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Histochemical Studies on Five Variants of *Viscum* L. (Loranthaceae)

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Abstract: The histochemical studies on the 5 variants of *Viscum* (A-E) showed important observation that only 3 different types of crystals are present. These are crystal sand, circular and rectangular crystals. Variants A has circular crystals at the leaf and crystal sand at the stem. Variant B has rectangular and circular crystals at the leaf and crystal sand at the stem. Variant C has circular crystals at the leaf and crystal sand at the stem. Variant D has rectangular and circular crystals at the leaf and crystal sand at the stem and finally Variant E has rectangular and circular crystals at the leaf and crystal sand at the stem. The similarities and variations in shapes of these oxalate crystals among the investigated variants of *Viscum* are discussed in relation to their biosystematic relevance.

Key words: Histochemistry, calcium oxalate, crystals, *Viscum*, variants, biosystematics

INTRODUCTION

The African mistletoes are parasitic plants that derive all or most of their nutrition from other flowering plants. They originated from the tropics where soils are typically poor in nutrition and competition between plants and microorganisms are very high. According to Takht and Zimmern (1996), the mistletoe plant belongs to the kingdom plantae, division Magnoliophyta, subdivision Magnoliophytina, class Rosopsida, subclass Rosidae, superorder santalanae, order santales, family Loranthaceae and genus *Viscum*. However, the taxonomic identity of the genus *Viscum* has been controversial as most authorities claim that it belongs to the family Loranthaceae, while others placed it in the family Vicaceae (Engler, 1964; Nickrent and Musselman, 2004). Hutchinson and Dalziel (1958) stated that the African countryman does not necessarily differentiate one specie from another, thus group name mistletoe tend to be used and critical distinction where necessary is made not between species but between hosts identified as a prefix or suffix to the group name or even just by the host name.

Juss (1808) recorded 75 genera and over 900 species of Loranthaceae, according to him, is the largest family of the Santales. However, Barlow (1964) provided compelling evidence from cytology and biogeography that supported the concept of 2 independent families in contrast to Linnaeus who recognized just one genus Loranthus for the family. Shukla and Mistra (1979) stated that Loranthaceae is divided into 2 subfamilies: Loranthoidea and Viscoidea. Based on the differences between the 2 subfamilies especially in floral structures and mode of development, Engler (1964) elevated the status of the subfamilies Loranthaceae and Viscaceae.

Several variants of *Viscum* are used in Christmas decorations, they are also used medicinally to treat various forms of cancer. For example, recombinant mistletoe Lectin (rmL) has been used to treat ovaries cancer (Robert and Gorter, 2002). Viscotoxins extracted from *Viscum* have immunomodulatory effects. The plants are used to counter sorcery, treat skin diseases, stiffness, phlebitis, fractured limbs, rheumatism, cough and chest conditions.

The importance of histochemistry in solving critical biosystematic problems is as popular as the use of other markers. According to botanical literatures, the use of histochemical characters in taxonomic conclusions is now a common practice. For example, the presence of calcium oxalte crystals in various plant families have been reported by various scientists. Mbagwu and Edeoga (2006) reported that the size and shape of calcium oxalate crystals though variable in each species showed enough interspecific differences that may be used for taxonomic inferences in vigna species. This has been done in other groups of plants such as Dioscoreaceae, Edeoga and Okoli (1995). Icacinaceae, Heintzelman and Howard (1948) Nyctaginaceae, Edeoga and Ikem (2002) and Verbenaceae, Mathew and Shah (1984). Histochemical studies showed that calcium oxalate crystals exist in different forms such as raphides, styloids, variously shaped prisms, crystal sand and druses (Al-Rais *et al*., 1971). Scott (1941) investigated the distribution of calcium oxalate crystals in *Ricinus communis* and attributed this to the pattern

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of tissue differentiation and presence of other ergastic substances. Stebbins *et al.* (1972) also reported the occurrence of crystals in apple stems, petiole and fruit tissues. Okoli (1988) made similar observation in Cucurbitaceae.

