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LOCALIZATION OF CALCIUM OXALATE CRYSTALS IN THE STARCH GRAINS OF EDIBLE COCOYAMS GROWN IN NIGERIA

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ABSTRACT

Tissues of the tubers of two edible species of cocoyams - Colocasia esculenta (L.) Schott and Xanthosoma sagittifolium (L) Schott were examined for the presence and nature of calcium oxalate crystals in their starch grains using light microscopy and histochemistry. The histochemical tests confirm, for the first time, the occurrence of varying shapes and sizes of calcium oxalate crystals in the parenchymatous tissues of the two species. The variation in the shapes and sizes of these crystals and the starch grains bearing them may be utilized for taxonomic purposes. The probable functions of the calcium oxalate crystals in these starch grains were further considered given their widespread utilization as a staple food.

***Key words**: Histochemistry, oxalate crystals and taxonomy.*

INTRODUCTION

Cocoyams (family: Araceae) especially *Colocasia esculenta* (L.) Schott and *Xanthosoma sagittifolium* (L) Schott are widely cultivated in Nigeria and other West African countries for the tubers which are used mainly as staple food and soup thickener (Onwueme, 1978). The cocoyam tubers are sources of carbohydrates and proteins in the diets and also used as livestock feed (Agueguia and Fatokun, 1987). Nigeria is reportedly the world's largest producer of cocoyams accounting for about 40% (3, 041. 58 million metric tones annually) of the total global production (APMEU, 1996). With increasing cultivation, several varieties and land races have emerged over time.

Starch grains are known to occur in many plants in varied morphology as have been reported in some species (Gaucher, 1902; Demiriz, 1969; Mahlberg, 1973, 1975). The usefulness of starch grain morphology in taxonomy is also fairly documented (Mahlberg, 1975; Biesboer and Mahlberg, 1981; Mahlberg, 1982).

Crystals of calcium oxalate are among the most widely occurring ergastic substances in plants as indicated by the extensive literature available on them. (Schneider, 1901; Solereder, 1908; Dormer, 1961; Price, 1970; Metcalfe, 1983; Okoli and McEuen, 1986; Okoli and Green, 1987; Okoli, 1988; Edeoga and Okoli, 1992, 1995; Edeoga and Ugbo, 1997; Osuji, *et al.,* 1997). Wattendorff (1978) reported the occurrence of crystals in the lumina cells of tissues of the bark and secondary xylem. Buttrose and Lott (1978) also found crystals to occur within seed protein bodies of some plants. Okoli and McEuen (1986) examined and reported the presence of varying types of crystals in the leaves, probracts, stems, flowers and seed coats of *Telfairia* species. In another study involving seven species of yams – *Dioscorea,* Okoli and Green (1987) reported the localization of calcium oxalate crystals in the starchy tissues of the tubers. Okoli (1988) also found

calcium oxalate crystals in different organs of nine out of the ten species of Cucurbitaceae investigated. Recently, Osuji, *et al* (1997) reported the occurrence of calcium oxalate crystals in the peels and pulps of unripe fruits of plantains and banana cultivars.

Intra-amylar localization of calcium oxalate crystals has been scantly reported (Okoli and Green 1987; Osuji, *et al.*, 1997). Despite the vast literature currently available, there is no known report involving the cocoyam species of *Colocasia* and *Xanthosoma.*

The probable functions of calcium oxalate crystals in plants are still uncertain although several suggestions have been made (Schneider, 1901; Edeoga and Okoli, 1992, 1995). Okoli and McEuen (1986) and Okoli (1988) suggested mechanical and protective functions. The observation of calcium oxalate crystals in seed protein bodies (Buttrose and Lott, 1978) and starch grains (Okoli and Green, 1987; Osuji *et al*, 1997) led the authors to suggest that these ergastic substances may be serving storage functions.

The present study was embarked upon in furtherance to the search for the occurrence of calcium oxalate crystals and their probable functions in starch grains using histochemical methods. The investigation is particularly significant given the fact that these starchy tubers serve as major staple foods and condiments in Nigeria and other developing countries. The variation in size and shape of the crystals and the starch grains bearing them may be further exploited for taxonomic purposes.

