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Epidermal and Cytological Studies on Cultivars of *Xanthosoma* (L.) Schott. and *Colocasia* (L.) Schott. (*Araceae*)

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Authors' contributions

This work was carried out in collaboration between both authors. Author JOO designed the study, wrote the protocol, managed the experimental process and analyses of the study and identified the species and cultivars of plants. Author PCN wrote the first draft of the manuscript managed the literature searches and performed the laboratory analysis. Both authors read and approved the final manuscript.

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ABSTRACT

Epidermal and cytological studies were carried out on the cultivars of *Colocasia* and *Xanthosoma* species to determine their taxonomic value within and between the accessions. Upper and lower epidermal membranes of the leaves were peeled and stained with 0.1% safranin solution. Young healthy roots (about 15mm long) were obtained and fixed in 3:1 ethanol: acetic acid for about 18-24 hours and stored in 70% ethanol. The root tips were squashed in FLP Orcein and observed under the microscope. Stomata were found on both upper and lower epidermis of both *Colocasia* and *Xanthosoma* spp. but were more abundant on the lower epidermis of both species. Papillae were present on the lower epidermal cells of *Xanthosoma* but absent on the lower epidermal cells of *Colocasia*. Details of the ultra structure of the papillae showed that intraspecific variations occurred in the epidermis of these species. The epidermal variations in stomatal index within the *Xanthosoma* and *Colocasia* cultivars reflect their ecological adaptation to variation in the degree of

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wetness of the environment. All accessions of *Xanthosoma* gave the somatic chromosome count 2n = 2x = 24 while all *Colocasia* cultivars gave the somatic chromosome count 2n=2x=42. The chromosomes varied mostly from metacentric to subacrocentric in both species. These observations form part of the baseline data needed in planning their improvement and germplasm conservation.

Keywords: Colocasia; chromosomes; epidermis; papillae; stomata; Xanthosoma.

1. INTRODUCTION

Cocoyam has remained relevant as a source of food. It belongs to the family *Araceae*, a family consisting of herbaceous, perennial wetland or terrestrial plant species. They are called aroids and have more than 3,300 species. They are mostly tropical and subtropical, useful as ornamentals and are sources of medicine and food for both man and animals. The major edible aroids are classified into two tribes and five genera: Lasioideae (*Cyrtosperma* and *Amorphophallus)* and Colocasioideae (*Alocasia, Colocasia* and *Xanthosoma*) [1]. *Colocasia* and *Xanthosoma* are however, the most commonly cultivated in the tropics, including Nigeria and are the most important.

Members of this family include food crops, ethnomedicinally invaluable genera and species, ornamental and other unexploited plants. Among the edible (i.e. crop) species of this family are the genera *Colocasia* and *Xanthosoma*. In Nigeria, *Colocasia* and *Xanthosoma* form important root crops whose leaves could be eaten as vegetable and corms and cormels eaten as sources of staple carbohydrate. Cocoyam research and development in Nigeria is still backward though in some other countries such as Cameroon, Ghana and America, research on the aroids is a little more advanced [2]. The cocoyam grown in Nigeria was for many years presumed to be *X. sagittifolium* but is now correctly classified as *X. mafaffa* [3]. According to [3], cocoyam reached West Africa between the 16th and 17th centuries and was spread further by traders, missionaries and other travellers. The taxonomic position of the Nigerian cultivated *Xanthosoma* species is unclear and in recent years the tendency has been to give the name *Xanthosoma sagittifolium* to all cultivated *Xanthosoma* varieties [4].

According to the opinion of Okeke [5] all Xanthosomas in Nigeria belong to the species *Xanthosoma mafaffa* and not *Xanthosoma sagittifolium*, based on inflorescence characters and vegetative morphological features. He also reported that the name *X. mafaffa* was published earlier for the West African and Nigerian species of *Xanthosoma* before the name *X. sagittifolium*. Unfortunately, most researchers in Nigeria and elsewhere have continuously used the nomenclature, *X. sagittifolium* to represent the Nigerian *Xanthosoma* germplasm.

Not much information is available on the micromorphology, anatomy, histochemistry, cytology and cytogenetics of these very important sources of staple food. Micromorphological and anatomical features have not been investigated in these genera to ascertain their value in the characterisation of the *Xanthosoma* and *Colocasia* germplasm in Nigeria. Occurrence of raphide bundles was reported in Nigerian aroids and their probable functions in various tissues were discussed [6]. The only cytological data on edible aroid genomes are counts of 2n=24 for *Xanthosoma* and 2n=42 for *Colocasia*. To date, information is lacking on the chromosome features, breeding behaviour, phylogenetics and molecular genetics of the cultivars. Good knowledge of the cocoyam genomes is very necessary in order to establish a proper approach to its improvement.

