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THE STOMATAL TYPES IN *SESBANIA BISPINOSA* (JACQ.) W.F. WIGHT SEEDLINGS

D. Khan and M. Javed Zaki
Department of Botany, University of Karachi, Karachi-75270, Pakistan.

ABSTRACT

Seedling and stomata types in variously aged seedlings of *Sesbania bispinosa* (Jacq.) W.F. Wight are described. The seedling, according to Garwood's (1996) scheme of seedling classification, was Phanerocotylar-epigeal- foliaceous type – cotyledons enclosed in seed coat are raised above the soil due to rapidly growing hypocotyl and later the cotyledons coming out of seed coat and undergoing expansion. After Vogel (1980) seedlings of *Sesbania bispinosa* may be referred to as Macaranga type (*Macaranga hispida*).

There appeared a greater degree of stomatal diversity in *S. bispinosa* – relatively higher diversity in cotyledons than in the leaves. In all, the seedlings exhibited the occurrence of anisocytic, anisotricytic, anomocytic, tetracytic, staurocytic, and infrequently the paracytic types of stomata. There were a few desmocytic stomata on the epicotylar stem only. Abnormal stomata included the stomata with common subsidiary (ies), contiguous stomata (placed juxtaposed or the two stomata placed more or less at right angle) and deformed stoma with more than one pores and stoma with no guard cells. Totipotency of guard cell was noticed in a cotyledonary stoma - division to give rise a new cell. The hypocotyl and dorsal and ventral surfaces of three-day old cotyledons of *S. bispinosa* had epidermal cells with straight or curvy anticlinal walls but 7-day old cotyledons showed curvy to sinuous anticlinal walls. Leaves exhibited curvy to sinuous anticlinal walls. Undulations were U-shaped. On primary simple leaf of the seedling, waviness of the epidermal cells averaged to 6.34 ± 0.206 wave crests per cell varying from 3 to 10 (CV = 27.24%). The number of undulations per cell was larger in larger cells. At least in case of cotyledons, the degree of waviness appeared to be influenced by the age of the cotyledons as undulations in the contour of epidermal pavement cells were only observed in 7-day old mature cotyledons. Three-day old cotyledons exhibited no undulations in the pavement epidermal cells.

Key words: *Sesbania bispinosa* (Jacq). W. F. Wight, Seedling type, stomatal diversity of seedlings.

INTRODUCTION

Sesbania bispinosa (Jacq) W.F. Wight (vern. Dhaincha; Family Papilionaceae) is a fast-growing, rain-fed, annual and multipurpose legume distributed in tropical Africa and Asia. It is used as fodder of sheep, goats and cattle. Leaves are used in poultry feed (Orwa *et al.*, 2009). It is also green manure for rice and is tolerant to soil alkalinity up to pH:10 and growing well in water-logged or non-irrigated conditions. It has recently been described as a potential source of pulpable material for paper making (Sarkar *et al*., 2017).

Seedling morphology is a less explored but emerging domain in plant science (Paria, 2014) which documents the morphological characters and the changes that occur during development from early stages to adult (Fogliani *et al*, 2009). Leguminosae is a diverse and one of the large families of dicotyledons. There is a very long list of publications relating to seedling morphology and the epidermal (stomatal) diversity of leguminous plants (citing few, Shah and Gopal, 1969; Bleckman and Hull, 1975; Shah and Kothari, 1975; Kothari and Shah, 1975; Gill *et al.*, 1982; Monteiro, 1984; Dave and Bennet, 1989; Nyawuame and Gill, 1990; Chou and Liu, 1992; Farooqui *et al.*, 1999; Veasey *et al.*, 1999a and b; Parveen *et al.*, 2000; Mukherji *et al.*, 2000; Idu *et al*., 2000; Stenglein *et al.*, 2003; Agbagwa and Okoli, 2006; Zarinkumar, 2007; Edeoga *et al.*, 2008; Agbolade *et al.*, 2011; Tripathi and Mondal, 2012 a and b; Nemato and Ohashi, 2012; Adeniji and Airwaodo, 2012; Albert and Sharma, 2013; Khan *et al.*, 2014; Sanyal and Paria, 2014; Khan *et al.*, 2015a &b; Khan *et al.*, 2016; Khan and Sahito, 2017 a and b; Khan *et al.*, 2017; Lobo *et al.*, 2014; Sarwar *et al*., 2015; Shreelalitha *et al.*, 2015; Alege and Shaibu, 2015; Aziagba *et al.*, 2017; Zareh *et al.*, 2017; Khan *et al.*, 2019; Owolabi and Adedeji, 2018). However, only few studies have been published on genus *Sesbania* in particular – to say Veasey *et al.* (1999) investigated germination in *S. exasperate, S. grandiflora, S. sesban, S. tetraptera and S. virgata*, Sarwar *et al.* (2015) investigated *S. bispinosa, S. cannabina* and *S. rostrata* for morphological characterization and biomass production – not micromorphology of the species and Shreelalitha *et al.* (2015) studied germination of *S. bispinosa* and *S. speciosa.* Parab and Vaidya (2016) while describing Pharmacognostic profile of leaves of *S. bispinosa*, only described stomatal size and density and not the stomatal types.

In the present paper we undertake to describe salient seedling characteristics of *Sesbania bispinosa* (Jacq.) W.F. Wight)- and its surface micromorphology especially stomatal diversity on hypocotyl, epicotylar stem, cotyledon and primary and secondary leaves. It appears to be pertinent in view of the fact that seedlings related studies are not only important taxonomically but also from conservation and restoration viewpoint particularly in tropical dry forests (Khurana and Singh, 2001).

MATERIALS AND METHODS

The seeds of *S. bispinosa* were provided by Dr. Anam, Dept. Botany, University of Karachi. They were germinated without any dormancy-breaking treatment in pots filled with garden loam soil maintained at 75% water holding capacity. Maximum germination was 50-60% achieved within a week. The seedlings were studied, for their morphological characters including stomatal types. Seedlings type was described according to Vogel (1980) and Garwood (1996). Hickey (1973) and LWG (1999) were followed for description of leaf architecture. Leaf epidermal impressions were made with clear nail polish (Wang *et al.*, 2006). Stomatal nomenclature suggested by Prabhakar (2004), being simple and based upon structure of stomata and not their ontogenetic pathways, was adopted to describe stomatal types.

