004) on epidermal and vegetable characteristics of the three species, more have only been reported on *C. moschata* (Okoli; 1984; Ndukwa, 1988; Ndukwa and Okoli, 1992). The few anatomical studies carried out on the species concentrated on seed-coat anatomy (Vaughan, 1970; Ndukwa and Okoli, 1992). These reports were not comparative and did not examine aspects of stem, leaf and petiole anatomy of the species.

The present study is based on comparative morphological and anatomical investigation carried out on the living samples of these species. It is intended that the result will provide additional evidence for the delimitation of species in the genus in Nigeria. Results from the

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Figure 1(a-d). Characteristic features of habit and fruits *Cucurbita* species studied. a-habit of *Cucurbita* species showing severely coiled tendrils represented with letter 'a'; b- three fruit varieties of *C. moschata*. ; these varietal forms are occasionally produced by one plant; c-fruit of *C. maxima*; fruit stalk is deeply depressed into the fruit forming a globose invagination ; d- fruit of *C. pepo*; fruit stalk is round, neither depressed nor sunken into the fruit.

anatomy will also provide a guide and basis for studies leading to further exploitation of these important vegetable species.

MATERIAL AND METHODS

Observations on morphological (vegetative and floral) characteristics were made on mature living plants grown in the University of Port Harcourt Botanic Garden. Also materials collected during field trips to different parts of Nigeria between 1998 and 2000 were examined. Photographs were taken of relevant morphological features. The tendrils were classified following Jeffrey (1964) for the Cucurbitaceae.

For anatomical studies, fresh seed, stem, leaves and petiole were fixed in FAA for 48 h, washed in several changes of distilled water, dehydrated through alcohol series (30, 50, 70, 100%), 2 h in each solution and embedded in wax. Sections were cut on a Leitz 1512 rotary microtome at thickness between 20- 24 μ m. The sections were dewaxed with pure xylene and rehydrated in alcohol series following Cutler (1978) with modifications. Staining was achieved by dipping the slides in 1% alcian blue for about 5 min, washed with distilled water and counter stained with 1% safranin for 2 min. The stained sections were dehydrated again through alcohol series and mounted permanently in Dpx (Dee Pex). Photomicrographs of the anatomical sections were taken with a

Leitz Diaplan photomicroscope fitted with Leica WILD MPS 52 camera.

RESULTS

Germination and flowering

The 3 species, *C. moschata*, *C. maxima* and *C. pepo*, germinated 4 days after planting. The first two leaves that emerged from *C. moschata* and *C. pepo* were light green and folded, while that of *C. maxima* was deep green. Flowering in *C. moschata* occurred 8 weeks after planting, and in *C. maxima* and *C. pepo* flowering occurred after 11 and 13 weeks, respectively.

Morphological studies

Table 1 shows the vegetative and floral morphological features of the three species. All results were based on 25 plants for each species. Figure 1(a-d) show some morphological characteristics of the three species. They

Table 1. Vegetative and floral morphological features of the *Cucurbita* L. species studied.

| | | <i>C. moschata</i> | <i>C. maxima</i> | <i>C. pepo</i> |
|------------------------------|-----------------------------------|---|---|---|
| Seed-coat | Number of seed coat layers | 4 | 4 | 4 |
| | Epidermal cell thickness | 1 cell layer thick | 1 layer of thick piliferous layer | 1 layer of thick piliferous layer |
| Leaf | Hypodermal cell thickness | 3 – 4 cell layers thick | 3 – 4 cell layers thick | 3 – 4 cell layers thick |
| | Sclerenchyma cell thickness | Thick columnar layer | Thick columnar layer | Thick columnar layer |
| | Chlorenchyma and parenchyma | 3 – 7 layers thick | 3 – 7 layers thick | 3 – 7 layers thick |
| | Leaf anatomical symmetry | Dorsiventral | Dorsiventral | Dorsiventral |
| | Nature of Epidermis | A layer of upper and lower piliferous epidermis | A layer of upper and lower piliferous epidermis | A layer of upper and lower piliferous epidermis |
| | Mesophyll layer thickness | 1 layer of palisade and several isodiametric spongy cells | 1 layer of palisade and several isodiametric spongy cells | 1 layer of palisade and several isodiametric spongy cells |
| | Hypodermal layer thickness | 3 – 4 layers thick | 2 – 3 layers thick | 3 – 4 layers thick |
| | Chlorenchyma layer thickness | 5 – 6 layers thick | 6 – 7 layers thick | 6 – 7 layers thick |
| | Number of vascular bundles | 7 surrounded by sclerenchyma cells | 7 | 7 |
| | Petiole | Nature of epidermis | Single layer, piliferous | Single layer, piliferous |
| Hypodermal layer thickness | | 4 – 5 layers thick | 5 – 7 layers thick | 6 – 8 layers thick |
| Chlorenchyma layer thickness | | 5 – 9 layers thick | 10 – 13 layers thick | 10 – 13 layers thick |
| Number of vascular bundles | | 10 bicollateral vascular bundles | 16 bicollateral vascular bundles, each surrounded by sclerenchyma cells | 14 bicollateral vascular bundles, each surrounded by sclerenchyma cells |
| Stem | Nature of epidermis | Single layer, piliferous | Single layer, piliferous | Single layer, piliferous |
| | Hypodermal layer thickness | 3 – 5 layers thick | 6 – 7 layers thick | 5 – 6 layers thick |
| | Cortical parenchyma layer | 3 – 4 layers thick | 2 – 3 layers thick | 2 – 3 layers thick |
| | Sclerenchyma layer thickness | 3 – 4 layers of thick cells arranged closely | 3 – 4 layers forming a thick continuous zone | 2 – 3 layers forming a thick continuous zone |
| | Number of vascular bundles | 10 bicollateral vascular bundles | 10 bicollateral vascular bundles | 10 bicollateral vascular bundles |

