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154Leaf Anatomy of Eight Species of *Indigofera* L.

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Abstract: Leaf anatomy of eight species of *Indigofera* of the tribe Galegeae was studied from Eastern Nigeria with the aid of light microscope. This is with a view of establishing taxonomic relationships among the species. Results from the investigation show that variation in leaf anatomical attributes were so obvious that they could be added to other taxanomic information and used in an attempt to determine the taxanomic importance of leaf anatomy. Taxonomically significant characters among the taxa investigated include the variation in shape of the epidermal cells-mostly rectangular and sinuous in *I. pulchra*, *I. senegelensis* and *I. stenophylla* while it varies from pentagonal in *I. paniculata*, *I. prieureana* and *I. terminalis* through hexagonal in *I. hirusta* and *I. ticntoria*. Other anatomical characters of the leaf epidermis that exhibit variation include number of palisade layer: 1-2 layers in *I. pulchra*, *I. senegalensis* and *I. stenophylla* to 4-5 layers in *I. prieureana* and *I. tintoria*, nature of epidermal cells: tubular in *I. hirsuta*, *I. prieureana* and *I. senegelensis* straight in *I. paniculata* and *I. tinctoria* while it is wavy in *I. stenophylla* and *I. terminalis*. Type of crystal present in the leaf epidermis is also variable among the taxa: raphides in *I. paniculata* and *I. tinctoria* but tanninferous bags in *I. senegelensis*. The biosystematic implications of these findings have been discussed in the light of current literature.

Key words: Anatomy, *indigofera,* leaf, leguminoseae, papilionoideae, taxonomy

INTRODUCTION

The *Indigofera L.* is one of the largest genus in the family Leguminosae-Papilionoideae. According to the taxonomic systems of Willis[1], there are 700 species of this genus which are found in the warm tropical and subtropical regions of the world. Bentham and Hooker[2],considered the order *Leguminales* as a whole to constitute one family Leguminosae among the dicotyledons with nine tribes. One of the tribes is Galegeae which consist of nine genera one of which is the genus *Indigofera.* On a global scale the genus *Indigofera* was further sub divided by Hutchinson and Daziel[3], into five sub genera. Hutchinson and Dalziel[3], further divided the sub genus *Indigofera* into three sections namely *Latestipulata, Paniculata* and *Indigofera*. The section *Indigofera* was further sub divided into seven sub sections Hutchinson and Daziel[3].

In west Africa Burkill[4], recognised 60 species of *Indigofera* while Hutchinson and Dalziel[3], identified 78 species two of which *I. heterocarpa* Welw.ex Bak and *I. variabilis* Berhaut are imperfectly known species. The reason for this confusion and discrepancies in estimation of the number of taxa in this group of plant could be due to the perceived similarities in structural and reproductive biology of these legumes. In Eastern Nigeria eight commonly occurring species of *Indigofera* were identified. These are *I. hirsuta* L. *I. paniculata Guill et Perr*, *I. prieureana Guill et Perr*, *I. pulchra Wild*, *I. senegelensis Lam. I. stenophylla Guill, Perr. I. terminalis Baker* and *I. tinctoria* L.

Indigofera species have been found to possess wide range of uses to mankind ranging from medicinal, ornamental, feed for livestock and as dye for commercial purposes. Furthermore, the crushed leaves are useful against skin itching. The fruit is reported to be used in Borno (Northern Nigeria) for ophthalmices[4,5]. A root decoction is applied in most parts of Nigeria to counteract various poisons[4].

Indigofera species are creeping, prostrate or erect annual, biennual or perennial herb or semi woody undershurbs and trees[6]. Stem may be slender, erect or conspicuously branched with bright green or tinged with red colour. The leaves are mostly alternate openly spaced on the stems around 10 cm long and may be roughly or velvety smooth to the touch[5]. The fruit is a pod usually smooth, reddish brown and cylindrical with 2-15 seeds in most species[7-10], The variation in the leaf anatomy is

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significant and showed that leaf anatomy possess many attributes of potential taxonomic importance that are diagnostic at the genus and species levels[11-13]. The contribution on the leaf anatomy of plants of various areas include those of[13,14]. Further contribution of anatomical features in systematic can be seen in the works of Gibson[15], in Anacardiaceae, Devadas and Beek[16], in Rosaceae and Leguminosae, Heo[17], in Monimiaceae, (Metcalfe and Chal[18], in Dicotyledons as a whole but no report specifically on the leaf anatomy of *Indigofera* species has been documented.

This study therefore reports the leaf anatomical attributes in eight species of *Indigofera* as observed with a light microscope. This study assesses the relevance of and discusses the extent to which the different leaf anatomical attributes might be utilised in the systematic consideration of some *Indigofera* species in view of many suggestions on this direction by different authors[3,4].