MATERIALS AND METHODS

The specimens used for the studies consist of freshly harvested mature tubers of *C. esculenta* and *X. sagittifolium* which were grown in the botanic garden of the University of Port Harcourt for about 8 weeks. Sizeable portions of each specimen were fixed in FAA (100ml of 40% formalin, 50ml of glacial acetic acid and 850 ml of 70% ethanol) for at least 48 hours. The specimens were dehydrated and embedded in wax following the methods of Ndukwu (1999). Sections of 15 – 20 uM thick were cut from the specimens with a rotary microtome. Histochemical staining for calcium oxalate crystals was performed following the methods of Silver and Price (1969). This involved treating the sections with 30% hydrogen peroxide and 5% silver nitrate under a bright light provided by a 60 watts electric lamp. The treatment lasted for about 30 minutes after which the sections were rinsed, dehydrated, passed quickly through xylene and made permanent in Dee Pex. Photographs were taken from the slides using a Leitz Laborlux-12 photomicroscope fitted with a Wild MPS-camera.

RESULTS

The studies indicate the occurrence of stable and well-developed starch grains in the tissues of mature tubers of the two species of cocoyams investigated. Variations were however observed in the size and shape of starch grains found in the two species. While the starch grains were generally circular in *X. sagittifolium* they were oval or spherical in *C. colocasia.* The starch grains of *C. colocasia* were generally longer (15-24 um) than those of

X. sagittifolium (12-18 um). There was no evidence of disintegration of the starch grains even with the process of micro-technique.

Calcium oxalate crystals of varying shapes, sizes and textures were also found to be present in the tissues of the tubers of the two species studied. The mostly solitary crystals were generally located in the stellar region (intra-amylar) of mature starch grains in both species. No raphide bundles or aggregate crystals were observed in any of the species.
In *X. sagittifolium,* the crystals were mostly granular in shape but generally irregular to tri-radiate in *C. colocasia.* The crystals of *X. sagittifolium* were also generally thicker and longer (3-12 um long) than those of *C. colocasia* (2-8 um long). These features are generally observable in photographs displayed as plates 1 A & B.

DISCUSSIONS

The variation in structure, size and shapes of the starch grains in the two species studied further lend credence to the value of these substances in taxonomic delimitation. This shows that the two cocoyam species can be distinguished using variations in the morphology of starch grains. The morphology of the starch grains as observed were obviously species specific. Already, Mahlberg (1982) had demonstrated the usefulness of starch grain morphology in the taxonomic delimitation of different varieties of *Euphorbia pulcherrima.*

The presence of intra-amylar calcium oxalate crystals in the tissues of the two species of cocoyam studied is being reported for the first time. However, these ergastic substances have been reported in starch grains of yams – *Dioscorea* species (Okoli and Green, 1987) and in *Musa* species (Osuji *et al.*, 1997).

The occurrence of the oxalate crystals within the parenchymatous starch grains of the cocoyam species suggests that these substances may indeed be playing storage functions. Okoli and Green (1987) and latter Okoli (1988) had posited that oxalate crystals may represent the storage forms of calcium oxalate. Their suggestion corroborates earlier reports such as those of Scott (1941) and Frenceschi and Horner (1980). The observation of calcium oxalate crystals in the seed protein bodies of *Eucalyptus jojoba* by Buttrose and Lott (1978) further strengthens the evidence that these substances may function as the storage form of either calcium or oxalate.

The variations observed in the shapes, sizes and textures of the crystals in the two species of cocoyam studied may be exploited for taxonomic purposes as has been shown in Icacinaceae (Heintzelman and Horward, 1948), Cucurbitaceae (Okoli, 1988), Verbanaceae (Matthew and Shah, 1984), Dioscoreaceae (Edeoga and Okoli, 1995) and *Commelina* (Edeoga and Ugbo, 1997). The occurrence of these intra-amylar crystals in the two species however confirms their phylogenetic relatedness despite belonging to different genera. A more comprehensive work involving the wild relatives of the two cocoyam species would be necessary to ascertain the taxonomic significance of intra-amylar oxalate crystals in the entire Araceae family.

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