The biosystematic importance and implications of histochemical features of ergastic substances, calcium oxalate crystals, nature of tannins and saponins have been investigated in various plant families such as Dioscoreaceae (Edeoga, 1991; Edeoga and Okoli, 1992, 1995). Commelinaceae (Edeoga and Ogbegbor, 1999). Leguminosae-Papilionoideae (Mbagwu and Edeoga, 2006). The contributions of the above scientists notwithstanding little or no information is available for the genus *Viscum* on histochemical studies hence the need for this investigation with the objectives of assessing the importance of and discussing the extent to which histochemical attributes could be utilized in the systematic consideration of five variants of he genus *Viscum*.

MATERIALS AND METHODS

Mature and fleshy parts (leaves and stems) of the five variants of *Viscum* were collected from samples from different parts of Imo state Nigeria. This investigation was conducted at the science laboratory of the Department of Plant Science and Biotechnology, Imo State University, Owerri, Nigeria in July 2007. These specimens were initially fixed in FAA (1:1:18) glacial acetic: 40% formaldehyde: 70% ethanol ($v\ v^{-1}$) for 48-72 h. The 48-72 h. The fixed materials were later sectioned using a Reichert rotary microtome at 26 µm following a slight modification of Cutler (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) were then taken from the slides using a Leitz Wetzler Ortholux microscope fitted with vivitar-V-335 camera.

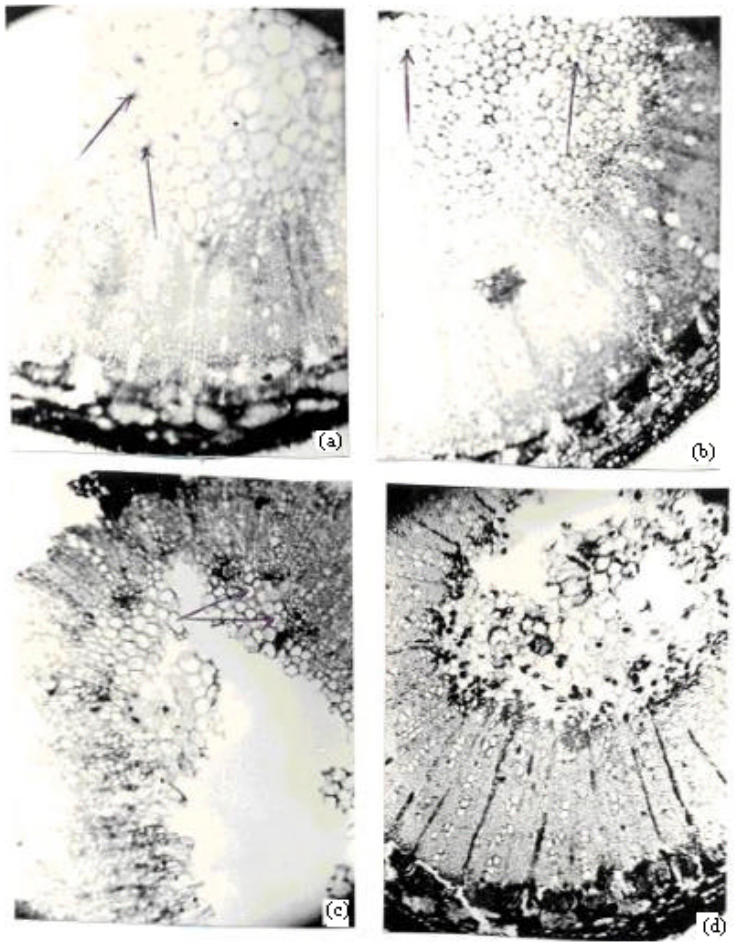

Fig. 1: TS of leaf, a): Variant A with circular crystals, b): Variant B with rectangular and circular crystals, c): Variant C with circular crystals and d): Variant D with rectangular and circular crystals (× 1000)