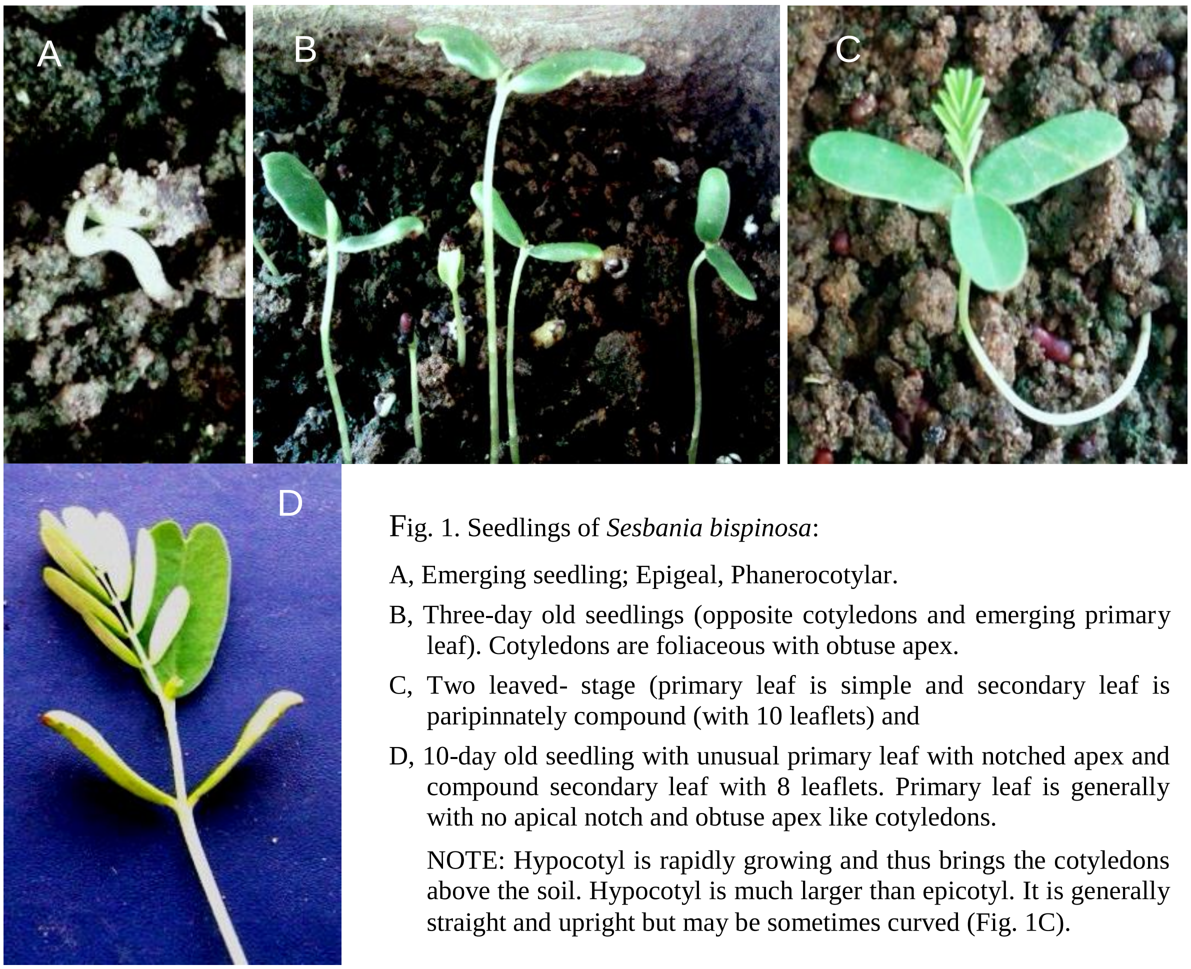


Fig. 1. Seedlings of *Sesbania bispinosa*:

A, Emerging seedling; Epigeal, Phanerocotylar.

B, Three-day old seedlings (opposite cotyledons and emerging primary leaf). Cotyledons are foliaceous with obtuse apex.

C, Two leaved- stage (primary leaf is simple and secondary leaf is paripinnately compound (with 10 leaflets) and

D, 10-day old seedling with unusual primary leaf with notched apex and compound secondary leaf with 8 leaflets. Primary leaf is generally with no apical notch and obtuse apex like cotyledons.

NOTE: Hypocotyl is rapidly growing and thus brings the cotyledons above the soil. Hypocotyl is much larger than epicotyl. It is generally straight and upright but may be sometimes curved (Fig. 1C).

RESULTS AND DISCUSSION

Seedling type

As per Garwood's (1996) scheme of seedling classification, the seedling of *S. bispinosa* was Phanerocotylar-epigeal- foliaceous type – cotyledons enclosed in seed coat are raised above the soil due to rapidly growing hypocotyl and later the cotyledons coming out of seed coat and undergoing expansion. Cotyledonary petiole small. They are green, photosynthesizing and foliaceous. Vogel (1980) puts seedlings of genus *Sesbania* in Macaranga type (*Macaranga hispida*) in his classification of dicotyledonous seedlings of Melesian taxa. He opines that Macaranga type is the most common type of seedlings seen in several families – Compositae, Cruciferae, Labiatae, Ranunculaceae, Rubiaceae, Umbelliferae etc. In this type cotyledons remain with the seedling for quite longer time. The cotyledons of *S. bispinosa* seedlings are opposite, sessile to subsessile and rounded at the apex and marginally entire (Fig. 1). They are glabrous, exstipulate, coriaceous. They are at times oriented obliquely with the axis, each forming an angle c 45^o with the axis (Fig. 1D).

The primary leaf is simple subsessile (petiole c 1mm in length), more or less like cotyledons in shape, wide elliptic in shape and obtuse (rounded) at the apex. Primary leaf may sometime be notched at the apex (Fig. 1D). Secondary leaf is unipinnately-paripinnately compound bearing 8-10 leaflets – small petiolate, leaflet blade obovateoblong, asymmetric, apex rounded, margin entire. The rachis is generally glabrous but sporadically some transparent delicate 1-2-celled trichomes may be seen (Fig. 2). The leaflet of secondary leaf may show, of course, rarely, a broad transparent and delicate multicellular acumen on the apex (Fig. 2). Some of the above-given characters resemble to those described by Sanyal and Paria (2014) for S. cannabina – now ranked as synonymy of S. *bispinosa*. Indeed, quite earlier Monteiro (1984) described genus *Sesbania* to be characterized with epigeal germination, long hypocotyl, short epicotyl, foliaceous cotyledons with very short petiole and escaping from seed coat while aboveground and second eophyll paripinnate and first eophyll is similar to cotyledon in shape.

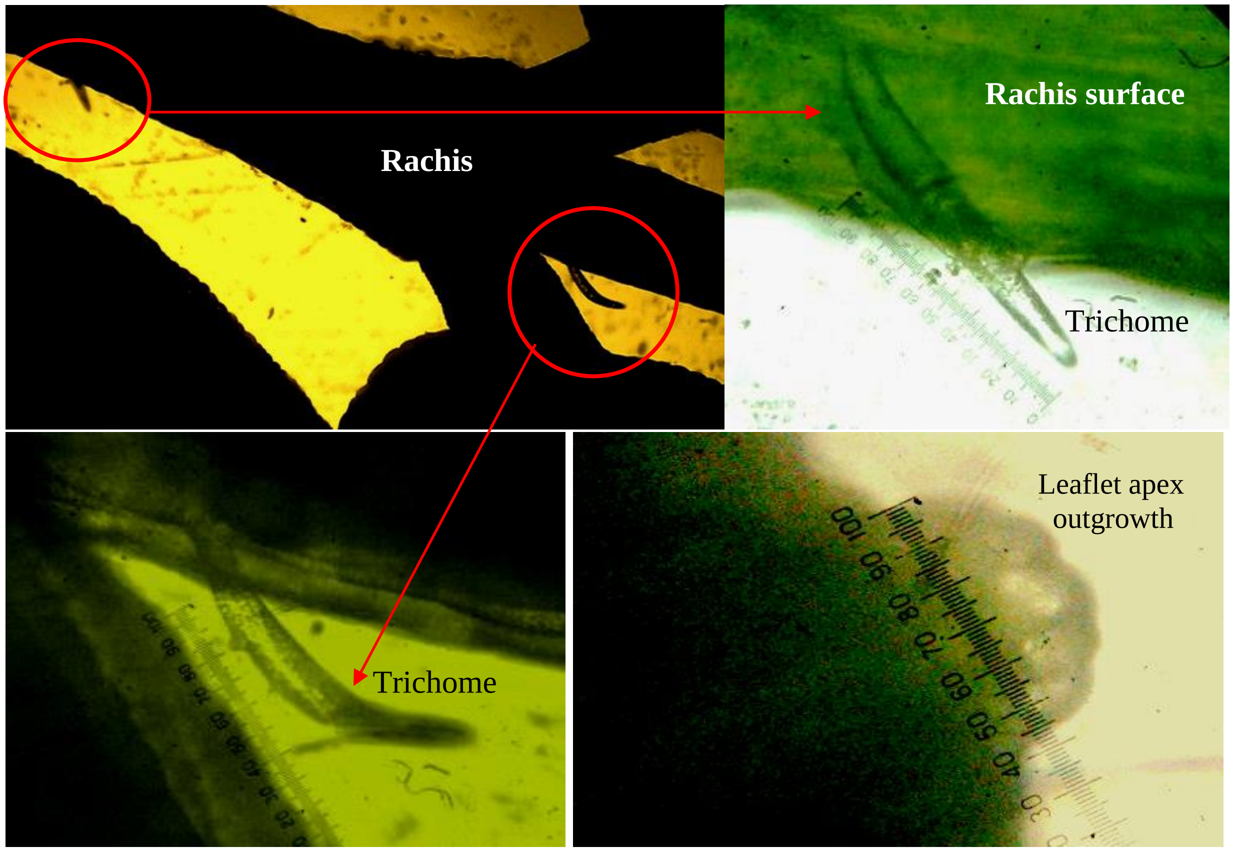


Fig. 2. Compound paripinnate leaf of S. *bispinosa* showing trichomes (distributed rarely on the rachis) and a translucent multicellular and arched outgrowth rarely seen on the apex of a leaflet.

