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Probable functions of calcium oxalate crystals in different tissues of the edible aroids (*Xanthosoma* and *Colocasia* spp.) in Nigeria

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Representatives of seven major edible aroid accessions were screened for calcium oxalate using standard histochemical methods. All the accessions were noted to contain calcium oxalate in the forms of raphide bundles and intra-amylar crystals. The crystals were widely present in all parts of the plants including spongy mesophyll of the leaves, laticifers in the midrib and petioles, cortex of the roots and in the starch granules. The crystals occurred in the starch granules as intra-amylar structures and in the vegetative tissues as raphide bundles located in parenchymatous idioblasts. The most remarkable aspect of the histochemistry of this ergastic substance in the edible aroids is the high concentration of the raphide bundles around the root apical meristems. This suggests that the crystals of calcium oxalate serve a protective function in the root of these taxa. Their occurrence in starch granules imply that they are stored products and as such of value to the plants.

Key words: Root apical meristem, calcium oxalate crystals, *Colocasia*, laticifers, mesophyll, raphide bundle, raphide idioblast, *Xanthosoma*.

INTRODUCTION

The family Araceae comprises more than one thousand, five hundred species made up mainly of herbaceous or occasionally climbing shrubs (Duta, 1979). The mesophytic species generally have normal fibrous roots but the climbers have two types of aerial roots, which include clinging roots that fix the plant to its support and hanging roots that grow down to the soil. Generally, they have underground stem, which could be in the form of sympodial rhizome, corm or erect rootstock. A distinguishing feature of members of this family is the inflorescence, which consists of a spadix subtended by large, often brightly coloured spathe. They are usually monoecious with female flowers at the base followed by neutral flowers and the male flowers at the apex of the spadix. In cultivation, they rarely produce flowers hence their vegetative features are mostly relied on for routine identification.

Members of the Araceae family include food crops, ethnomedicinally invaluable genera and species, ornamental and other unexploited plants (Etukudo, 2003). Among the edible genera of this family are *Colocasia* and *Xanthosoma,* which in Nigeria form important food crops whose leaves could be eaten as vegetable and corms and cormels eaten as sources of staple carbohydrate. In Nigeria, only one species each of *Colocasia* and *Xanthosoma* exist. These species *Colocasia esculenta* and *Xanthosoma maffaffa* have few cultivars each. The cultivars vary in their vegetative features and in the way

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they are processed or consumed based on acridity and taste.

Acridity in plants is believed to be connected with the occurrence of calcium oxalate. Calcium oxalate is a secondary metabolite, which has been reported widely in plants (Osuji and Ndukwu, 2005). In the plant tissues where they exist, they can be of several forms, including raphides, conglomerate, cystoliths etc. (Okoli, 1988). Calcium oxalates have been reported to play various roles in plants. Hence, their roles are believed to include storage and protection. They are often seen as ergastic substances that have no well defined attributes.

Studies on the occurrence and distribution of ergastic substances and other secondary metabolites have been carried out on several wild and cultivated plants (Buttrose and Lott, 1978; Okoli and Green, 1987; Tilton, 1978). Such studies on calcium oxalate have been conducted to evaluate their relevance to germplasm characterization as well as to elucidate their physiological relevance to plants (Osuji and Ndukwu, 2005). Osuji and Ndukwu (2005) showed that calcium oxalate gets translocated from old to young plant parts. This observation implies a storage value of this ergastic substance. Osuji et al. (1997) showed that crystals of calcium oxalate could play a taxonomic role since their quantity, frequency of occurrence and distribution could distinguish between related species and cultivars. To date, there is no such report on the edible aroid taxa in Nigeria or elsewhere.

The frequency of occurrence, quantity and distribution of oxalates of calcium are important taxonomic characters, which have been clearly used to delimit cultivars as well as characterize plant germplasm (Osuji et al., 1997; Osuji, 2006). The value of this secondary metabolite in the protection of young and delicate plant parts has not been investigated. Their implication as stored products or bye products of metabolism and their obvious translocation from older plant parts to younger ones have been reported on the genus *Musa* (Osuji and Ndukwu, 2005). In the edible aroids of Nigeria, the occurrence, distribution and taxonomic or biochemical implications for use and consumption of the crop has not received any attention. Hence, the objective of this work was to investigate the presence, distribution and diversity of calcium oxalate crystals in the Nigerian edible aroid, *Colocasia* and *Xanthosoma* germplasm.