Chromosomal data have proved invaluable in characterizing crop plants that are useful as breeding stocks as well as hybrids [7,8]. More recently, cytogenetic methods, especially using banding and fluorescence techniques have made possible a direct determination of the relative position and number of genic sequences on chromosomes [9]. Using both novel cytogenetic and molecular cytogenetic methods, several Nigerian food crops have been investigated and much data have been generated for their characterisation and genetic improvement [10]. This paper was aimed at reporting epidermal and cytological data useful in the proper taxonomic characterisation of the Nigerian edible aroids.

2. MATERIALS AND METHODS

The cocoyam cultivars used for this study were obtained from the field germplasm of the National Root Crops Research Institute, Umudike, Umuahia in February, 2011. The plant materials include five cultivars of *Colocasia* namely: 'Coco India' (*NCE 001*), 'Ede Ofe Green' (*NCE 002*),

'Ede Ofe Purple' (*NCE 003*), 'Ukpong' (*NCE 005*) and 'Akiri' (*NCE 0010*) and three cultivars of *Xanthosoma* namely: 'Ede Ocha' (*NXS 001*), 'Ede Uhie' (*NXS 002*) and 'Okorokoro' (*NXS 003*). *NCE* means Nigeria *Colocasia esculenta* while *NXS* means Nigeria *Xanthosoma* species. Corms of *Xanthosoma* and cormels of *Colocasia* were planted in plastic containers in the University of Port Harcourt Botanic Garden and regularly watered.

2.1 Epidermal Studies

Upper and lower epidermal membranes of the leaf were peeled out using forceps and scalpels. The thin layers of the peeled epidermis from the upper and lower sides of the leaves of the different cultivars were stained with 0.1 % safranin solution. The peels were then mounted with glycerine under No. 1 cover slips on clean glass slides for observation under the microscope.

2.2 Cytological Studies

Root tips (about 5-15 mm in length) from healthy sprouts were excised and collected in small specimen bottles for pre-treatment with 0.02M aqueous solution of 8-hydroxyquinoline for three hours during which they were refrigerated for the initial one hour to induce cold shock. The pre-treated roots were then fixed for 18-24 hours in freshly prepared Carnoy's solution. After fixation, the root tips were transferred to specimen bottles containing 70% ethanol and refrigerated until it was needed for squashing.

The root tips were placed in 9% aqueous hydrochloric acid for about 3-5 minutes to macerate their cell walls. About 1 mm tip of the root was excised on a clean glass slide and stained with a drop of FLP Orcein according to the method of [11], then covered with a 32 x 18 mm No. 1 cover slip and squashed using the head of a ball point biro pen. The slides were then observed under a Leica photographic microscope interfaced with a DELL computer at x 100. The micrographic images were read off the computer flat screen monitor and stored using wave Vision Pro LW – UTVFM.

3. RESULTS AND DISCUSSION

3.1 Epidermal Features

Intra-specific variations were observed in the epidermis of accessions of the two genera studied. The ordinary epidermal cells varied slightly in their shapes and sizes among the cultivar accessions and between the two species. The epidermal observations (Plates 1 and 2) revealed that there were stomata on both upper (adaxial) and lower (abaxial) leaf surfaces, though the number of stomata was higher on the abaxial surface than on the adaxial surface. The stomata of all the accessions of the two genera were mostly paracytic. In most cases the stomata appeared to be tetracytic but the cells at the tips of the guard cells were different from those beside the guard cell because they were more similar to the ordinary epidermal cells than the true subsidiary cells thus indicating that the stomata were paracytic. The stomata in all accessions of the two species were sunken though those on *Colocasia* accessions were more sunken than those of *Xanthosoma*. The stomata were all paracytic and slim with two guard cells but there was a brachyparacytic type in 'Ede Uhie' (Plate 1D). The stomata were more evenly distributed on the abaxial leaf surface than on the adaxial leaf surface where they occurred sparsely. The size and shape of the subsidiary cells varied between all the accessions studied. The sharing of similar stomata by related taxa indicates very close genetic background as shown in *Sphenostylis stenocarpa* (Hochst *ex* A. Rich) Harms by [12]. Brachyparacytic stomata were reported in the genus *Musa* L. [13]. The presence of brachyparacytic stomata in the abaxial leaf epidermis of 'Ede Uhie' and its absence in other cultivars indicate divergent advancement in its evolution.