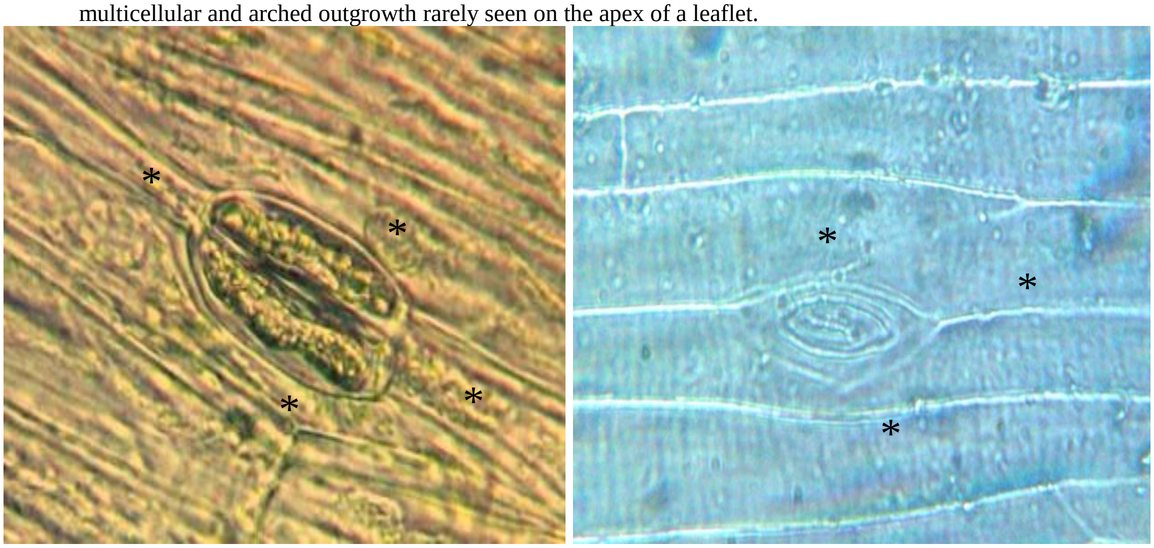


Fig. 3. A) Hypocotylar stoma surrounded by four subsidiaries (tetracytic type) on a three-day old hypocotyl – guard cells are thick bean shaped showing well-developed chloroplasts and B) Anisocytic stoma surrounded with three subsidiaries. Magnification: 45 x 10 X.

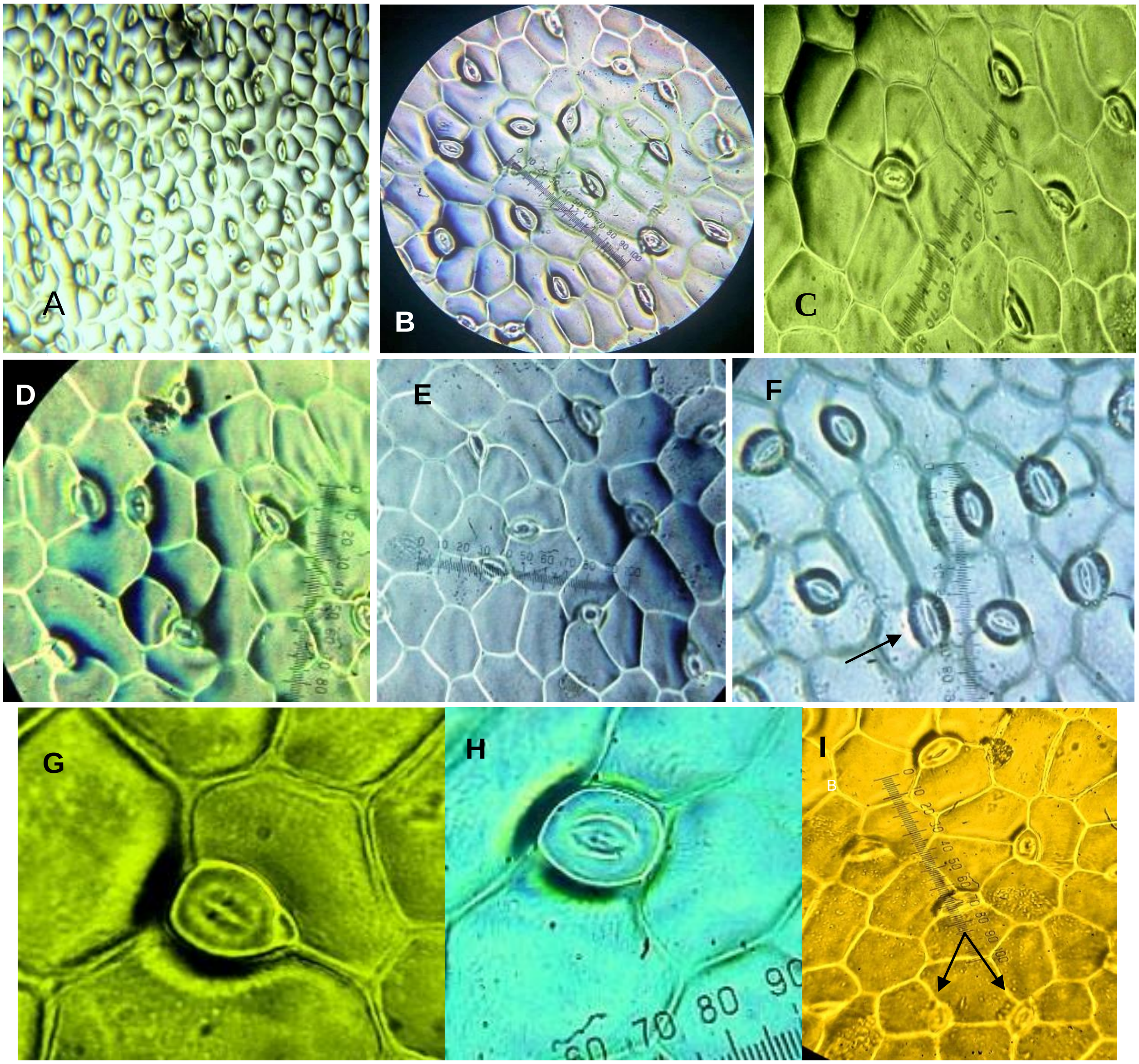


Fig. 4a. Dorsal surface of three-day old cotyledon of *S. bispinosa* seedling (A). Periclinal surface of epidermal cells is convex but stomata are in depression (B, C, D, G, and H). Anomocytic, anisocytic, tetracytic and staurocytic stomata (C, D and E), stomata with common subsidiaries (D and E), Paracytic, anisocytic, tetracytic and anomocytic (5-subsidiaries; shown by an arrow) stomata (F); Totipotent guard cell of an anisocytic stoma giving rise to a daughter cell (G); tetracytic stoma (H) and staurocytic and anomocytic stomata showing common subsidiaries between them (I, shown by arrows).

Stomata types

S. bispinosa seedlings exhibited a diversity of mature stomatal types (Table 1 and Fig. 3-10). Stomata were more diverse on upper surface of cotyledon exposed to sunlight than ventral surface. Generally, anisocytic type of subsidiaries' arrangement was more prevalent than other types Stomata were oval, wide elliptical to spherical. The leaves of *S. bispinosa* were amphistomatic. Several legumes are reported to be amphistomatic (Agbolade *at al.* 2011; Owolabi and Adedeji, 2018). The seedlings of *S. bispinosa* exhibited the occurrence of anisocytic, anisotricytic, anomocytic, tetracytic, staurocytic, and infrequently the paracytic types of stomata. There were a few desmocytic stomata on epicotylar stem only. Metcalfe and Chalk (1979) have enlisted anomocytic, paracytic, parallelocytic (*sensu* Payne, 1970) stomata from Papilionaceae beside stomata in groups (genus *Euchresta*) or surrounded by rosettes of more or less clearly defined subsidiaries, stomata restricted on adaxial surface in some genera of Papilionaceae and unusually small stomata in *Mundulea*. They reported no anisocytic stomata from Papilionaceae but from Leguminosae. They reported anomocytic and paracytic stomata from Caesalpiniaceae and only paracytic stomata from Mimosaceae. They reported no anisocytic and tetracytic stomata from Caesalpiniaceae and no anomocytic, anisocytic, tetracytic or parallelocytic stomata from Caesalpiniaceae and Mimosaceae.