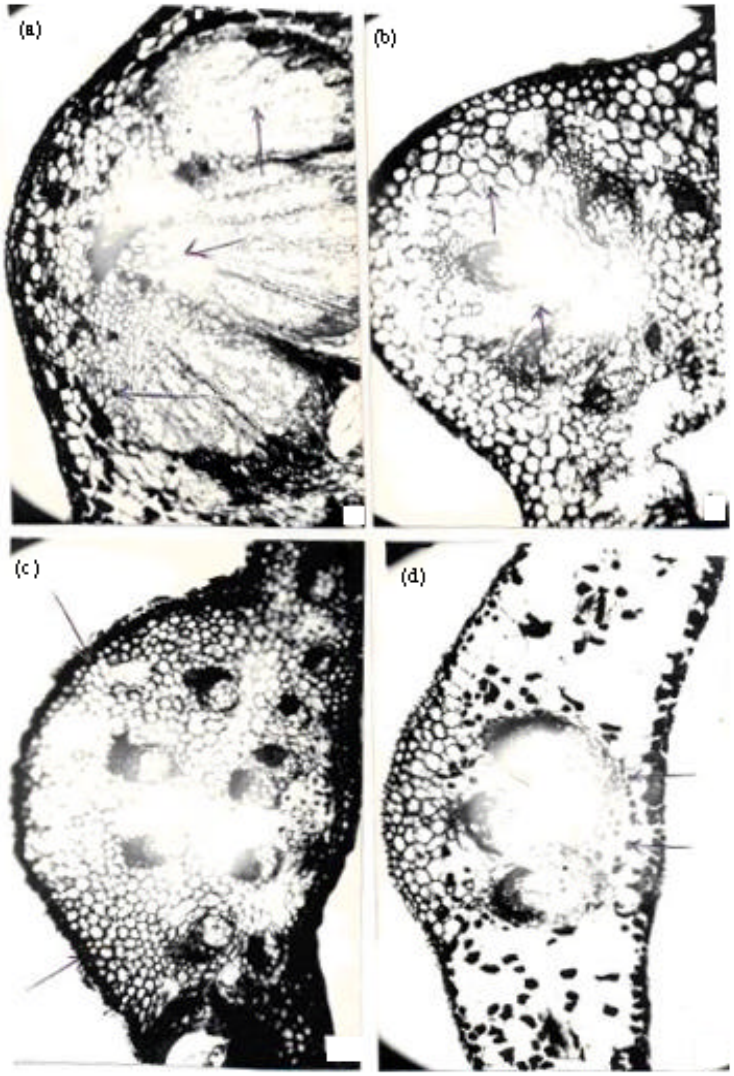

Fig. 2: TS of stem showing crystal sand crystals in all the variants

RESULTS

This investigation showed an important observation on these 5 variants with the presence of 3 different shapes of calcium oxalate crystals namely: Crystal sand, circular and rectangular crystals with crystal sand crystal appearing in the stem of all the 5 variants (Table 1 and Fig. 1). This result also showed that variant a posses circular crystals at the leaf and crystal sand at the stem, variant B possess rectangular and circular crystals at the leaf and crystal sand at the stem, variant C possess circular crystals at the leaf and crystal sand at the stem, variant D possess rectangular and circular crystals at the leaf and crystal sand at the stem and finally variant E possess rectangular and circular crystals at the leaf and crystal sand at the stem (Table 1 and Fig. 1). Another interesting aspect of the result is the appearance of starch grains inside the chloroplast in the leaves of all the variants (Fig. 2).

Table 1: Types of crystals found in the *Viscum* variants investigated

Plant parts	Variant A	B	C	D	E
Leaf	Circular	Rectangular and circular	Circular	Rectangular and circular	Rectangular and circular
Stem	Crystal sand	Crystal sand	Crystal sand	Crystal sand	Crystal sand

DISCUSSION

From these studies, the distribution and shape of the crystals among the investigated variants displayed

interspecific differences and similarities that are useful for the systematic grouping of these variants. The occurrence of crystal sand crystals in the stem of all the variants showed resemblance among the variants and reasons for all to be in the same genus. This study also, showed that variant A and C are more closely related just as variants B, D and E are also related. This is justified by the presence of the same type of crystals in those variants. So the use of crystals in solving taxonomic problems is not strange since some other workers have applied it in some plant families such as Mbagwu and Edeoga (2006) in Leguminosae-Papilionoideae; Edeoga and Okoli (1992, 1995) in Dioscoreaceae. The occurrence of these crystals within the leaves and stems of these variants is important to the role that these parts play in plant physiological processes. According to Mbagwu and Edeoga (2006), Edeoga and Okoli (1992, 1995) and Edeoga and Ugbo (1997), these ergastic substances could have nutritional, mechanical and transport roles in plants. These workers pointed that a close association of calcium oxalate crystals with the site of photosynthesis suggest that these substances could be involved in the synthesis of sugars.