The ordinary epidermal cells had convex surfaces with papillae. The papillae occurred mainly on the lower epidermis of the leaves but varied in size and shape. The papillae in *Colocasia* were rudimentary (Plate 2) though more developed ones were slightly cylindrical and rounded at the tip. Those on the foliar epidermis of *Xanthosoma* accessions were larger and more rounded-to-oval in shape. The subsidiary cells had no papillae. The surface of the subsidiary cells varied from nearly smooth in *Xanthosoma* leaves to wrinkle in *Colocasia* leaves. The presence or absence of papillae has been reported to have discriminative significance in *Musa* genus where its presence in the bracts of *M. sapientum* and absence from the bracts of *M. paradisiaca* was considered to be of diagnostic value [13]. The outline of the stomata was almost oval while the stomatal pore was narrowly elliptic with the anticlinal walls being straight in both species.

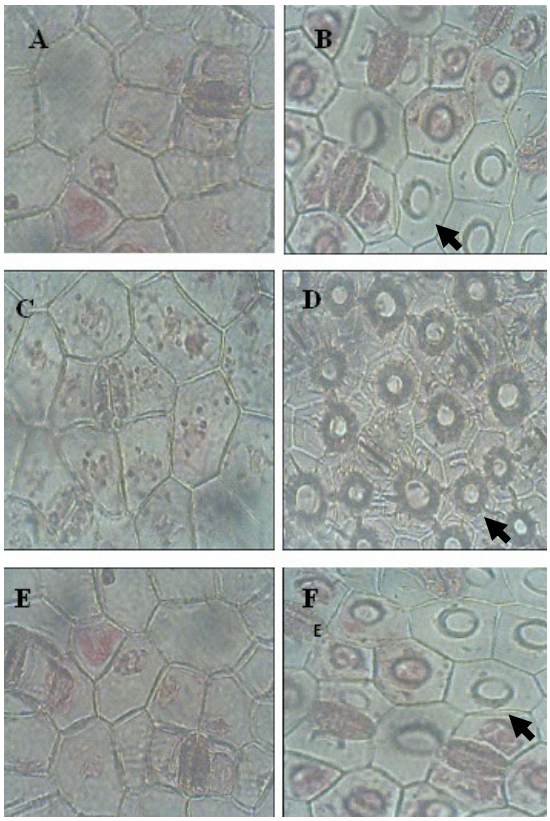

Plate 1. Epidermis of *Xanthosoma mafaffa* showing the presence of papillae (arrow) in the abaxial epidermis but none in the adaxial epidermis: A) Adaxial epidermis of *NXS 001*, 'Ede Ocha'; B) Abaxial epidermis of *NXS 001*, 'Ede Ocha'; C) Adaxial epidermis of *NXS 002*, 'Ede Uhie'; D) Abaxial epidermis of *NXS 002*, 'Ede Uhie'; E) Adaxial epidermis of *NXS 003*, 'Okorokoro'; F) Abaxial epidermis of *NXS 003*, 'Okorokoro'

The number and gross structure of subsidiary cells are of taxonomic value, especially when combined with other stomatal features. The number of epidermal cells per square millimeter varied from 11 to 24 while the wall pattern was straight. No appreciable variation existed in the shape of the guard cells and stomatal pore between the two species studied. The pair of guard cells per stoma was almost oval in outline while the pore was elliptic. Both *Colocasia* and *Xanthosoma* were amphistomatous. Similar observations were recorded for majority of the non-woody plants of the Nigerian legumes [14]. The maximum number of possible stomata on a square millimeter of the adaxial leaf surface was 2 whereas the maximum number of stomata on same area of the abaxial leaf surface was 4 (Table 1).

