HISTOCHEMICAL LOCALIZATION OF CALCIUM OXALATE CRYSTALS AND ITS IMPORTANCE IN BIOSYSTEMATICS.

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Abstract

Calcium oxalate is known to be a salt of oxalic acid. They are regarded as reserve materials and substances, which are produced and stored in plant cells, but they do not re-enter the metabolism of the plants, hence they are called ergastic substances. They are the most widely spread ergastic substances found in flowering plants. The distribution pattern of calcium oxalate crystals in tile leaf, Stein and loot of some plant species differed markedly and was reviewed to ascertain the taxonomic importance. The histochemically localized crystals of calcium oxalate varied in shape and region of localization depending on the vegetative parts of the plants under review. They appear as raphides, bundles of needles, styloids, elongated columnar crystals, druses, spheroidal aggregates of prismatic crystals etc. The variation in shape of calcium oxalate crystals is of taxonomic importance and could be used to distinguish and classify different plant species especially at lower taxonomic levels.

Introduction And Literature Review

Crystals of calcium oxalate, which occur in organs and tissues of many plant species, are among the most widespread ergastic substances known in angiosperms (Metcalf and Chalk, 1950). In plants, calcium oxalate many be present as the soluble salt or insoluble crystalline calcium oxalate. Calcium oxalate crystals assume different forms. They appear as raphides, bundles of needles, styloids, elongated columnar crystals, crystal sand, very small crystals usually in a mass: their shape ranges from prisms, to rectangular or pyramidal. According to AI-Rais et al (1971) the most commonly encountered forms of calcium oxalate crystals are raphides, styloids, variously shaped prisms, crystal sand and druses. (Fig I). Other shapes of calcium oxalate crystals appear to be variations of these forms. The occurrence of these substances in plants has received attention from anatomists for many years (Al - Rais et al 1971: Frey, 1925: Scurfield et al 1973). Taxonomic information on their location has been summarized in so far as it concerns - dicotyledonous plants by Solereder (1908) and by Metcalfe and Chalk (1950). Many scientists have demonstrated the importance of the distribution and types of calcium oxalate crystals in different groups of flowering plants. Scott (1941) examined the distribution of calcium oxalate crystals in *Ricimts communis*, relating this to the pattern of tissue differentiation and presence of other ergastic substances. Heintzelman and Howard (1948) made a comparative assessment of the morphology of the Icacinacceae with respect to the degree of pubescence and nature of crystals. Al - Rais et al. (1971) reported the presence of raphides and barrel-shaped crystals in the leaves of *Dioscorea alala.* Stebbins et al (1972) also reported the presence of crystals in apple stem, petiole, and fruit tissues. Francheschi and Horner (1980) made comparison of different groups of plants, as done by Okoli (1988) and Okoli and Mceuen (1986) for the cucurbitaceae and Okoli and Green (1987) for the Dioscoreaceae. Furthermore, the biological significance and implications of the histochemical localization of ergastic substances, including calcium oxalate crystals, has been highlighted in different plant families such as the Verbenaceae (Matthew and Shah, 1984, Dioscoreaceae (Ayensu, 1972; Edeoga, 1991; Edeoga and Okoil, 1995) and Commelinaceae (Edeoga and Ugbo, 1997). Edeoga and Ogbebor (1999) also described the distribution and forms of calcium oxalate crystals in the leaves, stems and roots of some Aneilema species common in Nigeria. In their-studies, different forms of calcium oxalate crystals have been observed in different organs of the Aneilema species as shown in table 1.

Taxa	Mesophyl l	Leaf Trichome	Stem Ground Tissue	Cortex	V Cortex	toot Endodermis	Exodermis
A. aequinoctiale	Irregular	Irregular & rliomboidal	Mostly rliomboida l	n . d	n . d	Irregular	n . d
A. beniniense	Irregular	Irregular & Rliomboidal	mostly rhomobida!	n. d	Irregular	n . d	n . d
A. Paludosum	Irregular	n . d	Irregular	n . d	Irregular	Irregular	Irregular
A. unbrosum	Irregular	Irregular	Irregular	n . d	Irregular	Irregular	Irregular

n. d = not determined because rarely occurring

SOURCE: Edeoga and Ogbebor 1999.

