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Leaf Anatomy of Some Nigerian Species of *Vigna* Savi (Leguminosae – Papilionoideae)

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Abstract: Leaf anatomical studies of eight *Vigna* species namely *V. ambacensis, V. gracillis, V. racemosa, V. reticulata, V. subterranea, V. triloba, V. unguiculata* and *V. vexillata* are reported in this study. Anatomical features of the leaves showed that these taxa possess useful taxonomic characters that can be used to establish interspecific relationships among the investigated taxa. An interesting aspect of the leaf anatomy is the presence of crystals inside the chloroplasts of *V. vexillata, V. unguiculata, V. ambacensis, V. racemosa* and *V. subterranea* which is different from the usual localization of crystals in the mesophylls of leaves. The presence of calcium oxalate crystals inside the chloroplasts of some of the *Vigna* species investigated is discussed in relation of their taxonomic significance.

Key words: Anatomy, leaf, crystals, chloroplasts, *Vigna,* taxonomy

INTRODUCTION

The genus *Vigna*s savi belongs to the dicotyledonous family, Leguminosae – Papilionoideae of the order Leguminales and tribe Phaseoleae. The family are mostly herbs but include also shrubs and trees[1]. They comprise one of the largest families of flowering plants, numbering some 400 genera and 10,000 species[2]. They are dicotyledonous plants bearing pods with one or more seeds whose pods dehisce along both dorsal and ventral sutures[3]. This family can be identified by the shape of their leaves and by structures called stipules. Their leaves, apart from being stipulate, are nearly always alternate, and range from pinnately or palmately compound to simple. Majority of them are normal plants while others are switch – plants with the principal photosynthesizing functions transferred to stems and leaves[4]. In recent years it has been observed that there is discrepancy in the number of species recognized by different authorities. Daniel[5], recognized 37 species, Hutchinson and Dalziel[6] recognized 25 species while Burkill[7] recognized 22 species. The reason for the contradictions in estimation of the number of taxa in these groups of plants is due to the perceived similarities in structural and reproductive biology.

The usefulness of utilizing vegetative anatomical features in the taxanomic and systematic consideration of different taxa has been reported[8-11]. In spite of the availability of these studies, no specific investigation has been conducted on the anatomical features of the leaf of *Vigna* sp. especially as it relates to the interrelationships of the *Vigna* sp.

This paper therefore reports the anatomical characters of the leaves of eight species of *Vigna* as observed with a light microscope. It assesses the relevance of, and discusses the extent to which, leave anatomical features might be utilized in the systematic consideration of the eight *Vigna* sp. in view of their perceived similarities in structural and reproductive biology.

MATERIALS AND METHODS

Mature and fresh leaves of the *Vigna* sp. were obtained from living samples collected from different parts of Eastern Nigeria. Sections of 26mm thick prepared from the leaves were fixed in FAA (1 : 1 : 18) glacial acetic acid: 40% formaldehyde: 70% ethanol (V/V) for 48 – 72 hours. These were then rinsed in several changes of distilled water and passed through alcohol series (30, 50, 70, 95 and 100%). The delydrated materials were infiltrated with wax by passing through different proportions of alcohol and chloroform (3 : 1, 1 : 1, 1 : 3 V/V). As the chloroform and wax gradually replaced the alcohol, pure chloroform and wax were put in the bottles to gradually infiltrate the tissue with wax which would be hard enough for microtomy.

The bottles were left on a hot plate (37 – 40°C) for 24 h before transferring to the oven (58 – 60°C). This step was designed to evaporate the chloroform. The wax having reached its melting point completely infiltrated the tissues in it. After a period of 2 – 3 days with constant addition of wax the specimens were embedded in paraffin melted wax. This was accomplished by a quick orientation

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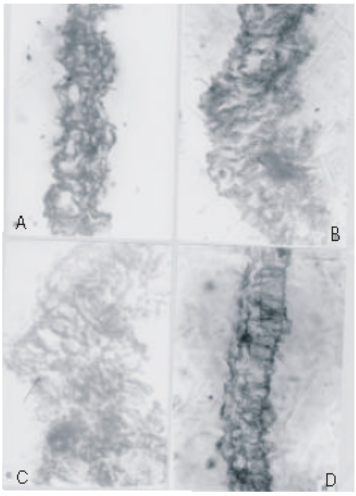