MATERIALS AND METHODS

The plant materials used for this work were obtained from the field germplasm of the National Root Crops Research Institute, Umudike, Umuahia. Corms and cormels of *Colocasia* and *Xanthosoma* accessions were obtained and cultivated in the experimental garden section of the Botanic garden of the University of Port Harcourt, Rivers State, Nigeria. Another set of the corms and cormels were allowed to set roots in an aerated humid chamber. Young roots were sectioned longitudinally and transversely while young leaves were sectioned only transversely. Transverse sections of the corms and cormels were also obtained after passing them through the microtometric method of Johansen (1940) as modified by Osuji and Uzogara (2003).

The specimens already fixed in FAA (1 part formalin, 1 part glacial acetic acid and 18 parts 70% ethanol v/v) were rinsed in deionized water and dehydrated through graded ethanol series in the order 30, 50, 70 and 90% for two hours in each solution and finally in absolute ethanol overnight. Dehydrated specimens were clarified in 3:1, 1:1 and 1:3 ethanol/chloroform solutions followed by pure chloroform each for 2 h. The samples were infiltrated in molten wax at 60°C for 7 h. Embedding of the specimens took place in plastic molds. The embedded materials were allowed to set on the bench for at least 10 h. Sectioning with Reichert rotary microtome took place at 15 to 20 μm thickness.

The sections were stained with hydrogen peroxide and silver nitrate for 5 min in bright light supplied by 100 W electric bulbs following the method of Silver and Price (1969) as modified by Osuji et al. (1997). The sections were stuck on slides and rehydrated through graded ethanol solution in the reversed order absolute, 90, 70, 50 and 30% ethanol solutions followed by deionized water, each for 5 min. Staining with 10% hydrogen peroxide (H_2O_2) for 2 min and silver nitrate ($AgNO_3$) for 4 min in bright light was conducted. The stained sections were mounted in DPX under No. 1 coverslip. The slides were observed under an OLYMPUS CX31 light microscope. Representative free-hand diagrams of features of the sections were drawn to scale.

Fresh leaves, petioles, corms and root tips of the *Xanthosoma* and *Colocasia* cultivars were obtained and fixed in freshly prepared FAA (1 part formalin, 1 part glacial acetic acid and 18 parts 70% ethanol v/v) for at least 24 h. The aroid materials were rinsed in deionized water before sectioning. About 5 mm length of the root apices was excised and free-hand-sectioned longitudinally and transversely. The sections were lightly stained with 0.1% safranin solution, mounted in glycerine under No. 1 18 x 18 cm coverslips. The slides were observed under an OLYMPUS CX31 light microscope fitted with an OLYMPUS E-330 Digital camera at X4, X10 and X20 magnifications. Photographs were taken of informative sections.

RESULTS AND DISCUSSION

The leaves of the accessions showed dorsiventral structure with uniseriate adaxial and abaxial epidermes, uni- to biseriate palisade and multiseriate spongy mesophyll. In the veins and midrib, the vascular tissues were lined immediately under both epidermes and subtend the ground tissue within which vascular bundles and laticifers occurred (Figure 1). In the leaf lamina, most of the raphide bundles occurred within the spongy mesophyll tissue (Figures 1 and 2). The roots showed a tetrarch vasculature with laticifers running along the cortical tissue.

Calcium oxalate was present in the leaves, corms, cormels and roots of all the accessions examined. The oxalate crystals occurred in the form of raphide bundles in all the vegetative tissues but in the form of intra-amylar crystals in the corms and cormels. The shapes of the intra-amylar crystals varied from solitary to triradiate in all the varieties. The bundles varied in size and each contained several solitary raphides, but averagely, the bundles in *Xanthosoma* varieties were thicker than those of *Colocasia* which were slimmer. Each raphide bundle was located in a special cell (raphide idioblast), which