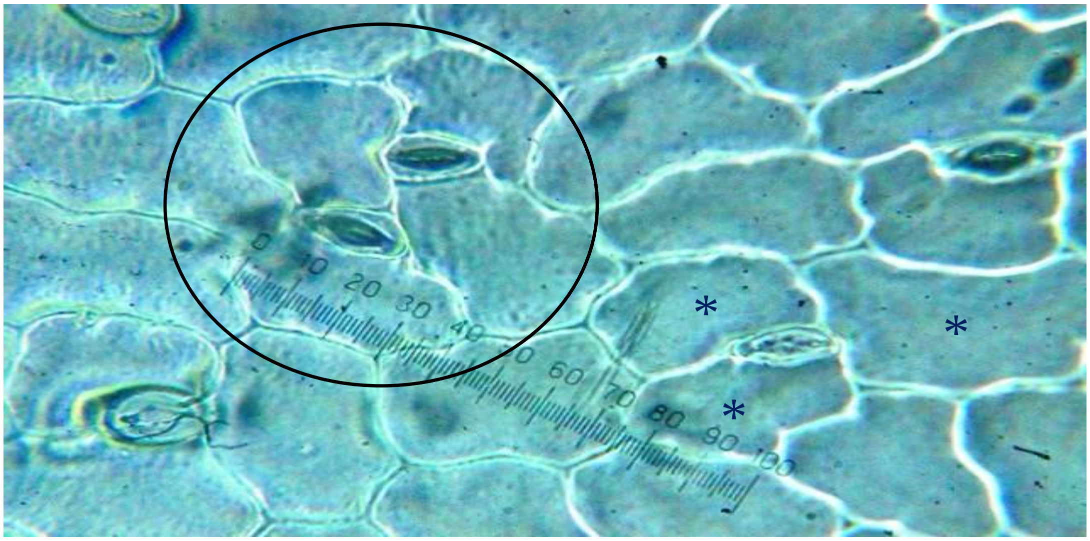


Fig. 4b. Dorsal surface of seven-day old cotyledon. Surface under high magnification (40 x 10 X) showing anisocytic and anisotricytic stomata sharing common subsidiaries (B). The anticlinal walls are straight to curvy.

Farooqui *et al.* (1999) reported that besides paracytic stomata, tricytic or tetracytic stomata do occur in papilionaceous *Dalbergia latifolia*, *D. sissoides* and *D. sissoo.* Idu *et al.* (2000) reported paracytic and anomocytic stomata in some hardwood species of Fabaceae. Adeniji and Airwaodo (2012) reported paracytic stomata in genus *Pericopsis* of Nigeria. Tripathi and Mondal (2012) described epidermal diversity in 45 legumes of India and described three stomatal types characterizing these legumes (paracytic, anisocytic and anomocytic – none of species showed diacytic stomata. They reported that *Sesbania aculeata* Pers. and *S. grandiflora* Pers. had anomocytic and anisocytic stomata and *S. sesban* Merrill. only anisocytic stomata (no paracytic stomata was reported from *Sesbania* spp. Studied). Yadav *et al*. (2010) and Venkateshwarlu *et al.* (2012) reported only anisocytic stomata from *S. grandiflora* leaves. Agbolade *at al.* (2011) reported paracytic (75%), anomocytic (16.67%) and anisocytic (8.3%) in 12 legumes accessions tested of *Vigna*, *Lablab* and *Sphenostylis stenocarpa*. No diacytic stomata in any of the 12 accessions of legumes were found. Gill *et al.* (1982) studied 74 species of legumes from Nigeria and found diacytic type of stomata by far the most common type. Owolabi and Adedeji (2018) when working with some papilionaceous species reported paracytic stomata to be main types in *Desmodium* (*D. tortuosum, D. seorpianum and D. adscendens*), *Mucuna pruriens*, *Calopogonium mucunoides*, *Cajanus cajan, Vigna unguiculata*, *Centrosema mole* and *Gliricidia sepium* with occasionally occurring anisocytic and / or tetracytic stomata. Main type of stomata in *Crotalaria retusa* were, however, anisocytic type and occasionally occurring paracytic type.

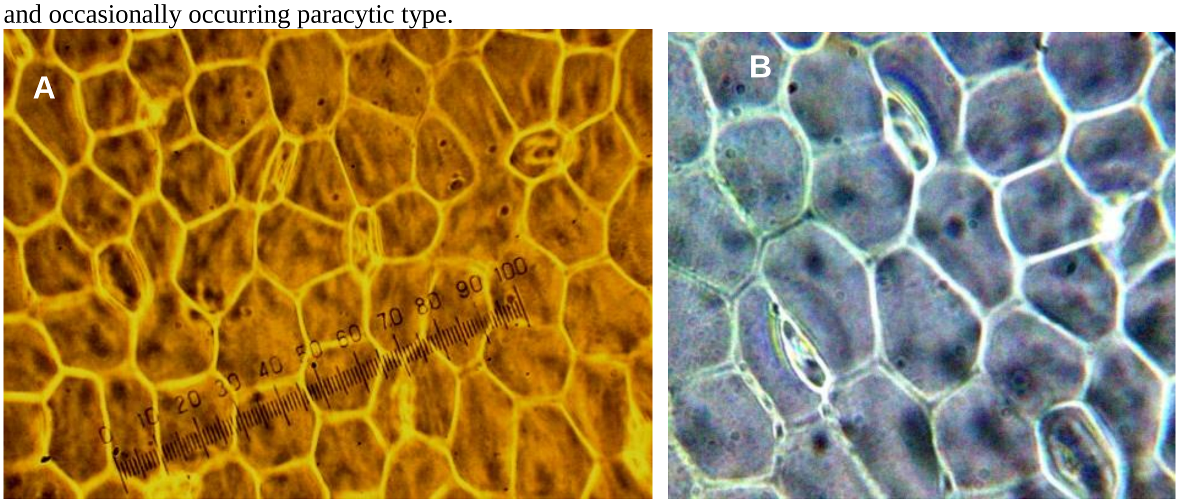


Fig. 5a. Ventral surface of cotyledon of three-day old seedling. The subsidiaries are straight-walled and unequal in size. Stomata are generally of anomocytic (A) and paracytic and tetracytic stomata (B) are present.

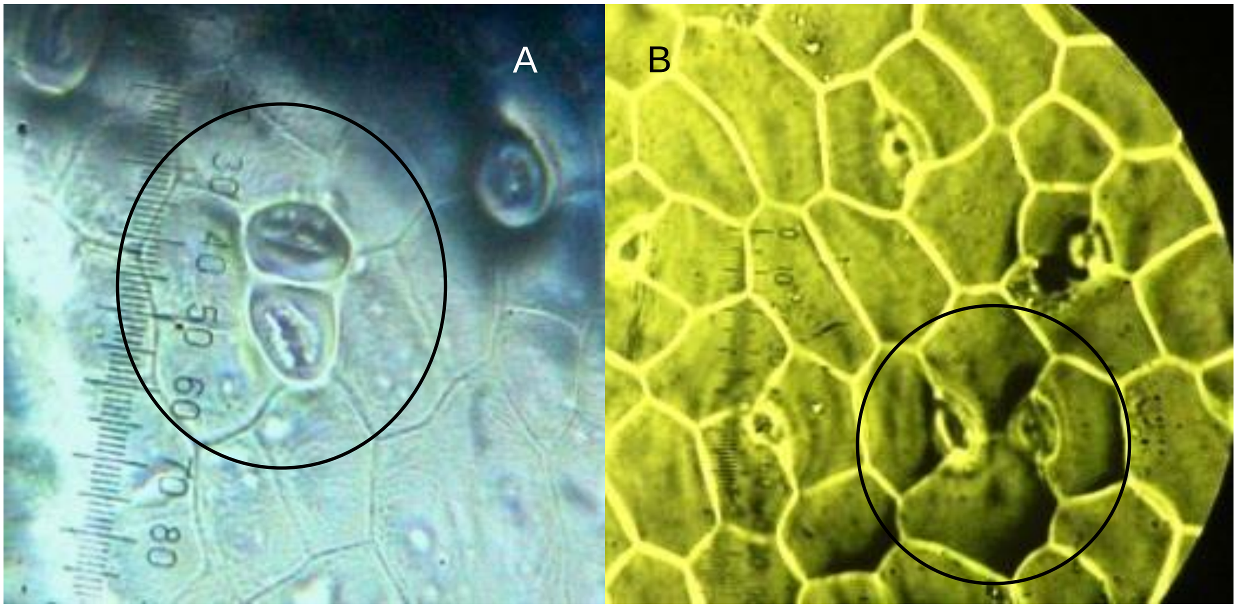


Fig. 5b. Contiguous stomata (differentially oriented pore axes, more or less at right angle) surrounded by six subsidiaries on ventral surface of cotyledon of three-day old seedling (A, within circle) and two anisocytic stomata sharing common subsidiaries (B, within circle). Magnification: 40 x 10X.

