Leaf Anatomical Characteristics Of Five Variants Of The Genus Viscum L. (Loranthaceae)

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Abstract: Leaf anatomical characteristics of five variants of five variants (A-E) of the genus *Viscum* (Loranthaceae) were investigated in this study to ascertain the usefulness of these characters and determine the intervariant relationships among the investigated variants. The anatomical features of the leaves showed that these variants possess useful biosystematic characters that can be used to establish intervariant relationships among investigated variants. An interesting aspect of this study is the presence of calcium oxalate crystals inside the chloroplast of variant A, C and D which differ from the usual localization of crystals in the mesophyll of leaves. Also the presence of uniseriate epidermal cells observed in variants B and C which differ from the multiseriate epidermal cells in A, D, and E coupled with the sunken stomata that characterized both variants are discussed in relation to their biosystematic significance. [Academia Arena, 2009;1(5):1-4]. ISSN 1553-992X.

Key words: Leaf, anatomy, characters, Viscum, variants, Loranthaceae

INTRODUCTION

The African mistletoes are parasitic plants that derive all or most of their nutrition from other flowering plants. Although many parasitic plants contain functional chlorophyll, they depend on their host plants for some of their carbon and other requirements. According to Takht and Zimmera (1996), the mistletoe plant belongs to the kingdom Plantae, division Magnoliophyta, class Rosopsida, order santales, genus Viscum and family Loranthaceae. However, Hutchinson and Dalziel (1958), placed the genus Viscum into the family Viscaceae. 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 to the family Viscaceae (Engler 1964, Nikrent and Musselman, 2004). Hutchinson and Dalziel (1958) stated that the African countryman does not necessarily differentiate one species 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.

Extracts from the genus *Viscum* impact both positively and negatively on human activities. For example, Recombinant Mistletoe lectin (rml) has been used to treat ovarian cancer (Robert and Gorter, 2002). Viscotoxins extracted from *Viscum* have immunomodulatory effects. In Africa, Mistletoes have magical and fetish values as their uses are primarily for illness thought to be of mystic origin. Their general uses include counter sorcery, mental conditions, fatigue, sterility and problems of urinogenital system. The leaves are used for treating skin diseases, stiffness, phlebitis, fractured limbs and rheumatism. This work is based on the hypothesis that the variations in this leaf anatomy are significant and revealed that leaf anatomy possess many attributes of potential taxonomic importance that are diagnostic at the genus and species levels (Mbagwu and Edeoga, 2006; Edeoga and Okoli, 2001, Nwachukwu and Mbagwu, 2007).

Although the usefulness of utilizing vegetative and anatomical features in the biosystematic considerations of various taxa have been reported (Edeoga and Okoli, 1998; Edeoga and Eboka, 2000; Edeoga and Ikem, 2001), there is no specific investigation conducted on the anatomical features of the leaves of these *Viscum* variants hence this paper reports the anatomical characters of the leaves of five variants of *Viscum*. It assesses the relevance of and discusses the extent to which leaf anatomical features might be utilized in biosystematic consideration of these *Viscum* variants.

MATERIALS AND METHODS

Fresh Leaves from the five variants of the Viscum species were collected from the Agricultural Garden of Imo State University, Owerri, Nigeria. This investigation was conducted at the Crop Science laboratory at University of Nigeria, Nsukka in January, 2007. The most healthy roots were collected and fixed in FAA (1:1:18) glacial acetic acid: 40% formaldehyde: 70% ethanol (v/v) for 48-72 hours. The roots were washed several times in distilled water then with two changes of 30% ethanol and dehydrated in the order 30%-50%-70%-95%-absolute alcohol. To infilterate wax into the specimens, they were placed for 3

hours in each of the following solutions containing a ratio of absolute alcohol to pure chloroform (v/v: 3:1, 1;1, 1;3) and then pure chloroform. At the stage of pure chloroform, wax pellets at 60°c melting point were added and the wax changed with new ones at intervals. The specimens were left in the oven for 2-7 days to remove the chloroform. To embed in wax, the contents of the vials were transferred into moulds and the specimens kept in place with hot needles. As the wax solidified, it was transferred to a cold water bath for hardening and later stored for two days in a refrigerator.

For sectioning, a reichert rotary microtome was used and 10-20 mm thick sections were made. The ribbons were placed on clean slides smeared with a thin film of Haupt's albumen, allowed to dry and drops of water added prior to mounting. The slides were placed on a hot plate at 40°c for few minutes for the ribbons to expand and were stored overnight. The slides were immersed in pure xylene for 2-5 minutes in a solution of xylene and absolute alcohol with 1;1 ratio (v/v) for few minutes. The slides were then transferred to another solution of xylene and alcohol in the ration 1:3 (v/v) for few minutes, to 95%, 90%, 70% and 50% alcohol. Drops of alcian blue were added on the specimens, washed off with water and counterstrained with safranin for two minutes, then dehydrated in 50% alcohol, 70%, 80%, 90% xylene/alcohol solution and mounted in Canada balsam. The slides were dried on a hot plate at 30°c. Then photomicrographs of the specimens were taken from the permanent slides (Figs 1 and 2) using a Leitz Wetzler ortholux microscope fitted vivitar-V-335 camera. (Cutler, 1978).