Fig. 1(a-d): T.S of Leaves of *V. vexillata*, *V. unguiculata*, *V.* racemosa and *V. subterranea*.

a: *V. vexillata* with calcium oxalate crystals inside the chloroplasts (arrowed)
b: *V. unguiculata* with calcium oxalate crystals inside the chloroplasts (arrowed)
c: *V. racemosa* with calcium oxalate crystals inside the chloroplasts (arrowed)
d: *V. subterranea* with calcium oxalate crystals inside the chloroplasts (arrowed)
(X 100)

of the specimens in the mould with a hot mounted needle and forceps and quick cooling on ice – block. The metal moulds were later trimmed and sectioned on Reichert rotary microtome at 20 -24 µm following a slightly modified method of Cutler[12].

The ribbons were placed on clean slides smeared with a thin film of Haupt's albumen and allowed to dry and drops of water added prior to mounting. The slides were placed on a hot plate at 40°C for a few minutes to allow the ribbons to expand and were stored overnight. The slides were immersed in pure xylene for 2-5 min in a solution of xylene and absolute alcohol with 1 : 1 ratio (V/V) for 5 minutes. The slides were then transferred to another solution of xylene and alcohol in the ratio 1 : 3 (V/V) for 5 min to 95, 90, 70 and 50% alcohol. Drops of alcian blue were added to the specimens for 5 minutes, washed off with water and counter – stained with safranin for 2 minutes, then dehydrated in a series of alcohol 50, 70, 80, 90% xylene / absolute alcohol solution (i.e. 1: 3 and 1: 1 V/V) and pure xylene at intervals of few seconds and mounted in Canada balsam. Photomicrographs of the specimens were taken from the permanent slides (Figs. 1a-d), using a Leitz Wetzler ortholux microscope fitted with vivitar -V- 335 camera.

RESULTS

The results of this investigation showed that *V. ambacensis* has large central cells with dark stained contents which are stains of the oxalate and the epidermal walls are uniseriate and sinuous. *V. gracillis* has sunken stomata and the spongy mesophyll is confined to the centre of the lamina with the wall architecture of the upper epidermal layer regular. In *V.* racemosa, the inner sheath is represented by thickwalled parenchymatous cells that are uniseriate and the chloroplast prevent the nature of the crystals (Fig. 1c). The wall architecture of the upper epidermal layer is regular with multiseriate cells in *V. reticulata* while in *V. subterranea*, the vascular bundles are composed of 1 – 3 tracheids and few phloem elements (Fig. 1d). In *V. triloba,* the vascular bundles are prominent with 4 – 6 layers of cells that are irregular in shape. An interesting aspect of this result is the presence of calcium oxalate crystals inside the chloroplasts of *V. vexillata, V. unguiculata, V. ambacensis, V. racemosa* and *V. subterranea* (Fig. 1a- d) which is reported for the first time and differ from the usual localization of crystals in the mesophylls of leaves.

DISCUSSION

The presence of calcium oxalate crystals inside the chloroplasts of *V. vexillata, V. unguiculata, V. ambacensis, V. recemosa* and *V. subterranea* (Fig. 1) is a new observation in these groups of plants. This is because crystals are usually observed in the mesophylls of leaves and not the chloroplasts. This localization of crystals inside the chloroplasts among these taxa is a good taxonomic character that can distinguish these taxa from the rest hence there is an interspecific relationships among these taxa. The presence of sunken stomata in *V. gracillis* could be an ecological advantage that enables this taxon to regulate its water loss.

Therefore the use of anatomical features in systematic consideration of different taxa is no more a rare event by different authors. The work of Anyensu[13] in Dioscoreaceae, Gibson[14] in Anacardiaceae, Decadas and Beck[15] in Rosaceae and Leguminosae, Heo[16] in Monimiaceae, Metcalfe and Chalk[8] in Diotyledons as a whole, Tomlinson[8] in Commelinales and Zingiberales are classical examples. The similarities in anatomical structures in these *Vigna* species showed reasons for these taxa being in the same genus while the differences indicate no relationship and reasons for the taxa being in different species.

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