CONCLUSION

This study is therefore, based on the hypothesis that histochemical studies have played a major role in establishing intervariant relationships among the variants but not enough to raise these variants to specie status.

REFERENCES

Al-Rais, A.H., A. Meyer and L. Waton, 1971. The isolation and properties of oxalate crystals from plants. Ann. Bot., 35: 1213-1218.

Barlow, B.A., 1964. Classification of Loranthaceae and Viscaceae. Proc. Linn. Soc. N.S.W., 89: 268-272.

Cutler, D.F., 1978. Applied Plant Anatomy. Longman, London, pp: 1-103.

Edeoga, H.O. and I.C. Ikem, 2002. Tannins, saponins and calcium oxalate crystals from Nigerian species of *Boerhavia* L. (Nyctaginaceae). South Afr. J. Bot., 68: 386-388.

Edeoga, H.O., 1991. Taxonomic studies on certain species of *Dioscorea* L. (Dioscoreaceae) in Eastern Nigeria. Ph.D Dissertation, University of Port Harcourt, Nigeria.

Edeoga, H.O. and B.E. Okoli, 1995. Histochemical studies in the leaves of some *Dioscorea L.* (Dioscoreaceae) and the taxonomic importance. Feddes Report, 106: 113-120.

Edeoga, H.O. and B.E. Okoli, 1992. Ergastic substances: Distribution in certain species of *Dioscorea* L. (Dioscoreaceae) and their taxonomic importance. J. Exp. Applied Biol., 4: 65-75.

Edeoga, H.O. and N.O. Ogbegbor, 1999. Distribution of calcium oxalate crystals in some Nigerian species of *Aneilema* R. Br. (Commelinaceae). Plant Biosys., 133: 193-198.

Edeoga, H.O. and H.N. Ugbo, 1997. Histochemical localization of calcium oxalate crystals in the leaf epidermis of some *Commelinai* (Commelinaceae) and its bearing on taxonomy. Acta phytotax. Geobot., 48: 23-30.

Engler, G.A., 1 964. Syllabus der Pflanzen Familien. 12th Edn. Revised. In: Melchior, H. (Ed.). Gebriider Borntraeger, Berlin, 2: 540-555.

Heintzelman, C.E. and R.A. Howard, 1948. The comparative morphology of the Icaciraceae and the pubescence crystals. Am. J. Bot., 35: 45-52.

Hutchinson, J. and M.J. Dalziel, 1958. Flora of West Tropical Africa. 2nd Edn. Crown Agents for Overseas Governments and Administrations, London, 1 (2): 144-154.

Juss, E.O., 1808. Classification of phylogenetique des Angiosperms cet article ou cette section necessittent, d'etre traduirs. Lentierment, ou non depuis l'article A en angulais.

Mbagwu, F.N. and H.O. Edeoga, 2006. Histo-chemical studies on some Nigerian species of *Vigna savi* (Leguminosae-Papilionoideae). J. Agron., 5 (4): 605-608.

Mathew, L. and G.L. Shah, 1984. Crystals and their taxonomic significance in some verbernaceae. Bot. J. Linn. Soc., 88: 279-289.

Nickrent, D.L. and L.J. Musselman, 2004. Introduction to parasitic flowering plants. The Plant Health Instructor, 13: 300-315.

Okoli, B.E., 1988. On the probable function and taxonomic value of calcium oxalate crystals in curcurbitaceae. Feddes Report, 99: 139-142.

Robert, W. and M.D. Gorter, 2002. Mistletoe extracts and cancer therapy. Washington Press, pp: 500-550.

Scott, F.M., 1941. Distribution of calcium oxalate crystals in *Ricinus communis* in relation to the tissue differentiation and presence of other ergastic substances. Bot. Gaz., 103: 225-246.

Stebbins, R.I., G.H. Dewey and V.E. Shull, 1972. Calcium crystals in apple stem, petiole and fruit tissue. Hortic. Sci., 7: 492-493.

Shukla, P. and S.P. Mistra, 1979. An introduction to the taxonomy of Angiosperms. New Delhii. Vikas, pp: 447-448.

Takht, L. and B. Zimmern, 1996. Angiosperms. Reveal, Phytologia, 4: 70-79.