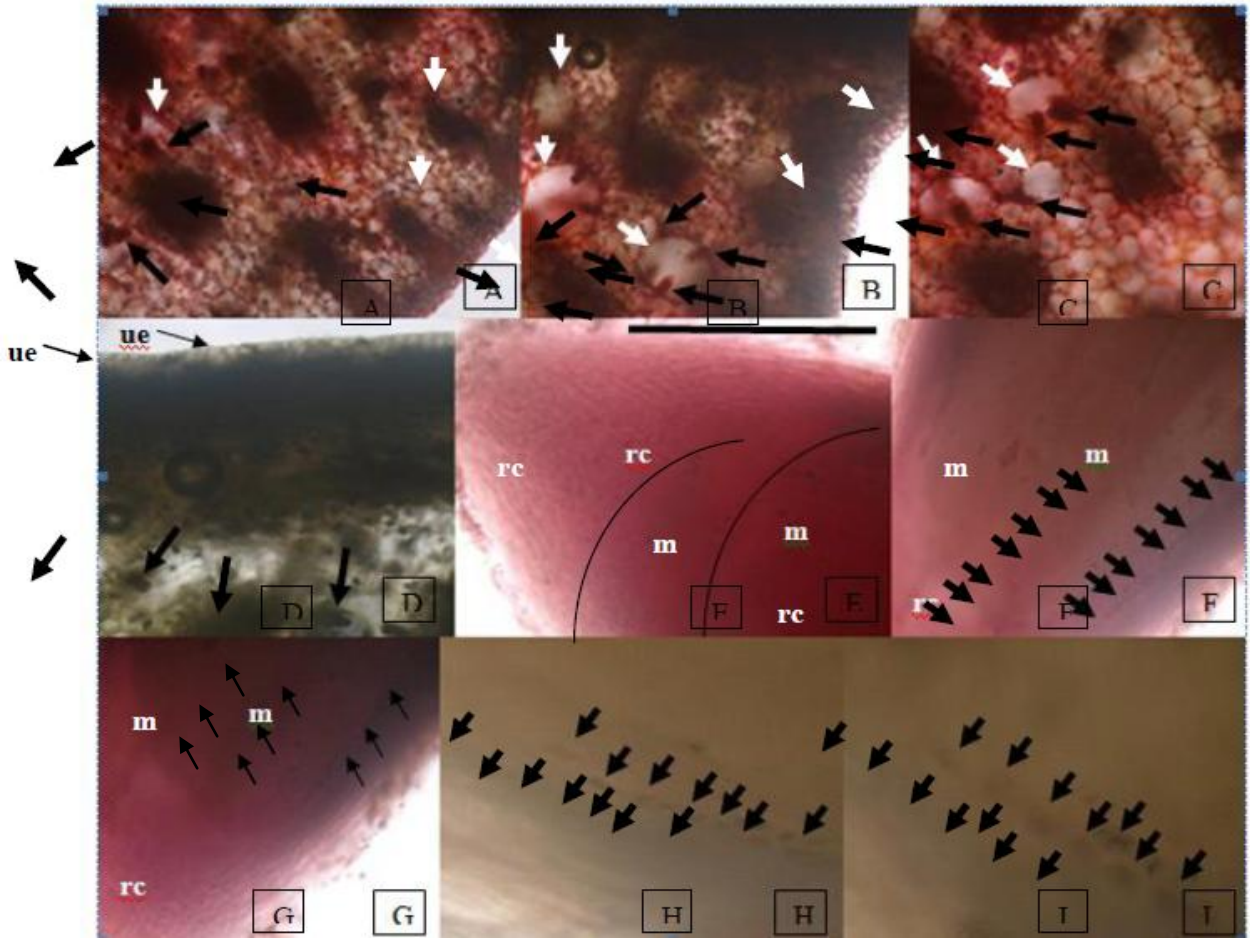

FIgure 1. Longitudinal Section (L.S.). of leaf, petiole and root apex of *X. maffaffa.* (A - D) Leaf showing some vascular bundles, laticifers (white arrow) and raphide bundles (black arrow) arranged around the laticifers, and (E - I) Root showing the regions of root cap (rc) and meristem (m, the boundary between the rc and m being marked in E with an arc). A) Petiole, B) side of the midrib, C) ground tissue of the midrib, D) leaf lamina showing convex adaxial epidermis and raphide bundle localized in the spongy tissue, E) root tip showing a funnel-shaped root cap tissue followed by the meristem, F - I) different magnifications of the cortical tissue of the root apex showing a longitudinal line of raphide idioblasts each of which contains 1-5 raphide bundles. Scale bar = 10 mm.

formed a storage compartment for it. However, the bundles were gathered within the laticifers in *Xanthosoma* midrib (Figure 1) but mostly positioned across linking contiguous laticifers (like interconnecting structures) in *Colocasia* (Figure 2). This implicates the raphide idioblast as a storage facility in which calcium and/or oxalates could be stored in the form of calcium oxalate (Okoli, 1988; Okoli and Green, 1987; Okoli and McEuen, 1986).