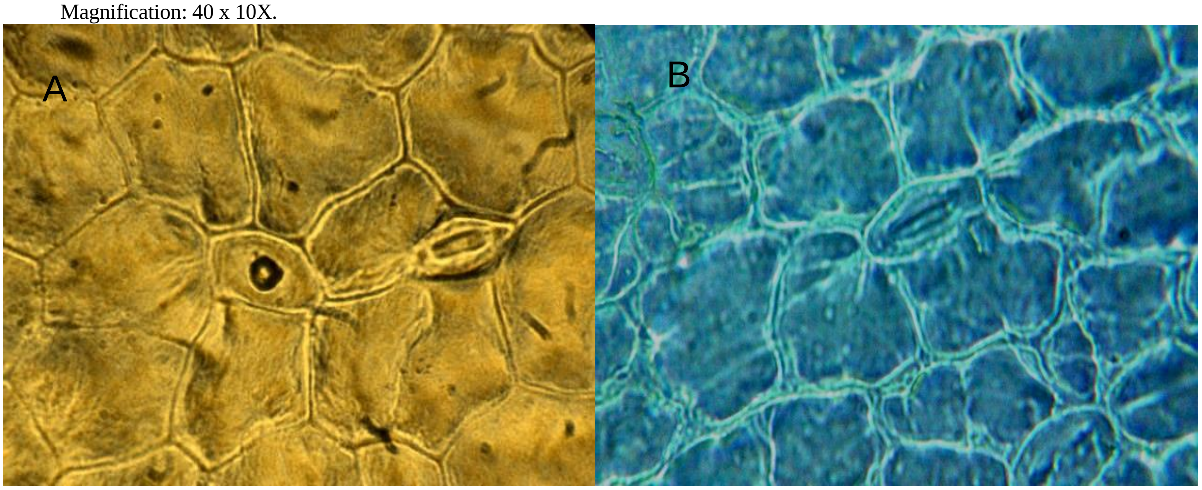


Fig. 5c. A, Ventral surface of cotyledon of 5-day old seedling of *S. bispinosa* showing two anomocytic stomata with common subsidiary cells between them, pore is deformed in one of them. B, Ventral surface of seven-day old cotyledon. Anomocytic stoma, 40 x 15 X.

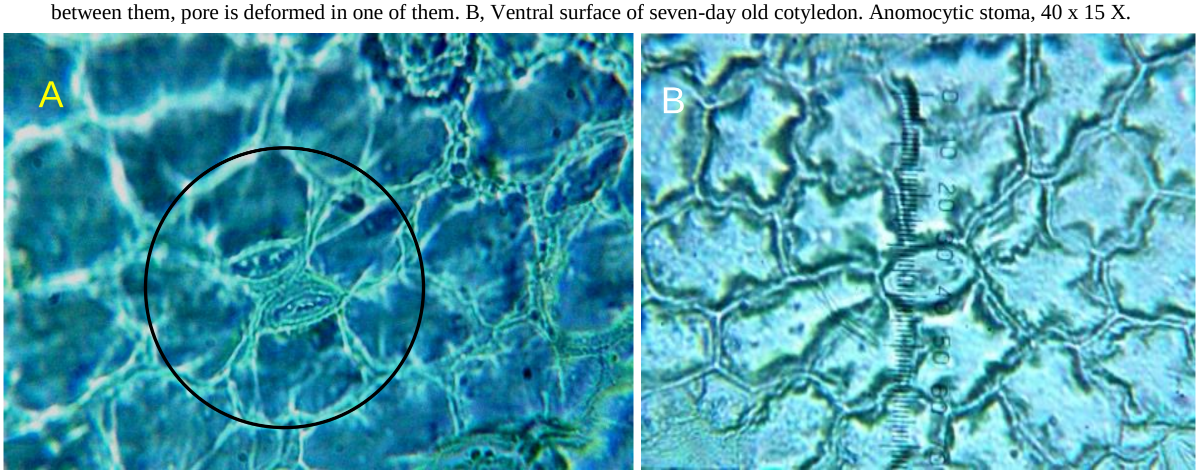


Fig. 5d. Ventral surface of 7-day old cotyledon – ventral surface. A, Contiguous stomata (juxtaposed, parallel orientation of pore axis); B, anomocytic stomata surrounded by seven subsidiaries. Anticlinal walls are sinuous.

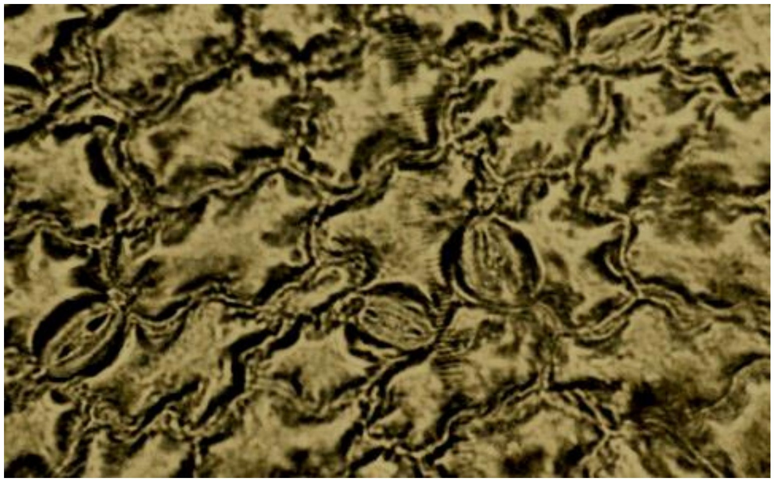


Fig. 5e. Two anisocytic stomata with common subsidiaries on ventral side of 7-day old cotyledon. 40 x 10 X.

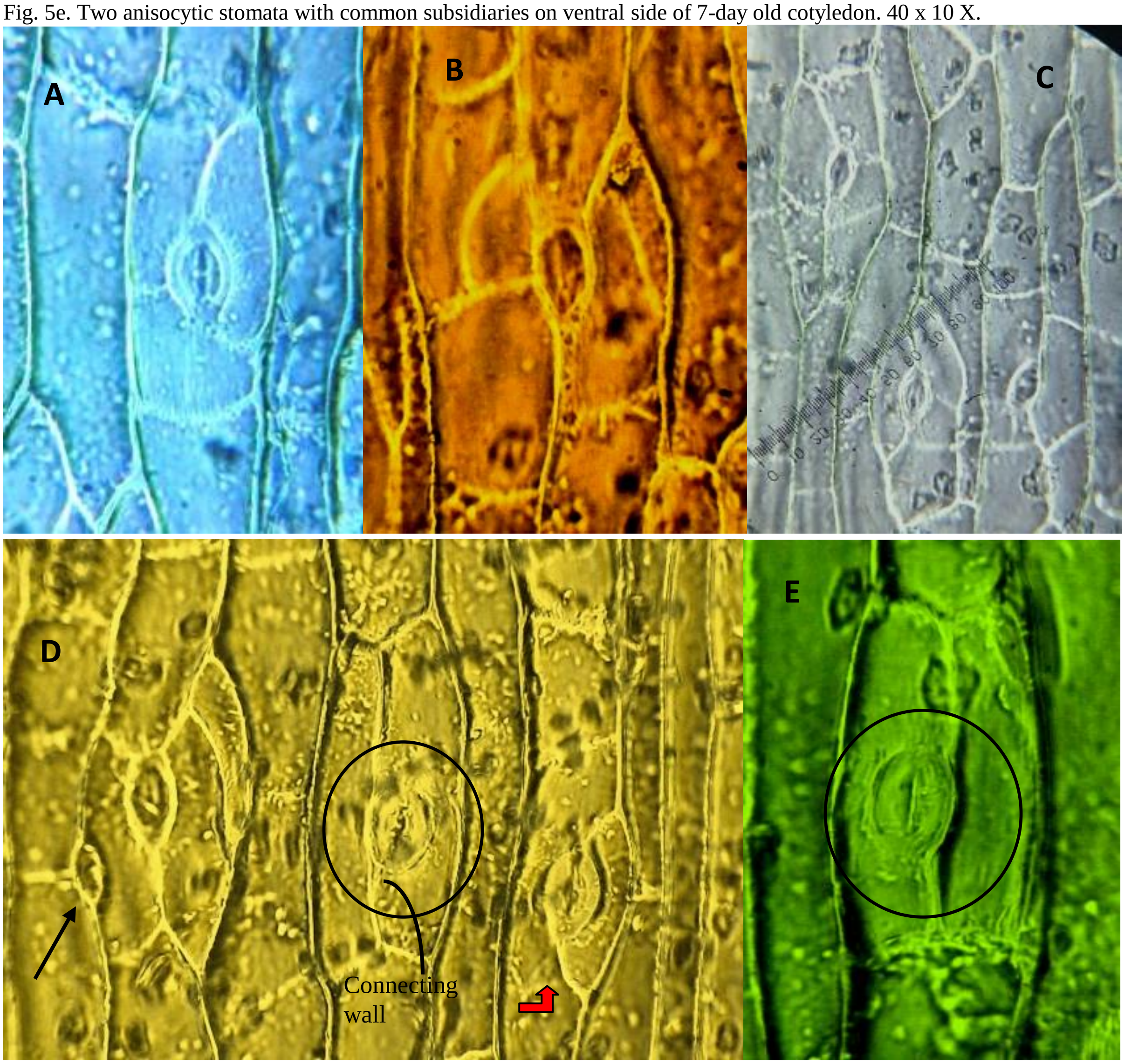
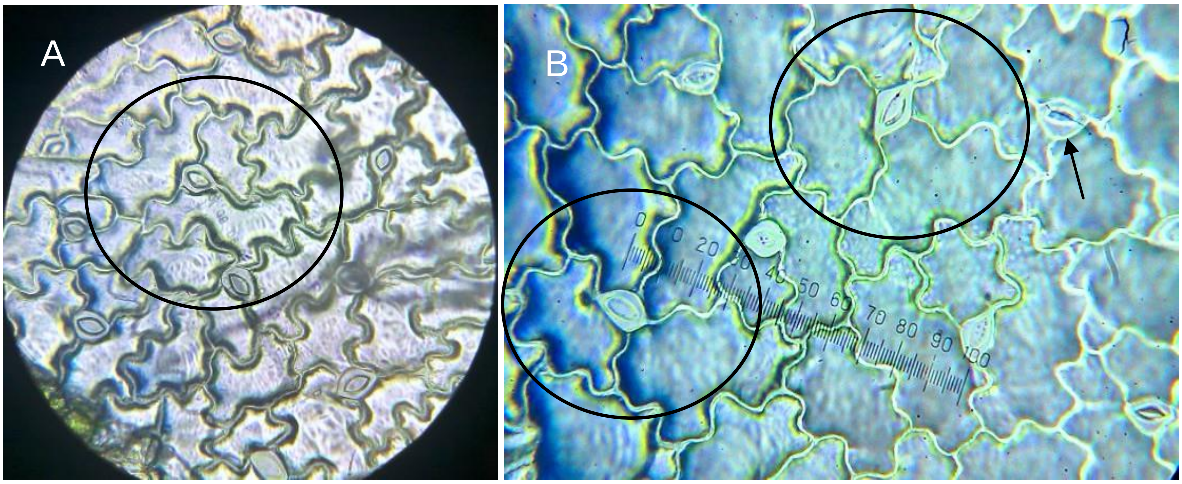


