Anatomy and Systematics in the Costus afer - C. lucanusianus Complex (Costaceae)

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Abstract. The anatomy of lamina, rhizome and root of three taxa of Costus was investigated with a view of establishing interrelationships among the previously confused species of the genus. Differences in features of the vegetative anatomy suggest that a separate specific status for C. afer and C. lucanusianus is justified as opposed to the conspecific treatment given to them by previous researchers. A hybrid collection of these Costus species does not merit a separate specific status, although it possessed distinct anatomical features. The systematic importance of this investigation is clear since a 3–4 layered spongy mesophyll of C. afer is different from a 4–5 layered spongy mesophyll of C. lucanusianus and the 2–3 layers of the putative hybrid. Similarly the hexarch, nonarch and polyarch vascular bundle of the root of C. afer, C. lucanusianus and the Costus hybrid respectively, are all distinguishable attributes of these taxa that are important in systematics.

Key words: anatomy, Costus, Costaceae, lamina, rhizome, root, taxonomy

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The genus Costus L. is a member of the ginger lily family, Costaceae (K. Schum.) Nakai, a former subfamily of Zingiberaceae, treated by Hutchinson (1959) merely as a tribe but which merits separate family status (Tomlinson, 1969). The Costaceae were first raised to the rank of a family by Nakai (1941). The family is one of the most distinctive and isolated members of the order Zingiberales (Dahlgren et al., 1985). Before the elevation to family status, Engler and Prantl (1930) recognised Costoideae as a subfamily under Zingiberaceae. Several anatomical and morphological features support this isolated position including often well developed aerial shoots with distinct, rigid and commonly branched stems. The leaves are inserted in a low spiral with divergences unlike any other flowering plants. The leaf sheaths are always tubular, and the plants are aromatic, rhizomatous except in Costus engleriatus. The production of new plants by asexual reproduction (apomixis) has recently been reported in Costus lucanusianus (Tomlinson, 1969; Edeoga and Okoli, 1991, 1996). The family Costaceae consists of four genera and approximately 200 species (Airy Shaw, 1973). The genus Costus is the largest in the family with about 150 species that are mainly tropical in distribution (Humphries, 1985; Hickey and King, 1981). In addition to its small size and distinctive morphology, Costus has a pantropical distribution especially in the forest

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and savanna regions. In West Africa, there are about 11 species of *Costus* with nine species recorded in Nigeria. There are three in the northern part of Nigeria and six, including *C. afer* Ker Gawl and *C. lucanusianus* J. Braun & K. Schum, in the southern part of Nigeria (Hutchinson and Dalziel, 1968). Some species of *Costus* from West Africa, namely, *C. afer*, *C. deistelli* and *C. lucanusianus*, are considered to constitute a species complex, the *Costus afer* complex by some workers including Oteng-Yeboah (1981). This is as a result of the reported occurrence of continuous variations in the morphological and cytological features of these plants. This systematic problem demands a thorough investigation into the lamina, rhizome and root anatomy in relation to systematics in this complex.

Tomlinson (1956, 1962, 1969) presented some reasons for treating the Costaceae as a separate family based on a number of anatomical characters of the vegetative organs. Some of these diagnostic characters were summarized by Tomlinson (1969). They include the uniseriate filamentous hairs, isodiametric, irregular, polygonal or transversely extended thin-walled epidermal cells, tetracyclic stomata (stomata with subsidiary cells), slightly asymmetrical nature of guard cells, colourless and continuous hypodermis, clearly differentiated cortex, well-developed vascular-plexus at each node, restriction of silica to internal tissues close to vascular bundles, solitary bodies stellately-spherical or druse-like, vessels restricted to roots, etc. Edeoga (1991) compared the epidermal morphology of the *Costus afer* - *C. lucanusianus* complex and assessed its relevance in systematics, noting that multicellular and unicellular trichomes differentiate the two taxa. Other available information on the morphology of *Costus* species include those of Morton (1968) and Edeoga and Okoli (1991, 1996). Apart from the preliminary investigation of Tomlinson (1969) on the anatomy of some members of Costaceae no other information on the lamina, rhizome and root anatomy of these *Costus* species have been documented to the best of our knowledge.