In roots, the raphide idioblasts and their enclosed bundles were most concentrated close to the root tips (Figures 1 and 2). The idioblasts, which were localized in the cortical tissue of the root, were very numerous and took the semblance of a mat or fence of raphides around the meristematic tissue in both *Xanthosoma* and *Colocasia* accessions and species. This concentration of the raphide structures around the meristem suggests protective function for these calcium oxalate structures. This agrees with the findings of Osuji and Ndukwu (2005) and Uno et al. (2001).

The raphide idioblast had the shape of a cylindrical tube, varied in size and comprised an elongated parenchymatous cell. Each raphide idioblast measured about 0.3-1.8 mm (4 to several cells) long in *Xanthosoma* and 0.3 to 1.4 mm in *Colocasia*. No contiguous idioblasts were observed in any of the tissues, organs or accessions. An idioblast contained one to five laterally adjoining raphide bundles (Figure 3).

The frequency of the bundles in an idioblast varied slightly between aroid species and accessions. The *Xanthosoma* accessions had larger raphide idioblasts and bundles than the *Colocasia* accessions (Figure 3). There was no evidence of variation in size of the raphide bundles between corms and cormels. The corms and

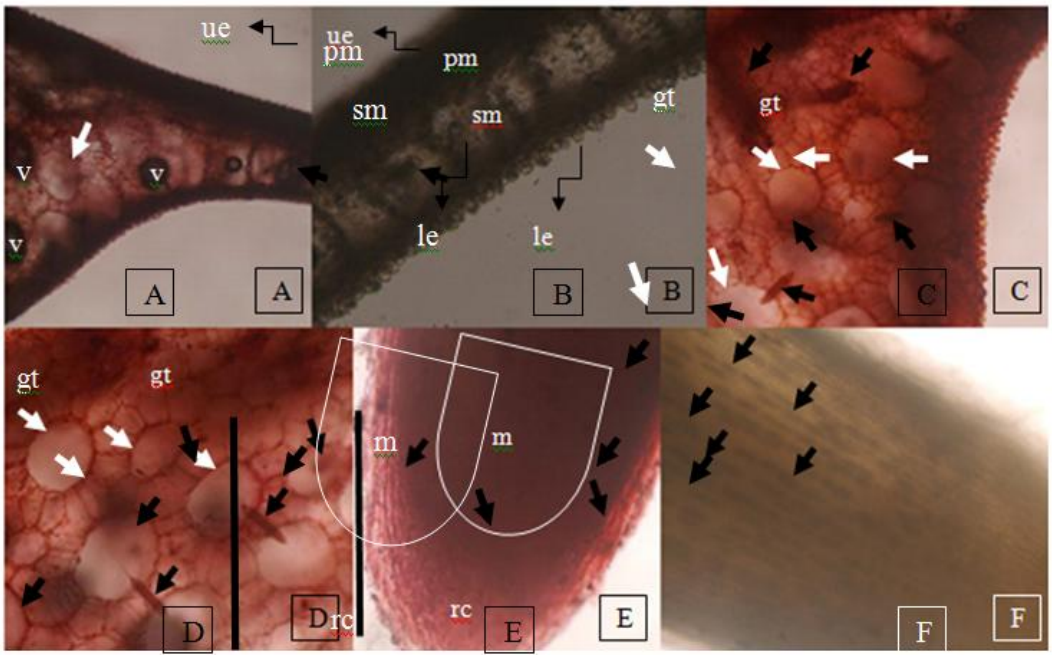