Fig. 6. Stomata types of epicotyl – stomata are generally of anisocytic type (A, C, D), tetracytic (B); a stoma lacking guard cells (shown with black arrow in vicinity of a anisocytic stoma, paracytic stoma (red solid arrow) and a stoma lying within the confinement of a cell – probably a desmocytic one (connecting wall visible) (D). E, A stoma similar to that as in D, but no connecting wall seen.



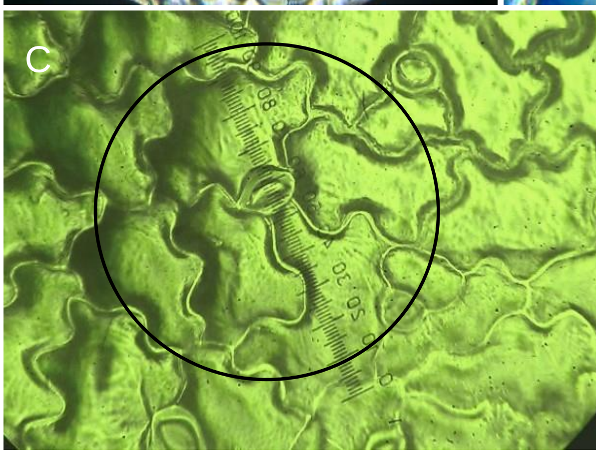


Fig. 7. Stomata of dorsal surface of primary simple leaf. Stomata anisocytic (A), staurocytic (B) and tetracytic types (C). Stomata are oval, wide elliptical to round. Guard cells rarely of unequal width. The anticlinal walls of the epidermal pavement cells are sinuous. The cells are with convex bulging surfaces.

Table 1. Diversity of stomatal types observed in seedlings of *S. bispinosa*.

Seedling organ	Stomatal type	Anticlinal cell wall
Hypocotyl surface (Fig.3)	Tetracytic and anisocytic. Guard cells bean-shaped with obvious chloroplast.	Straight
Cotyledon (Dorsal surface) - Fig. 4a and 4b.	Stomata in depression – Anomocytic, anisocytic, anisotricytic, tetracytic, staurocytic and paracytic. Stomata with common subsidiary. Periclinal surface of epidermal cells is slightly convex* and stomata are in depression. A guard cell producing new cell.	Straight to curvy in 3-day old cotyledons.
Cotyledon (Ventral surface) – Fig. 5a -5e.	Anomocytic, anisocytic, tetracytic and rarely paracytic. Stomata with common subsidiaries and contiguous stomata. Epidermal cells unequal in size. Epidermal cells may be slightly convex* (Fig. 5d).	Straight to curvy in 3-day old and sinuous in 7-day old cotyledons.
Epicotyl surface (Fig. 6.	Anisocytic, tetracytic, paracytic and desmocytic. A stoma without guard cells.	straight
Primary leaf (Dorsal surface) Fig. 7.	Anisocytic, staurocytic, and tetracytic. Epidermal cells may be slightly convex.	Sinuous.
Primary leaf (Ventral surface) – Fig. 8.	Anisocytic, tetracytic and anomocytic stomata and rarely stoma may be deformed with more than one pores.	Sinuous
Secondary leaf (Dorsal surface) Fig. 9.	Anisocytic, paracytic and staurocytic.	Straight to curvy.
Secondary leaf (Ventral surface) Fig. 10.	Anisocytic, tetracytic, staurocytic and rarely paracytic. Epidermal cells with convex* surface.	Straight, curvy to sinuous.

*, as per designations for convex cell outlines scheme of Barthlott *et al.* (2017).

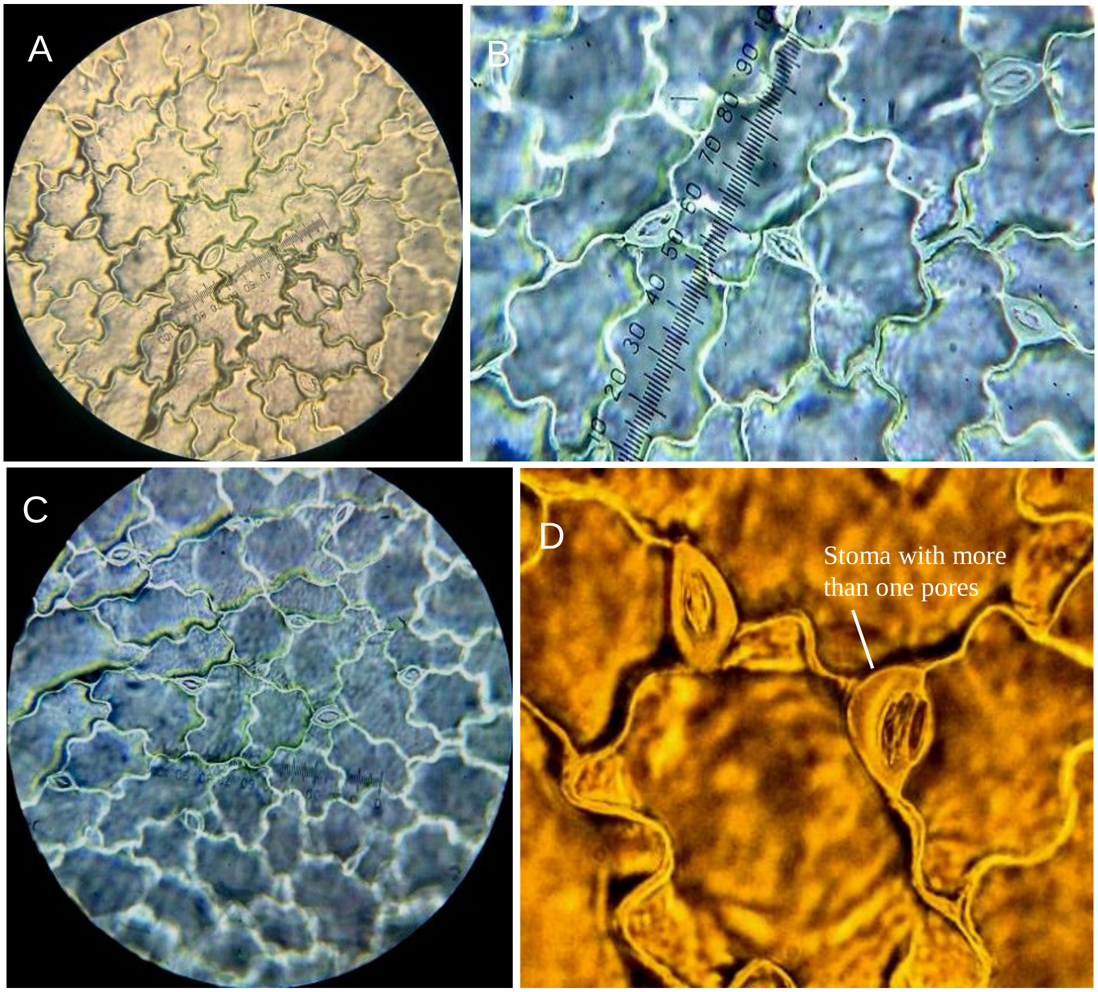


Fig. 8. Stomata of ventral surface of simple primary leaf. A, Anisocytic stomata; B, Anisocytic and tetracytic stomata; C, Tetracytic, anisocytic and anomocytic stomata; D, A abnormal stoma with single guard cell but more than one pores. The anticlinal walls of epidermal cells are sinuous with deep U-shaped undulations (D).