are generally annual herbaceous climbers with long-running vine; alternate leaves with cordate shapes and variously branched tendrils. Flowers are monoecious, acuminate, and pentamerous with different shades of yellow colour. Male flowers are generally more numerous and earlier than the female flowers. Corolla is thickly veined, campanulate, imbricate and acuminate. Ovary is inferior, unilocular, and parietal with placentae intruding deeply into it thus making it falsely trilobular. Fruits are true pepo.

Anatomical Studies

A summary of the anatomical features of the seed coat, leaf, petiole and stem of the three species are presented in Table 2. Figures 2 to 5 show photomicrographs of the anatomical sections. The seed coats in the three species have 4-tissue layers: epidermis, hypodermis, sclerenchyma and chlorenchyma. In each species, the hypodermal and chlorenchymatous parenchyma layers of the seed coat increase from the center towards the margin. In *C. moschata*, the hypodermis and chlorenchyma are both 3 – 4 layers each but increases to

6 – 7 and 4 – 7 layers, respectively. In *C. maxima*, the hypodermis and chlorenchyma are both 3–4 layers thick, but increases to 7 layers at the margin. In *C. pepo*, both have 3–4 layers at the center. However, the hypodermis increases to 6 layers while the chlorenchyma increases to 7 layers at the margin.

Transverse section through the anther-lobes of the 3 species showed that the lobes are made up of 15-chambers. These chambers occur in 3 group: 2 groups of equal size with 6 chambers each, and a third smaller group with 3 chambers. This is the same in all the 3 species except in some rare cases of *C. moschata* with 4 instead of 3 fused stamens.

DISCUSSION

The time of flowering of the 3 species from the date of planting varied from 8 weeks in *C. moschata*, to 11 and 13 weeks in *C. maxima* and *C. pepo*, respectively. A number of morphological features of the three species provide means for their easy identification. For instance, though the 3 species possess weak herbaceous climbing

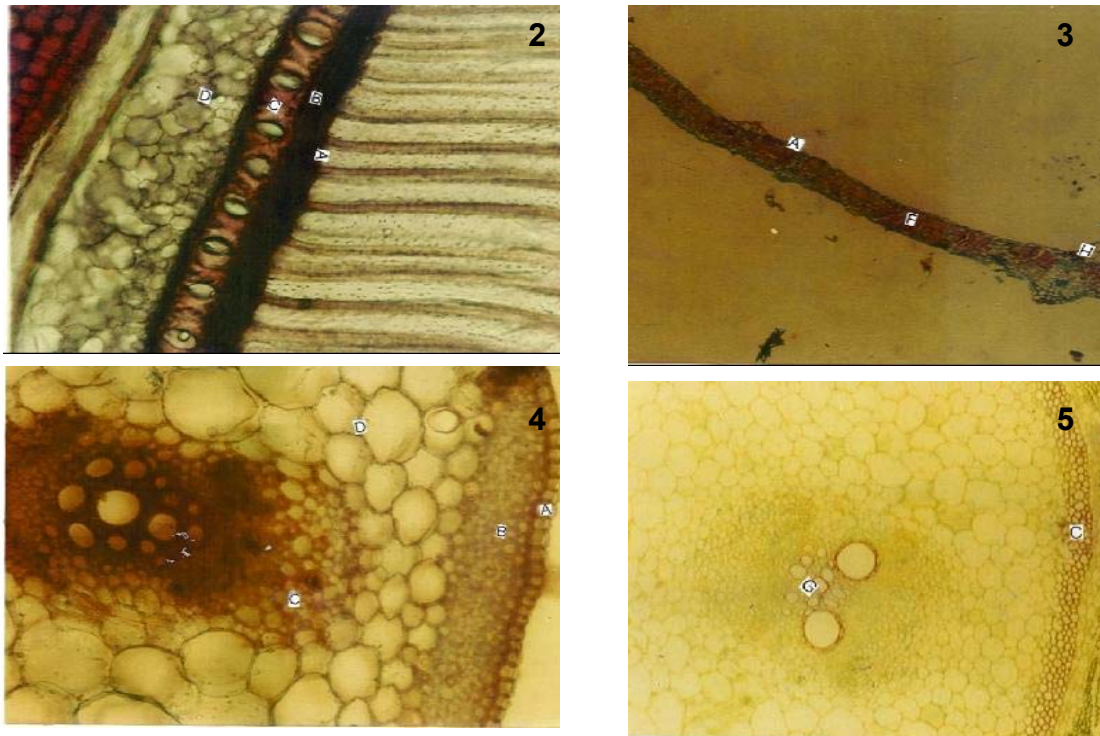
Table 2. Summary of anatomical features of the *Cucurbita* L. species studied.