As observed from the table, rliomboidal and ilrregular forms of crystals characterized the Aneilema species. Similarly, the Mesophyll region and basal cells of trichomes were associated with calcium oxalate crystals in the lamina of some of the Aneilema species they investigated. Most of the calcium oxalate crystals were found in the ground tissue region of the stems; crystals were common in the endodermis, pith and cortex of the roots of the Taxa investigated. This work aims at using the knowledge agained form the pattern of distribution of calcium oxalate crystals in the vegetative organs of the Aeilema species investigated to contribute to the apparent lack of information on these Taxa despite their biological and medicinal importance (Burkill, 1985).

The Role Of Calcium Oxalate Crystals In Plant Taxonomy

The works of Edeoga and Okoli (1992, 1995), Edeoga and Ugbo (1997) related the occurrence of clcium oxalate crystals to the metabolic activities of some plants and emphasized how the distribution and shapes of calcium oxalate crystals could be used for diagnostic assessment of different groups of plants. Hence, the different shapes and forms of crystals localized in the epidermal cells of their investigated Taxa make it possible to use them for diagnostic and taxonomic purposes. For example, the prismatic crystals found in the epidermal cells of the leaf in *Commelina erecta* subsp. *Erecta* and *C. eracta* subsp. *Livingstonii* were different the *need\e-shaped* crystals mostly found in the epidermal cells of the leaf in C. *benghalansis.* From this study, C. *erecta* subsp. *Livingstonii* could however be separated from C. erecta subsp. erecta in the acquisition of rliomboidal crystals in addition to the usual needle - like and prismatic crystals by the former. Similarly, the fact that the crystals were localized in different regions of the epidermal cells are equally of taxonomic interest. For instance in *C. benghalensis*, *C. africana,* and *C. erecta* subsp. *Livingstonii*, the crystals were vacuolar and crytoplasmic and present, close to the guard cells of the stomata.

The forms in which the crystals existed in the epidermal cells of these Taxa had some taxominic connotations. Thus the aggregated crystals in *Commelina diffusa* were peculiar to this taxon unlike in other Taxa where there were either single, needle-like, prismatic or rliomboidal crystals and raphide bundles. The histochemically localized ergastic substances had proved to be of great value in the identification of these Taxa. This was because, the observations made in this investigation had shown that distinct morphological biotypes of these *Commelina* species existed naturally despite the universal occurrence of oxalate crystals among there Taxa. Infact this universality in occurrence of oxalate crystal buttressed the fact that these were closely connected during the course of their evolution. Furthermore, the results from this study strongly suggested that *Commelina erecta* subsp. *erecta* and C. *erecta* subsp. *Livingstonii* could be separated and treated as separate Taxa. This was because of the fact that histochemical attributes of these two Taxa appeared quite distinct despite the high similarity in their morphology. The use of histochemica attributes of plants in solving critical taxonomic problems is now gaining wider popularity just as the use of DNA markers. A perusal through the botanical literature shows that the use of histochemistry in taxonomic conclusions is no more a rare exercise. It has been done in different groups of plants as could be seen in the works of AI - Rais et al, (1971), Watterdorf (1978), Francheschi and Horner

(1980), *Matthew* and Shah (1984), *Okoli* and Green (1987), *Okoli* (1988), Edeoga and *Okoli* (1992, 1995). The taxonomic significance of ergastic substances has been indicated in diverse plant families such as Verbenanceae (Matthew and Shah, 1984), Cucurbitaceae (Okoli, 1988).