RESULTS

The results showed that in variant A, the vascular bundles are not well developed. The epidermal cells are multiseriate. The central cells are with dark stained content believed to be stains of oxalate crystals (fig 1a). in variant B, the vascular bundles are well developed, about 3-5 arranged to form a ring with distinct xylem and phloem cells. The epidermal cells are uniseriate and characterized by well developed parenchyma and sclerenchyma cells(fig 1b). in variant C, the vascular bundles are well developed, 8-10 arranged to form a ring pattern within the cortex. The epidermal cells are uniseriate. There are also presence of oxalte crystals (fig 1c). in Variant D, there are presence of 2-3 distinct and well developed vascular bundles. These are stains of oxalate crystals and the spongy mesophyll are confined at the center of the lamina (fig 1d). in variant E, there is one large vascular bundles at the center of the cortex. Sinkers are lignified, projecting from the cortex into the central cylinder. The epidermal cells are 4-6 layers thick. There are presence of circular and crystal sand crystals scattered within the cortex (fig 1e). Both variants are characterized by sunken stomata and presence of starch grains inside stomata. (fig 1 a-e).

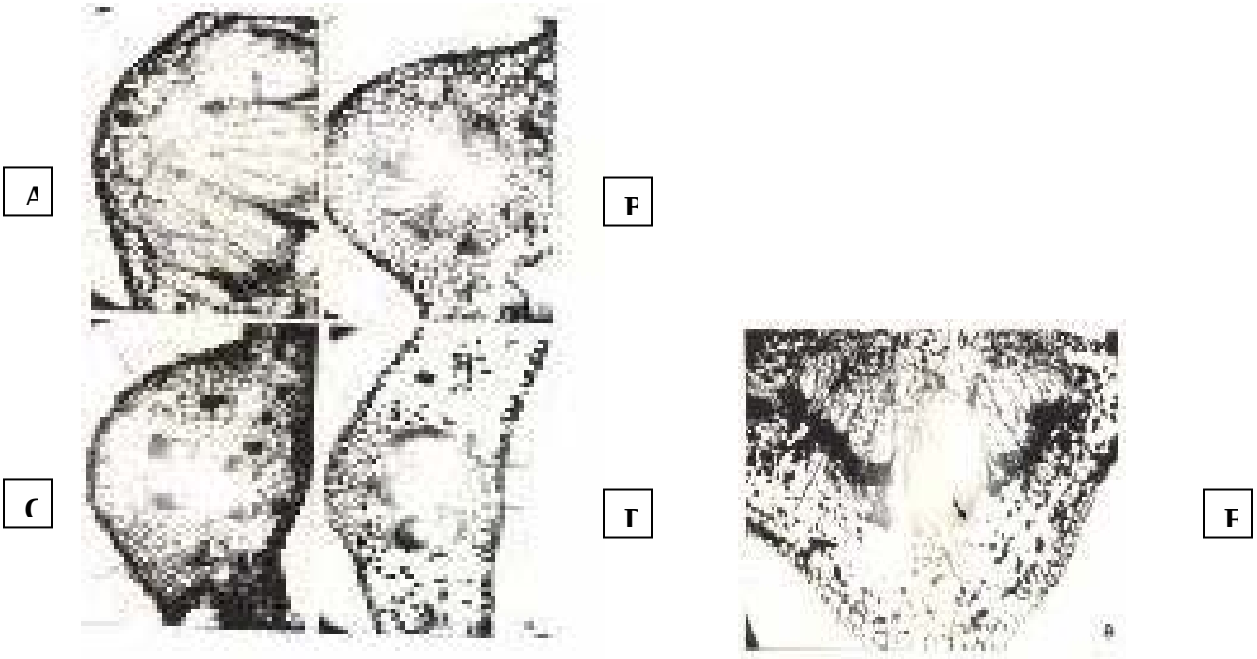

Figure 1. Both variants are characterized by sunken stomata and presence of starch grains inside stomata

DISCUSSION

The presence of calcium oxalate crystals inside the chloroplast of variants A, C and D is a new observation because crystals are usually observed in the mesophylls of leaves and not in the chloroplasts. This localization of crystals inside the chlorplasts of these three variants is a good taxonomic character that can be exploited to didtinguish these three variants from the other two hence there is an outstanding intervariant relationships among these three variants. Again, the presence of sunken stomata in both variants could be an ecological advantage that enables these variants to regulate water loss. This observation is in agreement with the work of Mbagwu and Edeoga (2006) who observed starch grians inside the chloroplasts of some *Vigna* species and used it to delimit these taxa.

Also the uniseriate epidermal cells as observed in Variant B and C compared to the multiseriate epidermal cells in other variants is distinct and serves as a good biosystematic character. Therefore, the use of anatomical features in systematic considerations of different taxa is no more a rare event by taxonomists. The work of Mbagwu and Edeoga (2006) in *Vigna* species, Nwachukwu and Mbagwu (2007) in Indigofera species, Mbagwu and Edeoga (2006) in the roots of some vigna species are classified examples. Although the differences in these variants are not enough to upgrade these variants into species rather the similarities in anatomical features showed reasons for both to be in the same genus *Viscum.* The overall findings support the principles, relationships and generalizations of other scientists that leaf anatomical features are useful tools in systematic botany.

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