The use of anatomical features in the systematic consideration of different taxa is no more a rare event by different authors. The work of Ayensu (1972) in Dioscoreaceae, Gibson (1981) in Anacardiaceae, Decadas and Beck (1972) in Rosaceae and Leguminosae, Heo (1996) in Monimiaceae, Metcalfe and Chalk (1950) in Dicotyledons as a whole, Tomlinson (1969) in Commelinales and Zingiberales are classical examples. Emphasis is on these *Costus* species due to the problems of identification by field collectors despite the medicinal and ornamental values of these plants elaborated by Oliver (1959), Williamson (1970) and Burkill (1985).

**Materials and Methods**

Mature and fresh lamina, rhizomes and roots of *Costus afer*, *C. lucanusianus* and a putative *Costus* hybrid (Edeoga and Okoli, 1996) obtained from living specimens of each plant were fixed in FAA (1:1:18 40% formaldehyde: glacial acetic acid: 70% ethanol (v/v)) for 48–72 hours. These were then rinsed in several changes of distilled water and
passed through alcohol series (30, 50, 95, 100%). The dehydrated materials were infiltrated with wax by passing through different proportions of alcohol and chloroform (v/v 3:1, 1:1, 1:3). As the chloroform gradually replaced the alcohol, pure chloroform and wax were added in the bottles. The idea was to gradually infiltrate the tissues with wax which would be hard enough for microtoming. The bottles were then left on a hot plate (37–40°C) for 24 hours before being transferred to the oven (58–60°C). This step was designed to evaporate the chloroform. The wax having reached its melting point completely infiltrated the tissue in it. After a period of 2–3 days with constant addition of wax by the use of metal moulds and melted wax. The moulds were later removed and the specimens with the wax-cube were trimmed and sectioned using a Reichert rotary microtome at 20–24 μm.

The ribbons were placed on clean slides, smeared with a thin film of Haupt's albumen, and allowed to dry and drops of water added prior to mounting. The slides were placed on a hot plate at 40°C for a few minutes to allow the ribbons to expand and were stored overnight. The slides were immersed in pure xylene for 2–5 minutes in a solution of xylene and absolute alcohol with 1:1 ratio (v/v) for a few minutes. The slides were then transferred to another solution of xylene and alcohol in the ratio 1:3 (v/v) for a few minutes, to 95%, 90%, 70% and 50% alcohol. Drops of alcian blue were put on the specimens for five minutes, washed off with water and counter-stained with safranin for two minutes, then dehydrated in a series of alcohol 50%, 70%, 80%, 90%, xylene/absolute alcohol solution (i.e. 1:3 and 1:1 v/v), and pure xylene at intervals of a few seconds and mounted in Canada balsam. Coloured photomicrographs were taken using a WILD-MPS camera fitted with Leitz Laborlux-12 microscope.

Results

The anatomical features of the lamina, rhizome and root of the Costus species and the putative hybrid are summarized in Table 1 and illustrated in Figs. 1 and 2. In outline, the leaf is isolaterally flattened with the midrib and major veins being prominent abaxially. The spongy mesophyll which is confined to the centre of the lamina is composed of 4–5 layers of cells which are irregular in shape in C. lucanusianus (Fig. 1b). The lamina in C. afer is characterized by well-developed sclerenchymatous cells (Fig. 1a) and the presence of mesophyll that is 3–4 cells thick outside the midrib and up to 8 cells thick within the midrib. The mesophyll cells in the hybrid are larger than in the putative parents and are 2–3 cells thick outside the midrib but up to 6 cells thick near the midrib. Both the upper and lower epidermis are made up of a single tier of cells in the three collections studied. The vascular bundles are bicolateral in the three taxa. The smallest bundles are composed of 1–3 tracheids and few phloem elements. In each bundle, the xylem pole consists of tracheary elements. The midrib is very conspicuous. The abaxial surface in C. lucanusianus is concave. In C. afer, the abaxial surface is also convex
TABLE 1. Anatomical features of the lamina, rhizome and root in the Costus species investigated.