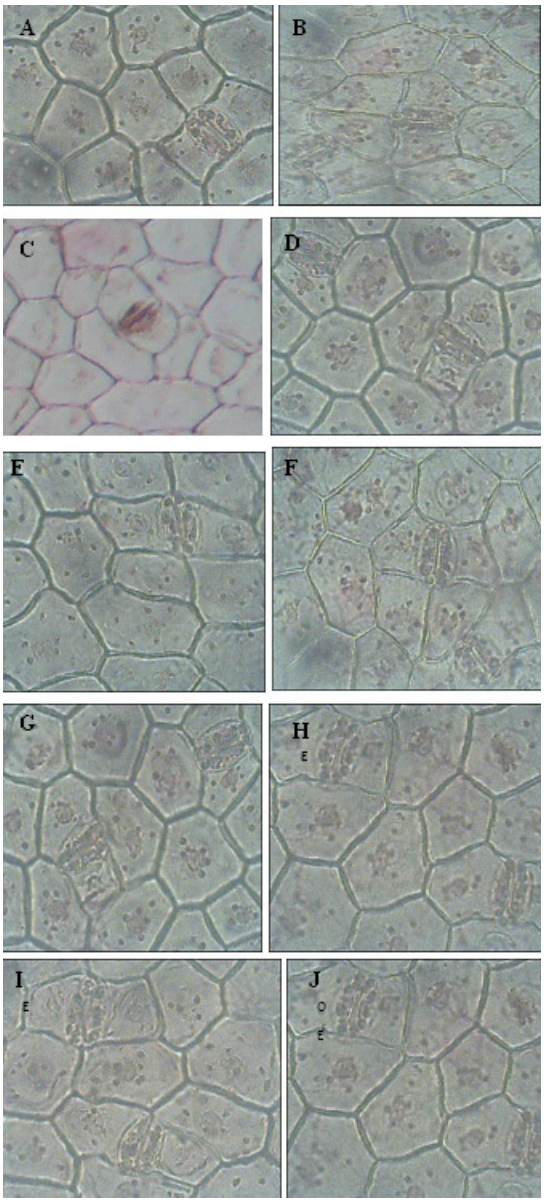

Plate 2. Epidermal features of *Colocasia esculenta*: A) Adaxial epidermis of *NCE 001*, 'Coco India'; B) Abaxial epidermis of *NCE 001*, Coco 'India'; C) Adaxial epidermis of *NCE 002*, 'Ede ofe green'; D) Abaxial epidermis of *NCE 002*, 'Ede ofe green'; E) Adaxial epidermis of *NCE 003*, 'Ede ofe purple' F) Abaxial epidermis of *NCE 003*, 'Ede ofe purple', G) Adaxial epidermis of *NCE 005*, Ede 'Ukpong'; H) Abaxial epidermis of NCE 005, Ede 'Ukpong'; I) Adaxial epidermis of *NCE 0010*, 'Akiri'; J) Abaxial epidermis of *NCE 0010*, 'Akiri'

Table 1. Summary of epidermal counts

Cultivar	Leaf surface	Mean number of stomata /sq mm	Mean number of epidermal cells	Stomatal index (%)
NXS	Adaxial	1	18	5.26
001	Abaxial	4	23	14.81
NXS	Adaxial	2	16	11.11
002	Abaxial	4	24	14.29
NXS	Adaxial	2	23	8
003	Abaxial	4	21	16
NCE	Adaxial	1	15	6.25
001	Abaxial	2	15	11.76
NCE	Adaxial	1	17	5.56
002	Abaxial	2	11	15.38
NCE	Adaxial	1	13	7.14
003	Abaxial	2	11	15.38
NCE	Adaxial	2	18	10
005	Abaxial	2	14	12.5
NCE	Adaxial	2	19	9.52
0010	Abaxial	2	13	13.33

Stomatal index = S/ (E+S) x100

Where

S = number of stomata per unit area while
E = number of epidermal cell in the same unit area

This suggested the occurrence of more stomata on the abaxial leaf surface of both species studied. The frequency of stomata seemed to vary with the distance away from the veins. It is plausible to state that the stomatal types in these species could also be of considerable taxonomic significance. The paracytic stomata were common to all the cultivars studied. This agreed with the observation of [15,16] on *Ipomoea*.

3.2 Cytological Features

Chromosome count 2n=24 was confirmed for all the *Xanthosoma* cultivars (Plate 3) while 2n=42 was confirmed for *Colocasia* cultivars (Plate 4) investigated. The chromosomes were relatively large. The chromosome counts recorded in this work agree with earlier counts [17]. This suggests that these two species may have evolved from a common progenitor, which has a basic somatic chromosome number n=x=6. Obviously, therefore, *Colocasia* may have arisen through polyploidisation. On the other hand, *Xanthosoma mafaffa* may have evolved through one step chromosome doubling.

There was slight cytoplasmic staining though this did not constitute a significant problem on microscopical observations. The chromosomes of *Xanthosoma* appeared slightly larger than those of *Colocasia.* Most of the chromosomes varied from metacentric to submetacentric in the two species. The micrograph was taken at x100.