| Characters | <i>Cucurbita moschata</i> | <i>Cucurbita maxima</i> | <i>Cucurbita pepo</i> |
|---------------|---|---|--|
| Habit | Annual: moderately strong and prostrate | Annual: prostrate and profuse | Annual: prostrate and profuse |
| Stem | Slightly 5-angled; pilose at ribs. | Soft, round, more or less; caducous aerial root at nodes | Hard, frequently 5-angled and hairy |
| Tendrils | Apically 3-fid | Apically 3-5- fid | Apically 3-4- fid |
| Leaves | 10 - 25cm by 13 - 35cm, cordate; shallowly 5-lobed, occasionally with white blotches; pilose. Petiole 10 - 30cm long | 9 - 13cm by 13 - 20cm, cordate; scarcely or shallowly lobed with white blotches; pilose. Petiole 7-9cm long | 12 - 15cm by 10 - 25cm, cordate; deeply 5-lobed with sinuse. No blotches. Petiole 10 - 13cm long |
| Inflorescence | Racemose; male flowers on pedunculate raceme with 10 – 15 flower heads or sometimes solitary with very long peduncule, more numerous and earlier than the female flower, female flowers solitary. | Same with <i>C. moschata</i> . Male flower bears 6 - 10 flower heads when racemose. | Same with <i>C. moschata</i> . Male flower bears 6 - 10 flower heads when racemose. |
| Flower | Yellow in colour; very tubular with petal 3-ribbed. Sepals acicular, deeply 5 - lobed, about 4.5-6.0cm long. | Light yellow in colour, very massive and tubular; petal 5 - ribbed. Sepal acicular 3 - 5cm long. | Light yellow in colour, very massive and tubular, petal 5-ribbed sepal very acicular, 1.2 - 1.5cm long. |
| Stamen | 3 or rarely 4 and fused; anthers yellow. | 3 fused stamens; anthers yellow. | 3 fused stamens; anthers yellow. |
| Fruit | 15 - 20cm by 10 - 12cm; round and of three colours: green, green with white, smooth patches and green with punctate blotches; 5-angled stalk, enlarged at fruit attachment and sunken | 25 - 32cm by 15 - 20cm, very large and massive with soft or hard leathery rind, mature stalk round, slightly soft, characteristically deeply depressed into the fruit forming a globose invagination. | 22 - 5cm by 12 - 20cm. Ellipsoid or oblong, hard - or soft-leathery rind; mature stalk is round, slightly enlarged at fruit attachment, neither depressed nor sunken into the fruit. |
| Seed | 1.1cm by 0.7cm, dull white or tan, sometimes brown; funicular attachment obtuse; seed margin scalloped. | 2.0cm by 1.2cm, fat, smooth, soft and leathery; brown in colour; funicular attachment acute; margin smooth. | 1.8cm by 0.9cm, smooth and white in colour; funicular attachment acute; margin smooth. |

stems, the characteristic angular shape of *C. moschata* stem distinguishes it from *C. pepo*, which is slightly 5-angled and *C. maxima*, which has a round or terete stem. This observation was similar to those of Purseglove (1968). Jeffrey (1980) had pointed out the value of tendrils in taxonomic delimitation of the Cucurbitaceae. Ndukwu and Okoli (1992) reported tendrils with distally 3-fid branching in *C. moschata*. While this is confirmed in this study, *C. pepo* characteristically possesses a mixture of 3-fid and 4-fid branching tendrils while *C. maxima* in addition has 5-fid tendrils.