<table>
<thead>
<tr>
<th>Characters</th>
<th>C. afer</th>
<th>C. lucanusianus</th>
<th>Costus hybrid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lamina:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spongy mesophyll</td>
<td>3–4 layers</td>
<td>4–5 layers</td>
<td>2–3 layers</td>
</tr>
<tr>
<td>Vascular bundle</td>
<td>Bicolateral</td>
<td>Bicolateral</td>
<td>Bicolateral</td>
</tr>
<tr>
<td>Rhizome:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sclerenchyma</td>
<td>Almost single layer</td>
<td>2–3 layers</td>
<td>2–3 layers</td>
</tr>
<tr>
<td>Xylem vessel</td>
<td>Small</td>
<td>Large</td>
<td>Large</td>
</tr>
<tr>
<td>Starch grains</td>
<td>Varied in shape</td>
<td>Mostly slipper and oval</td>
<td>Mostly slipper and oval</td>
</tr>
<tr>
<td>Root:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vascular bundle</td>
<td>Hexarch and small</td>
<td>Nonarch and large</td>
<td>Polyarch and large</td>
</tr>
<tr>
<td>Casprian strip</td>
<td>Poorly defined</td>
<td>Clearly defined</td>
<td>Clearly defined</td>
</tr>
<tr>
<td>Endodermis</td>
<td>Poorly differentiated</td>
<td>Well-developed</td>
<td>Well-developed</td>
</tr>
<tr>
<td>Passage cells</td>
<td>Absent</td>
<td>Present</td>
<td>Present</td>
</tr>
</tbody>
</table>

Figs. 1. Anatomical sections of lamina, rhizome and root in Costus. a. Transverse section (TS) of lamina of C. afer, showing well-developed sclerenchyma and bicolateral vascular bundle. ×130. b. TS of lamina of C. lucanusianus. Note the absence of palisade mesophyll. ×130. c. TS of root of C. lucanusianus showing nonarch vascular bundle. ×50. d. Section of root of Costus hybrid showing well-developed and delimited endodermis. Note presence of passage cells. ×130.

while the adaxial is concave. The same shape was also observed in the Costus hybrid.

The vascular bundles in the rhizomes of the three taxa are different from one another (Figs. 2a and 2b). The xylem vessels are smaller in
**Costus afer** and a sclerenchymatous layer surrounds the phloem and xylem. The vessels in the rhizomes of *C. lucanusianus* and the Costus hybrid are surrounded by a two-three layered sclerenchyma (Fig. 2b). The phloem in each taxon occupies a relatively small region of the vascular bundle. Interesting differences are also found in the shape of starch grains present in the rhizomes of these *Costus* species. In *C. afer*, simple, oval, slipper, circular and irregular shaped starch grains are present (Fig. 2a). In *C. lucanusianus*, slipper and oval shaped starch grains were predominant while the *Costus* hybrid is characterized by the presence of all the types of starch grains in the rhizome of *C. afer* and *C. lucanusianus*.

The root in *Costus afer* has six xylem strands alternating with six phloem strands (hexarch). The endodermis is not clearly differentiated. The vascular bundles in the root of *C. lucanusianus* are larger than those of *C. afer*. In *C. lucanusianus* the vascular bundles are nonarch, and the endodermis is well-developed with the Casparian strip clearly defined (Fig. 2c). In the Costus hybrid, there are 22 xylem strands alternating with 22 phloem (polyarch). The endodermis is well defined and clearly delimited from the rest of the root cells. The Casparian strip with passage cells are

![Figs. 2. Anatomical sections of rhizome and root in Costus. a. C. afer with a single vascular bundle in rhizome. Note that sclerenchyma is single layered and completely surrounded by the vascular bundle. ×130. b. C. lucanusianus with a single vascular bundle in rhizome. Sclerenchyma is 2–3 layered and completely surrounded by the vascular bundle. ×200. c. Section of root of Costus hybrid., showing that the Casparian strip and endodermis are well-defined, and passage cell conspicuous. ×200. d. Section of root of C. lucanusianus, showing the Casparian strip and well defined endodermis. ×200.](image-url)
conspicuously defined (Fig. 2d).