MATERIALS AND METHODS

Sections of mature and fresh leaves not longer than 1×0.5 cm of each of the eight species of *Indigofera* were put into labelled vials and fixed in FAA 1: 1: 18 40% formaldehyde: Glacial acetic acid: 70% ethanol (v/v) for 48-72 h. These were then rinsed in several changes of distilled water and passed through alcohol series (30, 50, 95 and 100%). The dehydrated materials were infiltrated with wax by passing through different proportions of alcohol and chloroform gradually replaced the alcohol, pure chloroform and wax were added in the bottles. The idea was to gradually infiltrate the tissues with wax which would be hard enough to microtoming. The bottles were then left on a hot plate (37-4°C) for 24 h before being transferred to the oven (58-60°C). This step was designed to evaporate the chloroform. The wax having reached its melting point completely infiltrated the tissue in it. After a period of 2-3 days with constant addition of wax by the use of metal moulds and melted wax. The moulds were later removed and the specimens with the wax cube were trimmed and sectioned using a Reichart rotary microtome at 20-24 μm.

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 min to allow ribbons to expand and were stored over night. The slides were then transferred to another solution of xylene and alcohol in the ratio 1: 3 (v/v) for a few minutes, to 95, 90, 70 and 50% alcohol. Drops of alcian blue were put on the specimens for 5 min, washed off with water and counter-stained with Safranin for two 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 a few seconds and mounted in Canada balsam. Photographs were taken using a Leitz Wetzlar Ortholux microscope fitter with a vivitar v-335 camera.

RESULTS

Anatomical features of the eight species of *Indigofera* studied are summarised in Table 1 and illustrated in (Fig. 1a-d and 2a-d). The pattern of the upper leaf epidermal wall in the taxa studied is highly variable in outline and slightly different from those of the lower epidermal cells that are less distinguishable. The cell wall pattern of the upper epidermis of *I. pulchra, I. senegalensis* and *I. stenophylla* (Fig 1d and 2a-b) is rectangular and sinuous, but pentagonal cells are found in *I. paniculata, I. prieureana* and *I. terminalis* (Fig. 1b,c and 2c) while it is hexagonal in *I. hirsuta* and *I. tinctoria* (Fig. 1a and 2d). The number of palisade mesophyll layer in the species studied is also variable. It is 4-5 layers in *I. prieureana* and *I. tinctoria* (Fig. 1c and 2d) but 1-2 layers in *I. pulchra*,(Fig. 1d) *I. senegelensis* (Fig. 2a) and *I. stenophylla* (Fig. 2b). This result is significant since this character could be used to separate these taxa into groups of closer affinity.

The nature of the epidermal cells show that it is tubular and are arranged in regular row extending length wise of the leaf in *I. hirsuta, I. prieureana , I. pulchra* and *I. senegelensis* (Fig. 1a-d and 2a) but straight in *I. paniculata* and *I. tinctoria* (Fig. 1b and 2d) while it is wavy in *I. stenophylla* and *I. terminalis* (Fig. 2b,c). Interesting differences are also found in the type and occurance of ergastic substances in the leaf of these *Indigofera* species. It appears as raphides in *I. paniculata* (Fig. 1b) and *I. tinctoria* (Fig. 2d) while in *I. pulchra*, *I. senegelensis* and *I. stenophylla* (Fig. 1d and 2a,b) the presence of crystal sands and tannin bag variously arranged (uniform in *I. senegelensis* scattered in *I. pulchra* and different sizes in *I. stenophylla*) on the palisade mesophyll layer was noticed. Table 1. This features reported for the first time are not common among other taxa studied hence it is remarkably diagnostic and could suggest affinity in these taxa. The stomatal characteristics of the eight *Indigofera* species studied are also of interest. The eight species of the *Indigofera* were amphistomatic. Anomocytic stomata were observed on both surfaces of *I. paniculata*

Fig. 1: *I. hirsuta, I. paniculata, I. prieureana and I. pulchra. -I. hirsuta*: Showing presence of wavy epidermal layer x100. *-I. paniculata*: Note the stomata within the epidermis x100. *-I. prieureana*: Indicating section of leaf cells with intercellular spaces x100. *-I. pulchra*: Showing presence of scattered tannins and crystal sand x100.

(Fig. 1b) only while the presence of anomocytic and anisocytic types were was observed on the upper and lower layers, respectively of *I. senegalensis* (Fig. 2a). The stomatal arrangement in the other taxa were less distinguishable. In general the stomata are longer than they are wide in the species were the occurred. There were no noticeable trichomes in the leaf anatomy of the species studied.