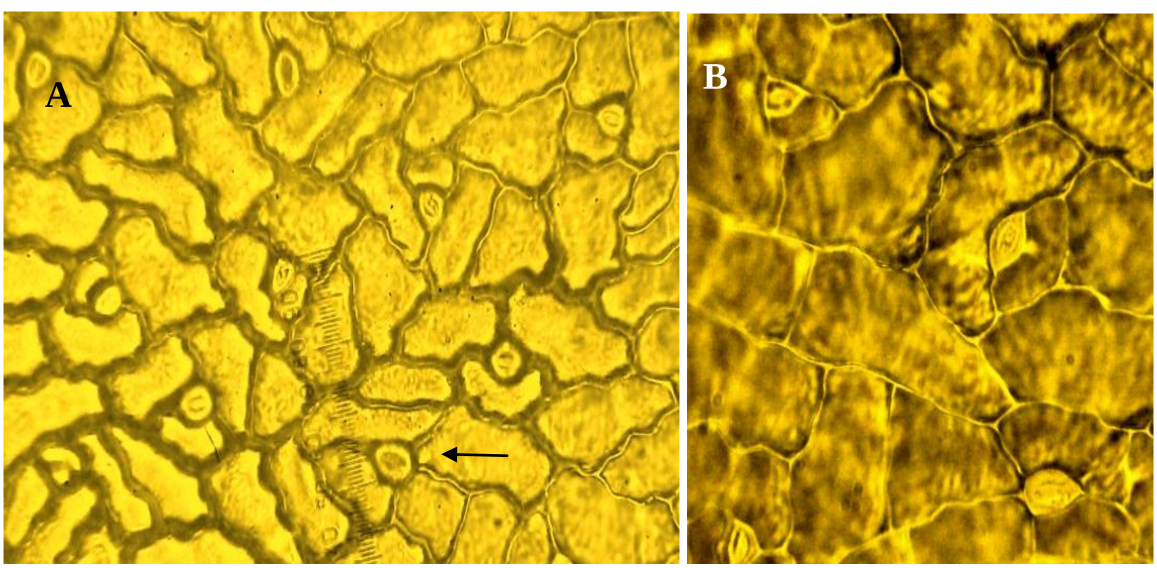


Fig. 9. Stomata of dorsal surface of leaflet of secondary leaf. Stomata are generally anisocytic, paracytic and staurocytic stomata (staurocytic stoma shown by an arrow). The anticlinal walls of the epidermal cells are straight, curvy to sinuous.

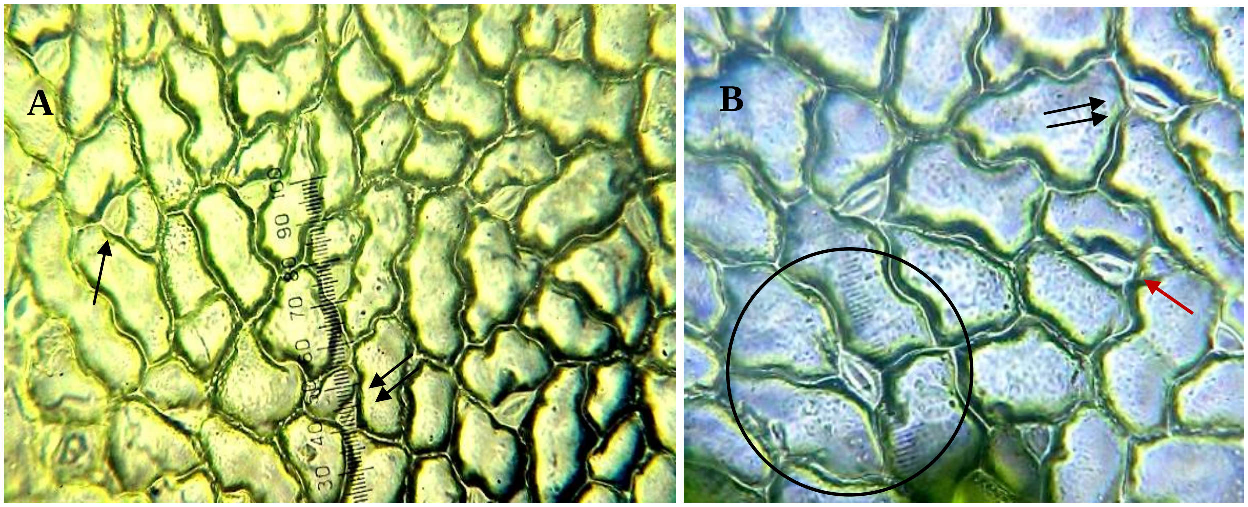


Fig. 10. Stomata of ventral surface of leaflet of secondary leaf. Anticlinal walls are curvy to sinuous. A, anisocytic (arrow) and tetracytic (double arrow) stomata; B, Staurocytic (within circle), rarely paracytic (arrow) and tetracytic (double arrow) stomata.

There appears a great degree of stomatal diversity in papilionaceae. Tribe Trifolieae is reported to exhibit paracytic (more frequent on stem and petiole), anisocytic, anomocytic (more frequent on leaflets) and haplocytic stomata (Shah and Kothari, 1975). Rashid *et al.* (2018) investigated 17 species of genus *Medicago* L., *Melilotus* Mill., *Trifolium* and *Trigonella* L. (Trifolieae) from various localities of Pakistan and reported anomocytic stomata (following Wilkinson's key in Metcalfe and Chalk, 1979) in these species. Among 40 papilionaceous species of tribe Hedysareae, the most frequent stomata were reported to be paracytic except *Zornia* where it was anisocytic and in *Aeschynomene, Alhagi* and *Taverniera* where it was anomocytic (Kothari and Shah, 1975). After study of some 20 papilionaceae species, Shah and Gopal (1969) reported four types of stomata in this family – paracytic, anisocytic, anomocytic and diacytic. In four *Abrus* spp. (*A. canescens, A. precatorius, A. pulchellus* and *Abrus* sp.) of Tropical West Africa, Agbagwa and Okoli (2006) reported five types of normal stomata paracytic (laterocytic), diacytic, anomocytic, anisocytic and staurocytic excepting *A. canescens* which was devoid of anomocytic stomata. Four types of stomata (anisocytic, anomocytic, paracytic and diacytic) were reported from 17 *Crotalaria* (leguminous) species of Eastern Ghats of India (Parveen *et al.,* 2000). Edeoga *et al.* (2008) recognized diacytic stomata in all *Mimosa* species studied. These stomata were generally the paracytic stomata as figures presented in their paper indicated. Zarinkumar (2007) examined 326 dicotyledonous species of Irano-Turanian protected area of which 48 species were papilionaceous. Stomata were found on both surfaces with the exception of *Astragalus glycyphyllus*. There was a variety of stomata in *Bauhinia* (Albert and Sharma, 2013) such as anisocytic, anomocytic and paracytic. Dominant type of stomata in *Astragalus* were anisocytic while in *Trifolium* and *Trigonella* anomocytic were most frequent. In *Lathyru*s and *Vicia,* laterocytic stomata were observed together with other type. The above discussion leads to conclude that there exists high stomatal diversity in papilionaceae and various types of stomata may occur on the same surface of an organ. This is obvious in case of *S. bispinosa* cotyledons and leaves confirming the earlier reports on the subject in papilionaceae (Pant, 1965; Pant and Banerji, 1965; Pant and Kidwai, 1964; Shah and Gopal, 1969, 1971). It is considered by some workers that occurrence of various types of stomata on the same surface reduces the importance of taxonomic importance of stomata as taxonomic marker.