Leaf architectural design as an essential tool in field and herbarium taxonomy has been highlighted by several workers (Davies and Heywood, 1973; Metcalfe and Chalk, 1979; Stace, 1980) and also specifically in the Cucurbitaceae by Jeffrey (1964, 1980). Some aspects of leaf morphological features such as shape, size, margin and colour were found diagnostic either at the genus or species level. The three species have cordate leaf shapes. *C. moschata* and *C. maxima* both have large shallowly lobed leaves occasionally with white blotches.

C. pepo has distinctly deeply-lobed leaves with acute sinuses. The shape and margin characteristics of the leaves in this genus distinguish it from other cultivated Cucurbitaceae genera like *Telfairia* and *Citrullus*, and in wild genera like *Momordica* and *Coccinia* (Okoli, 1984). The floral, fruit and seed variations are taxonomically valuable. Floral characters according to Stace (1980) have been and are still those mostly used in the classification of angiosperms. The type and nature of inflorescence in the three species are the same. All other floral characteristics explored show the close affinity of the species. There is however slight colour variation with *C. moschata* showing deep yellow colour, while *C. pepo* and *C. maxima* have bright yellow colours. Previous studies on the Cucurbitaceae have reported the presence of 3-syngenesious stamens for the species of *Cucurbita* (Hutchinson and Dalziel, 1954; Jeffrey 1964, 1980). It was observed in this study that *C. moschata* occasionally possesses 4 fixed stamens. The flowers of *C. maxima* and *C. pepo* are also massive and larger than those of *C. moschata*. The consistent occurrence of ribs originating



Figures 2-5. Anatomical features of *Cucurbita* species studied. 2 - seed-coat of *C. moschata* showing four distinct layers. Notice the thick columnar sclerotic layer 'C'. 3 - leaf anatomical features of *C. moschata*. This is similar to the other two species. 4- section through the petiole of *C. maxima*. 5 - stem section of *C. maxima*. The arrangement of tissues is similar to the other two species.

from the calyx-lobe (5 on each petal surface of *C. maxima* and *C. pepo*, and 3 ribs on those of *C. moschata*) is a character useful at species level delimitation.

Controversy exists with regards to the placentation of the Cucurbitaceae, whether axial or parietal (Dutta, 1979). For these species, the parietal placentation was established. Transverse section through several 5-day old developing fruits show that the confusion in placentation arises through the degeneration of the ovary at later stages of fruit maturity thus portraying an axial type. The establishment of the actual placentation type in the Cucurbitaceae therefore can only be made from a young ovary bearing in mind that the placenta detaches from the internal ovary wall at later stages, to give a false impression of axial placentation.

Though all the fruits are typically pepo, the shape, colour, surface texture and most importantly the nature of the fruit stalk are diagnostic and useful in field studies. In *C. moschata*, the fruit stalk is hard, 5-angled and enlarged at the point of attachment to the fruit. The stalk of *C. maxima* is round, slightly soft, not enlarged but deeply depressed into the fruit forming an invagination in the fruit. That of *C. pepo* is round slightly enlarged at fruit attachment, but characteristically neither depressed nor sunken into the fruit. Their seeds also show differences in size and colour but basically have the same shape. Thus

the variation in size and shape of the fruits together with the nature of the fruit stalk are very good species characteristics such that none of the three species have these characters in common. These differences provide easy identification guide to field officers and farmers.

Variation in the number of layers of the seed coat between taxa at different levels has been exploited in the Cucurbitaceae (Vaughan, 1970; Singh and Dathan, 1980; Jeffrey, 1980). The seed coat of the 3 species studied showed 4 distinct layers. These are piliferous epidermis, hypodermis, sclerenchyma, chlorenchymatous parenchyma. Jeffrey (1980) did not recognise the last layer in his review of the Cucurbitaceae, while Singh and Dathan (1980) included a fifth layer, the aerenchyma which undoubtedly exists in some cucurbits (Ndukwu, 1988) but not in the genus *Cucurbita*. The study proves that this genus belongs to the Cucurbitaceae of Singh and Dathan (1980). The lack of the aerenchyma layer, the variation in cell thickness, presence and nature of epidermal hairs are useful in taxa delimitation at the genus and species level.

Aspects of leaf, petiole and stem anatomy of these species confirm their affinity as they show marked similarities and overlaps in the number of layers of cell and tissues and also in their distribution and differentiation. However, the distinct numbers of petiolar vascular bundles varied in the three species with *C.*

moschata having 10, *C. pepo* 14, and *C. maxima* 16. This provides additional information for maintaining their distinction into three different taxa. This agrees with the taxonomic values of petiolar vascular bundle as earlier highlighted by Metcalfe and Chalk (1979).

The data from these studies has indeed provided further evidence for the taxonomic delimitation of species of *Cucurbita*, which are found in Nigeria. It is hoped that field officers, botanists and farmers will find these information helpful in distinguishing the species.

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