DISCUSSION

The eight *Indigofera* species investigated exhibit a wide variety of leaf anatomical features especially in the upper leaf epidermis. The variation in the cell wall pattern show single layer rectangular and sinuous in *I. pulchra, I. senegalensis* and *I. stenophylla* (Fig. 1d and 2a,b). Pentagonal cells in *I. paniculata, I. prieureana* and *I. terminalis* (Fig. 1b,c and 2c) while it Is hexagonal in *I. hirsuta* and *I. tinctoria* (Fig.1a and 2d). These variations are distinct among the eight species investigated and could therefore be used to separate the species into three groups based on the epidermal cell wall pattern which expresses close affinity between the species. Further separation of these taxa could be done through a combination of other characters such as stomatal arrangement, nature of epidermal cells and

Fig. 2: *I. senegalensis, I. stenophylla, I. terminalis and I. tinctoria, -I. senegalensis*, Showing tanninferous bags uniformly arranged x100. *-I. stenophylla*, Note variation in cell wall pattern x100. *-I. terminalis:* Wavy nature of epidermal layer x100. *-I. tinctoria*, Note hexagonal shape of cell and presence of crystals

Table 1: Leaf anatomical features of indigofera species studied

Attributes	*I. hirsuta*	*I. paniculatal.*	*I. prieureana*	*I. pulchra*	*I. senegalensis*	*I. stenophylla*	*I. terminalis*	*I. tinctoria*
Upper Epidermis								
Cell wall shape	Single layer and mostly hexagonal	Single layer and mostly pentagonal	Single layer and pentagonal	Single layer Rectangular and sinuous	Rectangular and sinuous	Single rectangular and sinuous	Single layer and pentagonal	Single layer andhexagonal
Number of palisade mesophyll layer	Less distinguishable	Less distinguishable	4-5 layers	1-2 layers	1-2 layers	1-2 layers	Less distinguishable	4-5 layers
Nature of epidermal cell	Tubular	Straight	Tubular	Tubular	Tubular	Wavy	Wavy	Straight
Presence of ergastic substances	-	Raphides	-	Scattered tannins and crystal sand	tannins bags	tannins	-	Raphides and tannins
Presence of Stomata		Stomata present	-	-	Stomata present	-	-	-
Trichome	Not present	Not present	Not present	Not present	Not present	Not present	Not present	Not present

presence of ergastic substance Table 1. This present finding is in line with the earlier works of Edeoga and Eboka[19], in the genus *Dissotis,* Edeoga and Osawe[20], in some species of *Senna*, Arroya[21], in the

family Tecophilaceae, Ahmed[22], in Acanthaceae, Olowokudejo[23], in Annonaeae, Edeoga and Ikem [24];in Leguminosae-Caesalpinoideae and Billore and Singh[25].

The number of Palisade mesophyll observed in the species of *Indigofera* studied is also diagnostic. The palisade mesophyll is 1-2 layers in *I. pulchra, I. senegalensis* and *I. stenophylla* (Fig. 1d, 2a and b) while it is 4-5 layers in *I. prieureana* and *I. tinctora* (Fig. 1c and 2d). The 1-2 mesophyll layers in *I. pulchra, I. senegelensis* and *I. stenophylla* stands these taxa out among the other taxa thereby further strengthening the closer affinity between these three taxa. In all the eight species of *Indigofera* studied the palisade mesophyll were well developed and the cells more or less isodiametric. The variation in the number of cell layers of mesophyll are equally remarkable attributes of these taxa hence the possibility of exploiting them for taxonomic purposes in the genus *Indigofera* in therefore a right approach. The variations in the types of ergastic substances reported for the first time in these taxa are remarkable and diagnostic. This is because Metcalf and Chalk[18,26], Al-Rais *et al.*[27], who studied the anatomy of some Leguminous species reported the presence of crystals and ergastic substances other than raphides and tannins hence this character can be used in the delimitation of these taxa. The stomatal features in the eight species of *Indigofera* studied show anomocytic stomatal arrangement on both layers of the leaf epidermis in *I. paniculata* (Fig. 1b) while a mixture of anomocytic on the upper layer and anisocytic on the lower layer were found in *I. senegelensis* (Fig. 2a). The existence of variable stomatal type on both the upper and lower layers of these taxa are diagnostic and remarkable. This is in support of the works of earlier authors who observed the presence of variable stomatal types in different groups of angiosperm but not any member of the genus *Indigofera*. Such reports include those of Petal and Shah[28], in Solanaceae, Shah and Gopal , in[29] Dioscoreaceae, Olowokudejo and Sheteolu[30], in Lamiaceae, Edeoga and Eboka[19], in Melastomataceae, Edeoga and Ikem,[24] in Nyctaginaceae, Okeke and Nwachukwu[31], in euphorbiaceae. In general the stomata are longer than they are wide in all the species where they occurred. This is the usual situation in most plants and so it in line with earlier reports by most workers in other taxa[32].

There were no noticeable trichomes in the leaf anatomy of the eight species of *Indigofera* studied. Similarly the spongy mesophyll as well as the vascular bundle arrangement did not show any deviation from the regular dicotyledonous leaf pattern.

It is obvious that the eight species of *Indigofera* from the eastern part of Nigeria investigated have foliar attributes that could be exploited in order to improve their recognition. Hence the need to incorporate information from the leaf anatomy in systematic consideration of *Indigofera* species is therefore incontestable.

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