Different types of stomata in papilionaceous taxa are known to largely follow the same sequence of development i.e. mesogenous (Shah and Gopal, 1969). According to them, plants of high stomatal diversity may be divided into two groups: (i) those in which different types develop in more than one way and (ii) those in which the different types follow a similar pattern of development. All the papilionaceous plants appear to fit in the second group. The value of such groupings will be enhanced when enough more information, based on a study of several families, is available (Shah and Gopal, 1969). In our opinion, relative abundance of stomatal types may be significant parameter to compare different papilionaceous or other taxa on numerical ground. It seems that data on abundance of various types of stomata on the same surface should be collected in a number of related taxa for numerical taxonomic investigations; mere presence or absence data should be of limited significance.

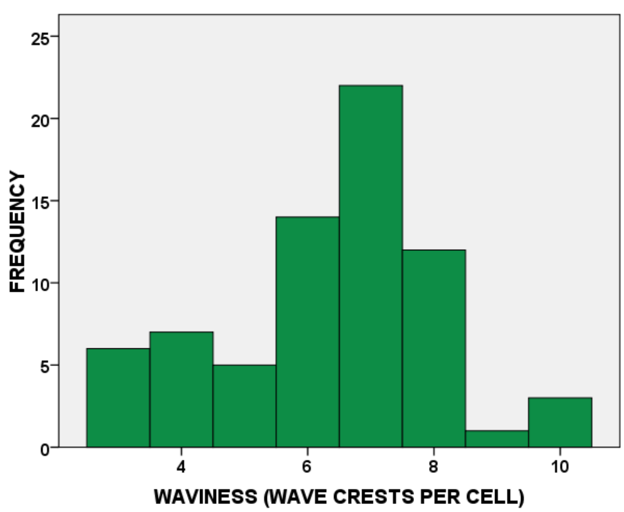
Mimosacean species such as *Leucaena leucocephala* and *Mimosa pudica* are reported to bear paracytic stomata (Shah *et al.,* 1972). Mature stomata in *Leucaena leucocephala* are paracytic but a few anisocytic type of stomata have also been observed (Nyawuame and Gill, 1990). Shah *et al.* (1972) opined that in Mimosaceae, the primary stomata are paracytic type which may secondarily give rise to anisocytic type due to transverse or oblique wall formation. (*Cassia fistula*), Khan and Zaki (2019) also demonstrated that the basic type of stomata are the one with paracytic arrangement of subsidiaries which may in later course of time turn into anisocytic type as a result of the development of a wall within a subsidiary as seen in process of making by Khan and Zaki (2019). Such a structure by further development of cell walls may change to anomocytic type. These results were in confirmation to the earlier speculation made by Stace (1966) who wrote - *"It seems that many of the genera may have basically paracytic subsidiary cells but that extra walls have usually developed, thus giving the appearance of an anomocytic state. The reported occurrence of anomocytic, anisocytic and paracytic stomata on one leaf of Anopyxis (Boodle and Fritsch in Metcalfe and Chalk, 1950) is probably explicable in this way."*

Abnormal stomata

In *S. bispinosa*, some abnormal stomata were also observed e.g. Stomata with common subsidiary (ies), contiguous stomata (placed juxtaposed or two stomata placed more or less at right angle) and deformed stoma with more than one pores, a stoma without guard cells and a new cell produced by the division of a guard cell. Abnormal stomata including stomata with single guard cell with pore, paired stomata having common subsidiary cell, stomata with aborted guard cells and contiguous stomata have been reported in papilionaceae earlier (Dave and Bennet, 1989; Mukherji, 2000). Contiguousness of abnormal stomata with single guard cell and those with arrested development is reported from tribe Trifolieae of papilionaceae by Shah and Kothari (1975). Recently, Agbagwa and Okoli (2012) reported contiguous stomata from some species of *Abrus* (Papilionaceae). Abnormal stomata are the feature from many families of plants such as Acanthaceae, Scrophulariaceae, Solanaceae etc. and contiguous stomata from families Bignoniaceae, Fabaceae, Ranunculaceae, Cruciferae (Ahmad, 1964; Rao and Inamdar, 1981; Mukherji *et al*., 2000). Pant (2003) has reported 'stomata without guard cells' in family Apocynaceae besides contiguous stomata such as juxtaposed and superimposed types. Ahmad (1964) has reported stomata with double pore in Fam. Cruciferae. Abnormal stoma with two apertures and three guard cells was found in endocarp of fruits of *Momordica dioica* by Thanki and Kothari (1980).

The contiguous stomata in *S. bispinosa* were either lying side by side (juxtaposed) or were oriented in different directions (more or less at right angle). Shah and Gopal (1969) have reported contiguous stomata to be opposite or juxtaposed in fam. papilionaceae. They were reported to be formed of two or more adjacent meristemoids. In the seedlings of Family Solanaceae, these stomata were also reported to be opposite or juxtaposed (Inamdar and Patel (1976).

The guard cells are diploid cells of unique specialization with no persistent loss of genetic integrity. They are totipotent and morpho-genetically very important as shown by Hall *et al.*, 1966) in *Beta vulgaris*. The division of a guard cell observed in cotyledon of *B. bispinosa* may be significant in development of contiguous stomata in this species. The formation of contiguous stomata by budding has been diagrammatically illustrated in *Lathyrus sativus* by Shah and Gopal (1969). Mutations do affect stomatal morphology.



N = 70 cells
Mean = 6.34 lobes
SE = 0.206
CV = 27.24%
Minimum = 03
Maximum = 10
K-S test * = 0.191
P < 0.0001
Shapiro-Wilk = 0.929
P < 0.001

Fig. 11. The waviness of epidermal cells on ventral surface of primary simple leaf in terms of number of wave crests per cell. Smaller cells had lesser number of lobes and larger cells had larger number of lobes. *, K-S test employed with Lilleifors correction.

Epidermal pavement cells

The hypocotyl and dorsal and ventral surfaces of three-day old cotyledons of *S. bispinosa* had epidermal cells with straight or curvy anticlinal walls but 7-day old cotyledons showed curvy to sinuous anticlinal walls. Leaves exhibited curvy to sinuous anticlinal walls. In Nigerian papilionaceae, the anticlinal walls were reported to be straight to curved in *Pericopsis elata* and only straight in *P. laxifolia* (Adeniji and Airwaodo, 2012). Dave and Bennet (1989) studied epidermal morphology in some fruits of Papilionaceae; epidermal cells were described by them to be polygonal and straight walled. Agbagwa and Okoli (2012) reported the anticlinal walls of foliar epidermis of some *Abrus* spp. to be irregular, wavy or arcuate.

The pavement cells of epidermis in leaf in *S. bispinosa* were quite intricate in shape with U-shaped undulations and they fit like the pieces of the Jigsaw puzzle. The protrusions or lobes of one cell fitting in the indentations or concavities of the adjacent neighbouring cell i.e. the lobes were perfectly interlocking. Waviness of the epidermal cells on primary simple leaf averaged to 6.34 ± 0.206 wave crests per cell varying from 3 to 10 (CV = 27.24%) (Fig. 11). The waviness of contour varied with the size of the cells. The smaller cells had lesser number of lobes and larger cells had larger number of lobes. Watson (1942) has presented the earlier work done in Germany on waviness of epidermis. It is known since Areschoug (1897) and Anheisser (1900) that there may be differences in waviness in sun and shade leaves. Watson (1942) proposed that waviness was determined by the cells outer cuticle with cell expansion being limited at regions of the cell wall that have a hardened cuticle, but not at regions where the cuticle is still hardening. The depth of undulation increases with shade (Watson, 1942) and waviness decreases from base of plant to the tip of *Sinapis alba* (Rippel, 1919). There appears greater tendency toward waviness on the lower side of leaves with few exceptions (Watson, 1942). Misra (2009) also reiterated that undulations are more pronounced on the lower side of leaf than upper surface. This waviness appears to be affected by the environmental conditions prevailing during leaf development.

The formation of undulations in the epidermal cells is considered to be regulated by sub-cellular self-organizing components – subcellular cytoskeleton organization of microtubules, cellulose microfibrils and actin (Panteris *et al.*, 1994; Jacques *et al.*, 2014; Sapala *et al.*, 2018). The wavy contours in epidermal pavement are considered to be of biomechanical benefits (Jacques *et al.*, 2014; Sapala *et al.*, 2018).

In *B. bispinosa,* the degree of waviness of the anticlinal walls of the epidermal cells appears to be influenced by the age of the cotyledons as the undulations of the contour of epidermal pavement cells were only observed in 7-day old mature cotyledons. Three-day old cotyledons exhibited no undulation in the pavement epidermal cells.

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