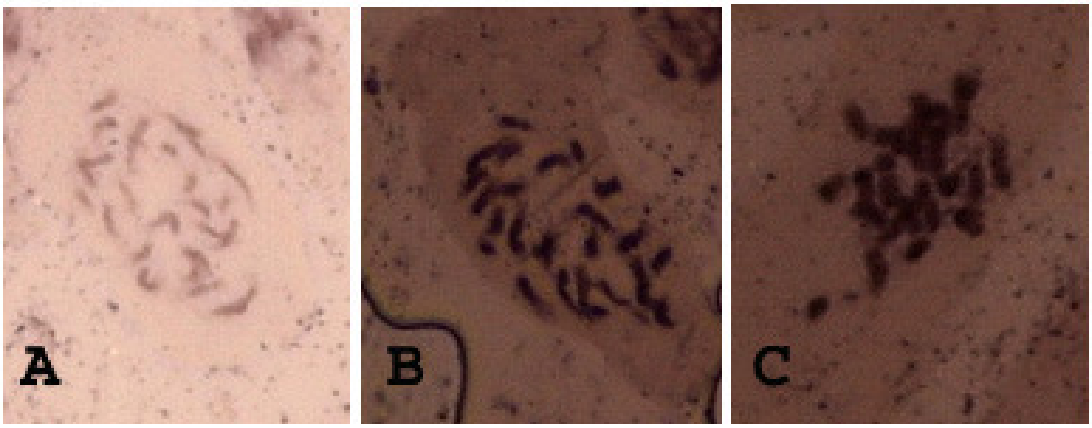

Plate 3. Mitotic chromosomes of *Xanthosoma mafaffa*: A) Mitotic cells at various stages of cell division. NXS 001 ('Ede Ocha'); B) Mitotic cells at various stages cell division. NXS 002 ('Ede Uhie'); C) Mitotic cell at prometaphase stage of cell division. NXS 003 ('Okorokoro')

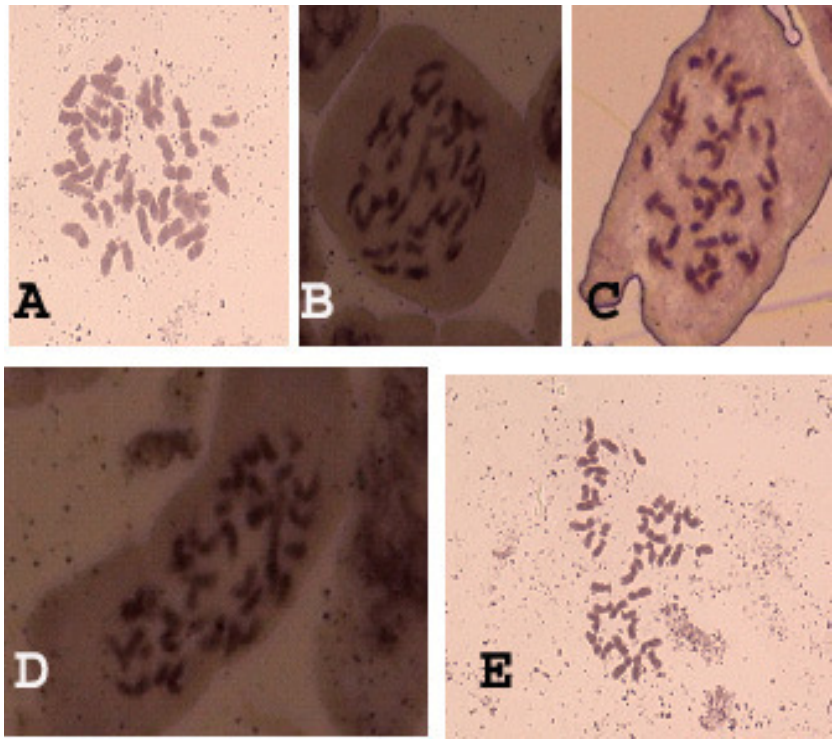

Plate 4. Mitotic chromosomes of *Colocasia esculenta*: A) Mitotic metaphase cell of *NCE 001* ('Coco India') showing a somatic count 2n=2x=42; B) Mitotic cells at various stages cell division of *NCE 002* ('Ede ofe green'); C) Mitotic cell at prometaphase stage of cell division *NCE 003* ('Ede ofe purple'; D) Mitotic cells at various stages of cell division *NCE 005* ('Ukpong'); E) Mitotic metaphase cell of *NCE 0010* ('Akiri') showing a somatic number 2n=2x=42

4. CONCLUSION

Based on the results obtained from these studies on the two genera, *Colocasia esculentum* and *Xanthosoma mafaffa,* it is plausible to state that all the *Colocasia* accessions though closely related have developed some degree of divergent morphological and cytological discontinuities whereas in the *Xanthosoma* accession, 'Ede Ocha' (*NXS 001*) which was significantly different by possession of brachyparacytic stomata should be considered a putative variant. It is also important to state that epidermal variations in stomatal index within the *Xanthosoma* and *Colocasia* cultivars reflect their ecological adaptation to variation in the degree of wetness of the environment.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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