Figure 2. L.S. of leaf, petiole and root apex of *C. exculenta.* (A - C) Leaf showing upper epidermis, 'ue'; lower epidermis, 'le'; vascular bundles, 'v'; palisade mesophyll, 'pm'; spongy mesophyll, 'sm; laticifers (white arrow), raphide bundles (black arrow) arranged around the laticifers and meristem 'm'. A) leaf showing the interface between the midrib and the lamina, B) lamina, C) side part of the midrib, D) ground tissue of the petiole showing raphide bundles lying across contiguous laticifers, E) root apex showing the root cap tissue 'rc' and meristem 'm' occupying the marked out region, and F) an L.S. section through the cortical cylinder of the root showing a mator fence of raphide idioblasts, with more concentration being towards the root tip (that is, upper left direction). Scale bar = 10 mm.

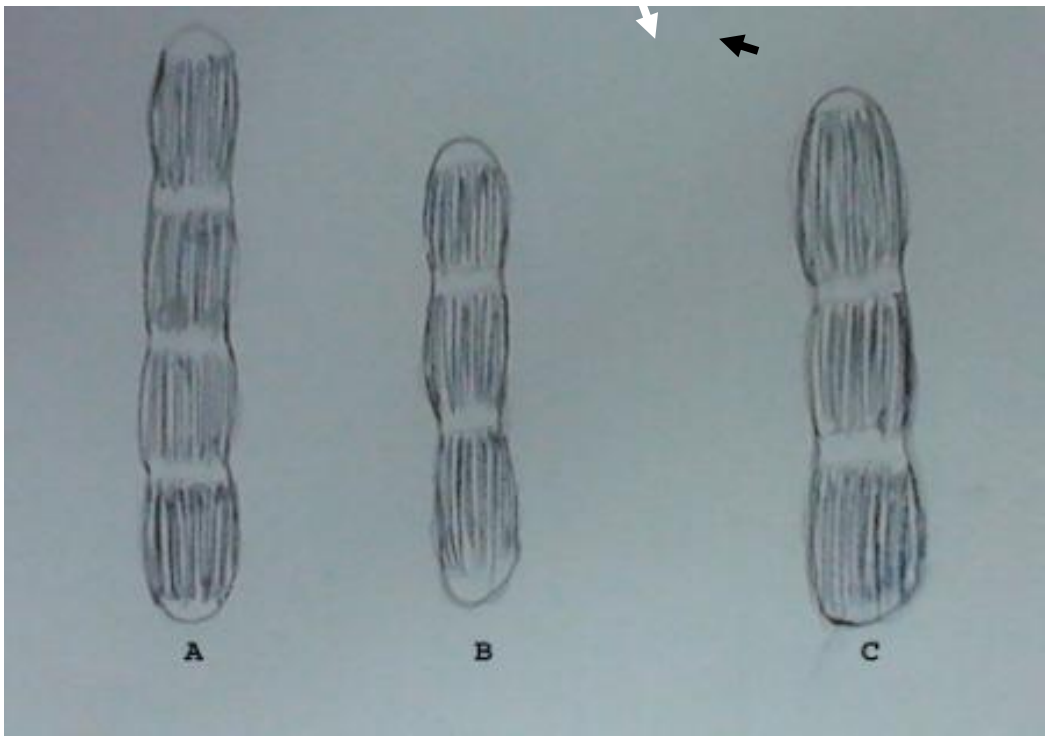

Figure 3. Raphide idioblasts in edible aroids containing bundles of calcium oxalate crystals. A and B) *Colocasia esculenta* and C) *Xanthosoma maffaffa*.

cormels that were already sprouting had less intra-amylar crystals than non-sprouting ones. Ergastic substances in plants are known to be objects of defensive mechanism (Uno et al., 2001). While most of them are very limited in their occurrence, some have proved to be of wide distribution among plants. Some of these widely distributed secondary metabolites have been a source of material and information useful in the improvement of perception, value and taxonomic characterization of plants. Among the ergastic substances that belong to this widely distributed category, tannins and calcium oxalate are notable examples. Their occurrence and distribution in the living tissues of plants have made them very useful in germplasm characterization and classification (Osuji et al., 1997).

