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# Histochemical Studies on Some Nigerian Species of *Vigna* Savi (Leguminisae-Papilionoideae)

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**Abstract:** Histochemical studies on eight species of *Vigna* namely *V. ambacensis*, *V. gracillis*, *V. racemosa*, *V. reticulata*, *V. subterranea*, *V. triloba*, *V. unguiculata* and *V. vexillata* was investigated. Analysis of this study showed that there are presence of crystal sand in the leaves of *V. a mbacensis*, *V. racemosa*, *V. subterranea* and *V. vexillata*. In the nodes, *V. gracillis* and *V. racemosa* possess aggregate crystals and crystal sand, *V. ambacensis*, *V. unguiculata* and *V. vexillata* possess crystal sand while *V. reticulata* and *V. triloba* possess aggregate crystals, respectively. In the petiole anatomy, there are crystal sand in *V. ambacensis, V. triloba* and *V. unguculata*, aggregate crystals in *V. gracillis* and rectangular and crystal sand in *V. racemosa*. There are rectangular crystals and crystal sand in the stems of *V. reticulata* and *V. subterranea*, crystal sand in *V. ambacensis* and *V. vexillata* while only the root of *V. subterranea* possess crystal sand. The diversity in shape of calcium oxalate crystals among the investigated species of *Vigna* is discussed in relation to their taxonomic significance.

**Key words:** Histochemistry, calcium oxalate, crystals, *Vigna*, taxonomy

## INTRODUCTION

The bean plants belong to the genus *Vigna* Savi (Willis, 1985). The genus *Vigna* belongs to the family Leguminosae-Papilionoideae and tribe *Phaseoleae* which is made up of about 80-100 species that are tropical especially in Africa and Asia. In recent years, it has been observed that there is discripancy in the number of species recognized by different authorities. Daniel (1960) recognized 37 species, Hutchinson and Dalziel (1954) recognized 25 species while Burkill (1995) recognized 22 species. The reason for the confusion and discrepancies in estimation of the number of taxa in these groups of plants is due to the perceived similarities in structural and reproductive biology of these bean plants.

The *Vigna* species are valued as forage, cover and green manure crops in many parts of the World (Singh *et al*., 1970). The economic *Vigna* species exhibit a number of attributes that make them particularly valuable for inclusion in many types of croping systems. They can be grown successfully in extreme environments such as high temperatures, low rainfall and poor soils with few economic inputs (White, 1972). *Vigna* food products exhibit many excellent nutritional attributes and these products produce a needed complement in diets comprised mainly of roots, tubers or cereals (Padulosi, 1997; Pasquet, 1996). They are not only used as sources of food for humans and livestock but also for soil conservation and fertility maintenance (Cobinna, 1992). Most *Vigna* species such as *Vigna unguiculata*, *Vigna radiata*, *Vigna subterranea* etc are sometimes called "the poor-man's meat" owing to their uses as a primary protein source (Chopra and Swamy, 1975).

The use of histochemical attributes of plants in solving critical taxonomic problems is now gaining wider popularity just as the use of other characters. A perusal through the botanical literature shows that the use of histochemistry in taxonomic conclusions is now a common practice. For example, the occurrence of calcium oxalate crystals in various plant families have been reported by various authors. Edeoga and Okoli (1995) showed that the size and shape of calcium oxalate crystal though variable within each species showed enough interspecific differences that may be utilized for taxonomic inferences in Dioscoreaceae. This has been done in other groups of plants such as in Icacinaceae by Heintzelman and Howard (1948) and Verbenaceae by Mathew and Shah (1984). Calcium oxalate crystals exist in different forms such as raphides, styloids, variously shaped: prisms, crystal sand and druses (Al-Rais *et al*., 1971). Other shapes exist but they are variations of these forms. Scott (1941) investigated the distribution of calcium oxalate crystals in *Ricinus communis* and attributed this to the pattern of tissue differentiation and presence of other ergastic substances. According to Al-Rais *et al*. (1971) there are presence of raphides and barrel-shaped

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crystals in the leaves of *Dioscorea alata*. Stebbins *et al.* (1972) also reported the presence of crystals in apple stem, petiole and fruit tissues. Francheschi and Horner (1980) made comparisions among a variety of groups of plants, as did Okoli (1988); Okoli and McEuen (1986) for the Cucurbitaceae and Okoli and Green (1987) for the Dioscoreaceae.

The biological significance and implications of the histochemical characters of ergastic substances, including calcium oxalate crystals, nature of tannins and saponins, has been highlighted in different plant families such as in the Verbenaceae (Matthew and Shah, 1984), Dioscoreaceae (Ayensu, 1972; Edeoga, 1991; Edeoga and Okoli, 1992, 1995). Commelinaceae (Edeoga and Ugbo, 1997; Edeoga and Ogbebor, 1999). Nyctaginaceae (Edeoga and Ikem, 2002) and Curcurbitaceae (Okoli, 1988; Okoli and Mc Euen, 1986). Despite the fact that some of these scientists have demonstrated the important role of calcium oxalate crystals and other ergastic substances in the biology of different groups of angiosperms, little or no information is available for the genus *Vigna*. This study therefore assesses the relevance of and discusses the extent to which calcium oxalate crystals and other ergstic substances could be utilised in the systematic consideration of the eight *Vigna* species.