Discussion

The Costus species investigated exhibit a wide variety of lamina, rhizome and root anatomical features. C. afer, C. lucanusianus and the hybrid collection each have a characteristic vegetative anatomy that could be used to distinguish it from other members of the Costaceae. The Costus species studied, however, appear to be a homogenous entity united by a series of lamina, rhizome and root anatomical characters, including isolaterally flattened lamina, bicollateral vascular bundle, presence of starch grains, and presence of tracheary elements. These features of the lamina, rhizome and root anatomy endorse the opinion of Oteng-Yeboah (1981) that some species of Costus from West Africa constitute a species complex, the Costus afer complex. Differences in vegetative anatomy among such members of Costaceae as Costus and Tupeinochilus (Tomlinson, 1956) have received some emphasis in phylogenetic discussion. As regards epidermal and vegetative morphology, the Costus species investigated are heterogenous. Variation in the epidermal and floral morphology of C. afer, C. lucanusianus and the putative hybrid (Edeoga, 1991; Edeoga and Okoli, 1991, 1996) has also received too much attention from systematic point of view. Most probably, these differences are related to differences in habitat, C. afer preferring extremely to shady environment than C. lucanusianus and the putative hybrid.

The anatomical structures of the lamina, rhizome and root of the Costus hybrid strongly resemble that of C. lucanusianus. Moreover, in both taxa the shapes of the starch grains are almost similar. Thus vegetative anatomy of the Costus hybrid suggests that C. lucanusianus and the hybrid should be accommodated in the same supraspecific taxon. Further studies are needed to show whether this will be at a subspecies level within C. lucanusianus or within a separate species of Costus. Edeoga and Okoli (1996) have suggested that apomictic event observed in the C. afer - C. lucanusianus complex has led to intermediacy of floral features among the Costus collections they investigated. The opinions of different authors based on differences in morphological and anatomical structures also point at the fact that such taxa in which this type of intermediary exists should be treated as either sister taxa or even contaxa, i.e. conspecific, congeneric, etc. (Oteng-Yeboah, 1981; van Heel, 1992). However, in as much as a separate specific status of C. lucanusianus and C. afer is justified, it is not justified for the Costus hybrid. Apart from macromorphological similarities, the three taxa of the Costus vary in their basic lamina, rhizome and root anatomy especially in the nature of spongy mesophyll, number of vascular bundles in root and the morphology of starch grains (Table 1).

Tomlinson (1957, 1962, 1969) presented some reasons for treating Costaceae as a separate family from Zingiberaceae, based on vegetative anatomy of some members of the Scitamineae. The lamina, rhizome and root anatomy of these Costus species investigated seems to agree with the
Tomlinson's (1956, 1962, 1969) reasons for supporting the raising of the genus to the rank of family by Nakai (1941). However, more studies are still required in such areas as cytology, palynology and phytochemistry before generalizing and upholding all the observations and suggestions arising from our present investigation. The systematic importance of this study could be seen since the 3–4 layered spongy mesophyll of C. afer is different from the 4–5 layered spongy mesophyll of C. lucanusianus and the 2–3 layered spongy mesophyll of the putative hybrid. Similarly, the hexarch vascular bundle of the root in C. afer is different from the nonarch and polyarch of C. lucanusianus and the Costus hybrid respectively. Hence each taxon could be distinguished from one another on the basis of these anatomical characters. Tomlinson (1969) outlined some diagnostic features of the leaf, stem, rhizome and root anatomy in the Zingiberales as a whole but did not specify how these could be used in resolving critical systematic problems as in the presently investigated C. afer -C. lucanusianus complex. The need to incorporate information on the lamina, rhizome and root anatomy in systematic consideration of Costus species is therefore incontestable.

References


摘 要

H. E. Edeoga¹-B. E. Okoli²: Costus afer - C. lucanusianus complex (オオホザキアヤメ科) の解剖と分類

これまで分類が混乱していたオオホザキアヤメ属の3分類群の関係を明らかにするために、葉身、根茎、根の比較解剖をおこなった。解剖学的な違いによって、これまで同種としてきた考えに反して、Costus afer と C. lucanusianus は別種とすることの妥当性が示された。しかし、これらの種の交雑個体は、解剖学的な差異はあるものの、別種とする必要はない。この研究によれば、葉肉の厚さは Costus afer では3~4細胞層で、C. lucanusianus では4~5細胞層で、推定交雑個体では2~3細胞層であった。根の維管束系は C. afer では6原型、C. lucasianus では9原型で、交雑個体では変原型で、この形態は種を区別する重要な特徴を示す。

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