Edeoga and Okoli (1992, 1995) and Edeoga and Ugbo (1997) had suggested that these ergastic substances could have nutritional, mechanical and transport roles in some *Dioscorea* and *Commelina* species. A close association of the calcium oxalate crystals with the site of photosynthesis suggests that these substances could be involved in the synthesis of carbohydrates. The calcium oxalate crystals could also offer some elements of rigidity within the mesophyll region thereby providing mechanical support to the cells of this tissue. Also important was the variation in the distribution patterns of the oxalate crystals among the taxa investigated. Thus, the distribution and shape of the crystals, though variable even within each species, displayed interspecific differences that could be used for taxonomic purposes. This has been done in other taxa including Icacinaceae (Heintzelman and Howard, 1948); Verbenaceae (Matthew and Shah 1984); Discoreaceae (Okoli and Green, 1987; Edeoga and Okoli, 1992, 1995), Cioscoreaceae (Okoli and Green, 1987: Edeoga and Okoli, 1992, 1995); Cucurbitaceae (Okoli, 1988: Okoli and Mceuen, 1986), and Commelinaceae (Edeoga and Ugbo, 1997). The taxonomic importance of calcium oxalate crystals found in the leaves, stems and roots of these taxa lies in the variation in their shape and localization. For example, the rhomboidal crystal *of Aneilema acquinocliale* and *A. umbrosum.* From these findings, these taxa could be separated on the basis of crystal shape. This agrees with the previous observations that mussile-like crystals of calcium oxalate were peculiar to *Dioscorea rolundata* and *D. birtiflora* while slightly elongated crystals of calcium oxalate were common in /). *hulbiferea* (Edeoga and Okoli, 1995). The localization of crystals within the root cortex is also important as this region, in addition to its storage function, offers protection against desiccation. The persistent association of the crystals with the ground tissue (supportive), cortex and mesophyll region (nutritional) clearly attests to the possible roles of calcium oxalate in the taxonomy and classification *of Aneilema* species. These observations are no more controversial since (1941), *Buttrese* and Lott (1978), Scurfield et al (1973), Edeoga and Okoli (1995) and Edeoga and Ugbo (1997) made similar observations in different groups of plants. Turning to more specialized examples of taxonomic value of crystals, Metcalfe and Chalk (1983) recalled that fibres derived from *Corchorus capsularis* and *Corchorus olitorius* can be recognized and nearly always distinguished from *Malvaceae* substitute fibres such as "Kenaf" of "Bilimbi" jute *(Hibiscus canabinus)* and "Roselle" *(Hibiscus sabdariffa)* by the presence of chains of solitary crystals.

Some Taxonomic Merits Of Calcium Oxalate Crystals As A Taxonomic Character

The use of calcium oxalate as a taxonomic character has helped greatly to solving so many taxonomic problems. For instance, the controversy and confusions on the systematic identities of the family constaceae as a whole was resolved to some extent through the work of Edeoga and Okoli (1997) on histomorphology of ergastic substances in the leaves and rhizomes of two *Costus* species (Costaceae). Also, Metcalfe and Chalk (1983) recalled that the jute fibers derived from *Corchorus* capsularis and Corchorurs olitorius can be recognized and neatly always distinguished from Malvaceae substitute fibers by the presence of chains of solitary crystals. Again the variation in structure of calcium oxalate crystals was consistent with the recent finding by Edeoga and Okoli (1992, 1995) and Edeoga and Ugbo (1997) in some *Dioscorea* and *Commelina* species hence these authors agreed that the form of crystals can be used as an aid to classification in Dioscoreaceae and Commelinaceae. Also, Ayensu (1972) made remarkable contributions on the anatomy and taxonomy of some, *Dioscorea* species including some of the West African subregion and these led to the reclassification of the whole members of the Dioscoreaceae and the establishment of the order Dioscoreales. Although, the size and shape of the crystals are variable within each species, but they show enough interspecific differences that may be utilized for taxonomic inferences. This has been done in other groups of plants such as in Icacinaceae by Heintzelman and Howard (1948) and Verbebaceae by Matthew and Shah (1984). Furthermore, the results from the study of Dioscoreaceae strongly supports the merging of *D cayenensis* with *D. rolundata* as reported by Lowe and Soladoye (1990). This is due to the fact that histochemical attributes of these two taxa appear quit similar despite the slight difference in their morphology.

Conclusion And Recommendation

The histomorphology of calcium oxalate crystals should not be overlooked when making taxonomic inferences. Thus, the recognition of the different morphoforms of calcium oxalate crystals as relevant markers, that could be considered along with other taxonomic markers, that could be considered along with other taxonomic markers in the classification, characterization, identification and naming of plant species is therefore apparently important. It is also necessary to collect taxonomic evidences from other biological disciplines such as floral and epidermal morphology, anatomy cytology, palynology, Genetics, etc, together with calcium oxalate crystals for a reliable taxonomic decision.