Calcium oxalate had been reported as a storage product (Okoli, 1988; Okoli and McEuen, 1986) and as waste product in plants (Stace, 1980). Osuji and Ndukwu (2005) reported calcium oxalate as stored and useful products of metabolism, which are recycled by translocation from old to young plant parts. This indicates that they are not waste but storage metabolic products. Hence, they are translocated from locations where they are stored to domains in young parts of plants, where they are required, probably for their protective value (Okoli and Green, 1987).

Plants unlike animals cannot run away from herbivores. Hence, many protective or defensive mechanisms have been developed by plants against herbivores. One of the defensive means of warding off or discouraging herbivores is the acquisition of irritating components as well as other components that would make plant parts unpalatable (Uno et al., 2001). In the aroid germplasm studied, the wide distribution of raphide bundles and the occurrence of intra-amylar calcium oxalate reflect the importance of calcium and/or oxalate to the crops. Hence, the crop accumulates calcium oxalate and continues to retain it in the form of raphide bundles.

While calcium remains useful as a macro-element to the crop plants, the oxalate helps the plants to ward off herbivores. It is long known that the occurrence of oxalates of calcium enable plants to cause itching when contact is established with them. Therefore, the plants or their parts that contain oxalates are avoided by herbivores and especially, browsing insects and animals.

In addition, the occurrence of these crystals in the form of tiny needles makes chewing of tips of cocoyam roots difficult. In the case of man, processing has helped formidably to solve the problem by reducing the irritable condition caused by exposure to oxalate. Hence the edible aroids are known as a major part of the diet of Southern Nigeria.

REFERENCES

Buttrose MS, Lott JNA (1978). Calcium oxalate druse crystals and other inclusions in seed protein bodies: *Eucalyptus* and jojoba. Can. J. Bot. 17:2083-2091.

Duta AC (1979). Botany for Degree Students. 5th Edition. Calcutta Oxford University Press, Delhi, Bombay, Madras. P. 909.

Etukudo I (2003). Ethnobotany: Conventional and Traditional Uses of Plants. The Verdict Press, No. 20 Akpakpan Street, Uyo, Akwa Ibom State. P. 191.

Johansen H (1940). Plant Microtechnique. New York.

Okoli BE (1988). On the probable function and taxonomic value of calcium oxalate crystals in Cucurbitaceae. Feddes Repertorium 99:139-142.

Okoli BE, Green BO (1987). Histochemical localization of calcium oxalate crystals in starch grains of yams (*Dioscorea*). Ann. Bot. 60:391-394.

Okoli BE, McEuen AR (1986). Calcium-containing crystals in *Telfairia* Hooker (Cucurbitaceae). New Phytologist 102:199-207.

Osuji JO (2003). Cytogenetics techniques. In: Onyeike EN and Osuji JO (eds.). Research Techniques in Biological and Chemical Sciences. Springfield Publishers Ltd, Owerri, Nigeria. pp. 70-83.

Osuji JO (2006). Microstructural characters of the inflorescence bracts distinguish between *Musa sapientum* L. and *M. paradisiaca* L. Intl. J. Bot. 2(1):11-16.

Osuji JO, Ndukwu BC (2005). Probable functions and remobilization of calcium oxalate in *Musa* L. Afr. J. Biotechnol. 4(10):1139-1141.

Osuji JO, Okoli BE, Ortiz R (1997). Histochemical localization of calcium oxalate crystals in fruits of plantain and banana cultivars. Fruits 52(1):5-10.

Osuji JO, Uzogara SG (2003). Histochemical Techniques. In: Onyeike EN and Osuji JO (eds.). Research Techniques in Biological and Chemical Sciences. Springfield Publishers Ltd. Owerri, Nigeria. pp. 60-69.

Silver VL, Price JL (1969). Demonstration of calcium oxalate crystals in plant tissues by Pizzolato ($AgNO_3$-H_2O_2) method. Stain Technol. 44:257-259.

Stace CA (1980). Plant Taxonomy and Biosystematics. Edward Arnold, London.

Tilton VR (1978). A developmental and histochemical study of the female reproductive system in *Ornithogalum caudatum* Ait. Using light and electron microscopy. Ph.D. Dissertation, Owa State University, Ames.

Uno G, Storey R, Moore R (2001). *Principles of Botany*. McGraw Hill Companies Inc. Boston Burr Ridge, New York, London. P. 552.