## MATERIALS AND METHODS

Mature and fleshy parts (leaf, node, petiole, stem and root) were collected from samples from different parts of Eastern Nigeria. This study was conducted at the Science Laboratory of Micheal Okpara University of Agriculture Umudike Umuahia, Abia State Nigeria in October 2005. These specimens were initially fixed in FAA (1:1:18) glacial acetic acid: 40% formaldehyde: 70% ethanol (V/V) for 48-72 h. The fixed materials were later sectioned using a Reichert rotary microtome at 26 μm following a slightly modified method of Culter (1978). Anatomical staining was done by initially staining with few drops of alcian blue for 5 min and counter stained with safranin solution for 2 min. The specimens were later made permanent on slides by mounting in Canada balsam.

Photomicrographs of the anatomical features (Fig. 1 and 2) were then taken from the slide using a Leitz Wetzler ortholux microscope fitted with vivitar-V-335 camera.

## RESULTS AND DISCUSSION

An important observation made on these taxa is the presence of three different shapes of calcium oxalate crystals namely:- crystal sand, aggregate and rectangular crystals with crystal sand crystal appearing in all the parts investigated (Table 1, Fig. 1 and 2). Another interesting aspect of the result is the occurrence of crystal sand crystal and starch grains inside chloroplast only in the root of *V. subterranea* and leaf of *V. unguiculata*, respectively (Table 1, Fig. 1 and 2). The existence of two types of crystal in an organ is also an interesting feature of this investigation.

From these studies, the distribution and shape of the crystals within these species, displayed interspecific differences and similarities that could be used for systematic purposes in these taxa. The occurrence of the same type of crystal(s) in different taxa showed interspecific relationship among the taxa whereas the presence of different type of cryatal(s) showed no relationship. So the use of crystals in solving taxonomic problems is not a new thing since some other workers have applied it in some plant families such as Heintzelman and Howard (1948) in Icacinaceae; Okoli (1988) in Cucurbitacea; Okoli and Green (1987) in *Dioscoreaceae*; Mathew and Shah (1984) in Verbenaceae and Edeoga and Okoli (1992, 1995) in *Dioscoreaceae*.

The presence of these crystals within the leaf, node, petiole, stem and root of these taxa is important due to the role that these parts play in physiological processes of these taxa. According to Edeoga and Okoli (1992, 1995) and Edeoga and Ugbo (1997), these ergastic substances could have nutritional, mechanical and transport roles in plants. These scientists opined that a close association of the calcium oxalata crystals with the site of photosynthesis suggests that these substances could be involved in the synthesis of carbohydrates. The crystals could also offer some elements of rigidity within

Table 1: Types of crystals found in the species of *Vigna* studied

| Part of Plant | *V. ambacensis* | *V. gracillis* | *V. racemosa* | *V. reticulata* | *V. subterranea* | *V. triloba* | *V. unguiculata* | *V. vexillata* |
|---|---|---|---|---|---|---|---|---|
| LEAF | Crystal sand | - | Crystal sand | - | Crystal sand | - | Starch grain Inside chloroplast | Crystal sand |
| NODE | Crystal sand | Aggregate and Crystal sand | Aggregate and Crystal sand | Aggregate | - | Aggregate | Crystal sand | Crystal sand |
| PETIOLE | Crystal sand | Aggregate | Rectangular and Crystal sand | - | - | Crystal sand | Crystal sand | - |
| STEM | Crystal sand | - | - | Rectangular and Crystal sand | Rectangular and Crystal sand | - | - | Crystal sand |
| ROOT | - | - | - | - | Crystal sand | - | - | - |

Fig. 1: TS of Stem, (a) *V. gracillis* with big vessels and crystal sand (arrowed), (b) *V. vexillata* with trichome and crystal sand (arrowed), (c) *V. triloba* with glandular trichome (arrowed). (X 100)

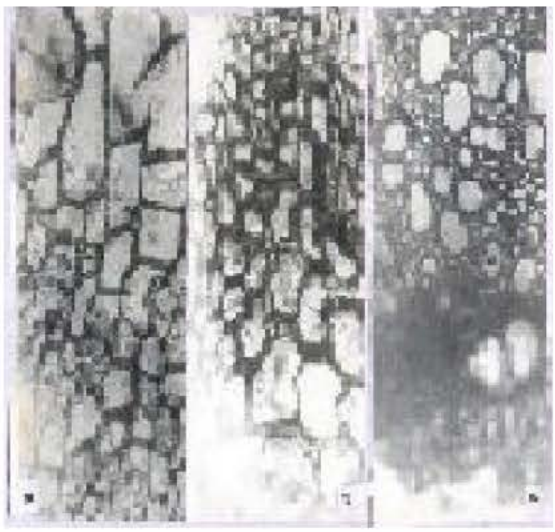

Fig. 2: TS of Node and Root, (a) *V. gracillis* with aggregate and crystal sand (arrowed), (b) *V. triloba* with aggregate crystals (arrowed), (c) *V. subterranea* with crystal sand (arrowed). (X 100)

the mesophyll regions thereby providing mechanical support to the cells of these tissues. This is not surprising because calcium is known to perform this function in plant cells. This study is therefore based on the principle that histochemical studies has played a major role in the identification, characterization and classification of plants.

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