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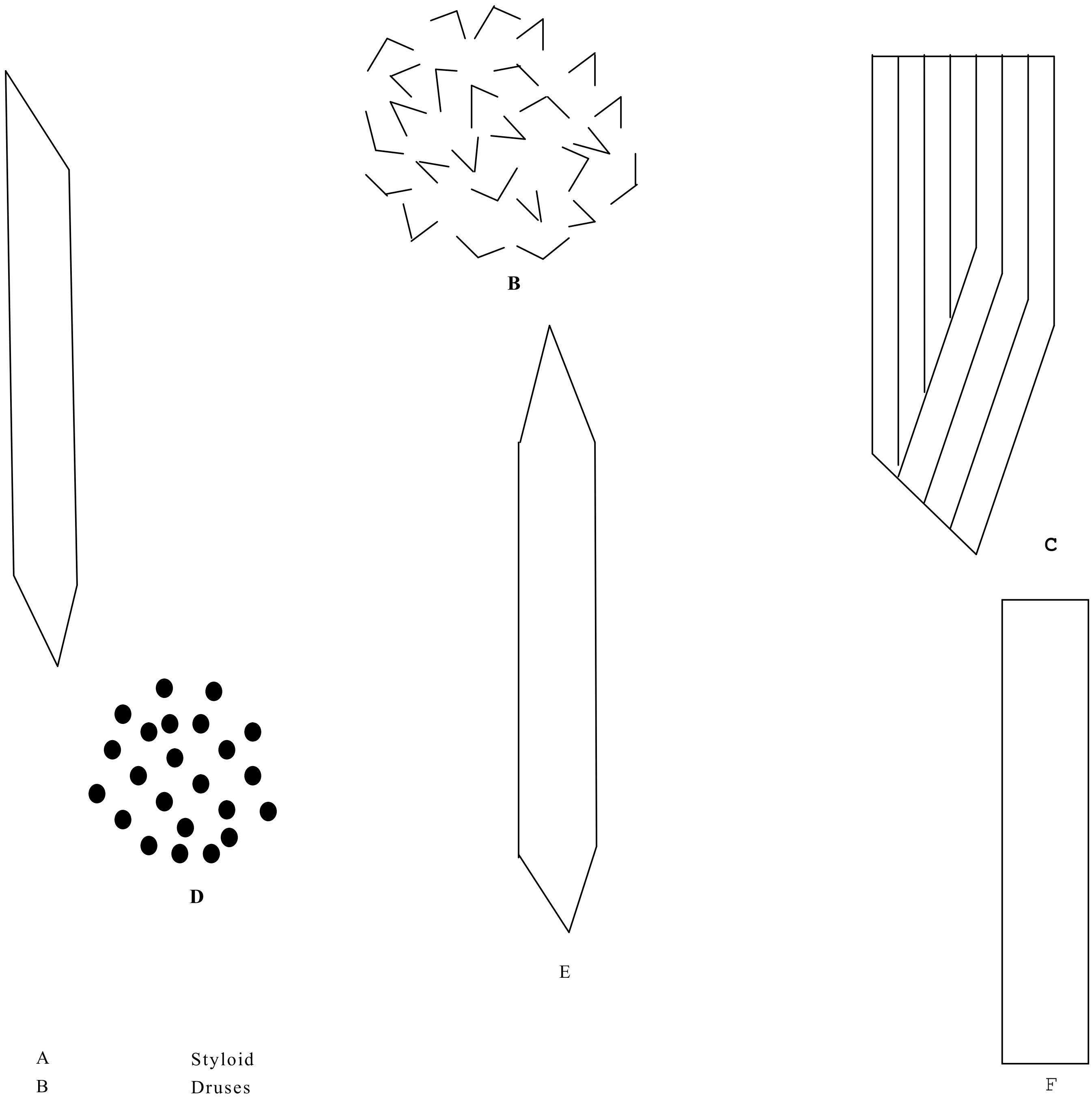
DIFFERENT SHAPES OF CALCIUM OXALATE



A	Styloid
B	Druses
C	Raphides (Aggregate)
D	Crystal sand
E	Rhomboidal Prismatic
F	

SOURCES: Plant Anatomy by C. Esua 1977 and The importance of calcium oxalate crystals in plant taxonomy by Mbagwu F. N. 2001.