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SEEDLING CHARACTERISTICS OF *CASSIA FISTULA* L. (CAESALPINIACEAE)

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ABSTRACT

The seedlings of *Cassia fistula* L. were studied for their morphological characters including stomatal and epicuticular wax crystalloids types. Germination in *C fistula* is Phanerocotylar-Epigeal-Reserve (≈PER) type. Cotyledons in *C. fistula* are isocotylar, opposite, flat leathery, sessile to sub-sessile, coriaceous and oblong-obovate in shape. Both apex and basal angles are obtuse. Cotyledons exhibited expansion during early days. Cotyledons of 1-day old seedlings were 74 to 94 mm^2 in size which increased to 255 to 267 mm^2 in size in 5-day old seedlings. There was apparently no further increase in cotyledonary size in older seedlings. Cotyledons were c 242 mm^2 in size in 40-day old seedling presumably being consumed with age when epicotyl develops. Epidermal cells polygonal in shape. An anticlinal wall of the epidermal cells was straight, arcuate or sinuous. Trichomes are present on lower hypocotyl, epicotyl, stipules and leaf. They are unicellular, unbranched, generally short, sometimes long, non-glandular, straight or curved (sword-like), hooked and with round basal cell, on veins as well as lamina. The trichome density on young leaflet (1.2 cm^2 in area) was found to be 85.12 ±2.55 trichomes per mm^2. *C. fistula* leaflets were amphihypostomatic. Stomatal outer ledges formed protective elliptical rim around stomatal pore. Taken together all of the organs (hypocotyl, cotyledons, epicotyl and leaflets) diverse type of stomata (nomenclature based on Prabhakar, 2004) were seen - paracytic, anisocytic tetracytic, 1½ cyclic tetracytic, staurocytic, anomocytic and abnormal contiguous stomatal type were seen in *C. fistula* seedlings. Basic type of stomata appeared to be the one with paracytic arrangement of subsidiaries which, however, appeared to change into anisocytic type as a result of the development of a wall within a subsidiary. Such a structure by further development of cell walls appeared to change to anomocytic type. The cotyledonary stomatal density was somewhat higher on upper surface (233.49 ± 4.085 stomata per mm^2 than lower surface (212.85 ± 4.99 stomata per mm^2). The stomatal density on ventral surface of leaflet averaged to 176.94 ± 5.38 stomata per mm^2 varying around 33.3%. In *Cassia fistula*, the wax crystalloids were composed of clearly discernible irregular platelets (rosettes of platelets) as per terminology of Barthlott *et al.* (1998) – also known as "Faballes" type.

Key Words: *Cassia fistula* L., Seedling type, stomatal type, stomatal density, Epicuticular wax crystalloids

INTRODUCTION

In the domain of seedling morphology, a host of marvelous publications have appeared – to cite a few, Lubbock (1892), Compton (1912). Duke (1969), Burger (1972), Vögel (1980), Smith (1981,1983), Smith and Scott (1985), Nenggan (1983-84), Deb and Paria (1986), Balasubramanyan and Swarupanandan (1986), Garwood (1995, 1996), Das and Paria (1999), Wright *et al.* (2000), Zanne *et al.* (2005), Lack *et al*. (2008), Miller and Miller (2011), Sinjushin and Akopian (2011), Nemoto and Ohashi (2012), Adeniji and Ariwaodo (2012), Barbosa *et al.* (2014), Lobo *et al.* (2014), Feitoza *et al.* (2014), Khan *et al.* (2014, 2015a and b, 2017a and b) etc. - several of which dealt with Family Leguminosae. Still seedling morphology is a less-explored, however, emerging domain in the field of 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). Data on seedling morphology of tropical tree species is still insufficient (Garwood, 1995).

In this paper we undertake to describe the seedling characteristics of *Cassia fistula L.* (Latin name *Cassia* comes from the Greek word "Kassia" meaning fragrant) with respect to its germination type, seedling growth and morphology and leaf architecture and ornamentation (stomata, trichomes and epicuticular waxy crystalloids) under local arid environmental conditions of Karachi, Pakistan. Despite the importance of legumes and their stomatal apparatus taxonomically, reports on such aspects are meagre or incomplete in many species (Gill *et al.*, 1982). It also 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). *C. fistula* is ornamentally attractive (Ali, 1973), medicinally very useful (Pandya *et al.*, 2012), acaricidal (Sunil *et al.*, 2013) and soil ameliorative (Roy and Datta, 2014).

MATERIALS AND METHODS

The seeds of *C. fistula* were collected from its mature large tree growing in the campus of University of Karachi. Since unabraded seeds of *C. fistula* didn't germinate for quite long time (Soliman and Abbas, 2012; Babaloba *et al.*, 2014), its 50 seeds abraded with sand paper were sown (at depth c 1.5cm) in pots filled with garden loam soil maintained at 75% water holding capacity. Islam *et al*. (2010) have reported optimum depth for *C. fistula* to be 1-2.5 cm). Seedling started emergence after three days of incubation. Maximum germination (90%) was achieved within 5 days of incubation. The seedlings were studied, for their morphological characters including stomatal types and epicuticular wax crystalloid types. Seedlings type was described according to Garwood (1996). Hickey (1973) and LWG (1999) were followed for description of leaf. 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 pathway was adopted to ascertain stomatal types. Length and width of stomatal pores was measured in µm with calibrated micrometer. The data was analyzed statistically (Zar, 2010). For scanning electron microscopy (SEM), air-dried plant material was mounted on brass stubs and coated with a 250 ^{o}A gold layer with JFC-1500 gold coater. SE micrographs were made at 15kV with JEOL JSM-6380A electron microscope at various magnifications. The images were saved digitally on computer. Elements detector system (EDS) based on Energy dispersive X-ray spectroscopy (EDS) attached to SEM was employed for element detection and quantitative elemental analysis. By this system, single shelled elements are not detected. Epicuticular wax crystalloids were identified after Barthlott *et al.* (1998) at three magnifications: 2700X, 7500X and 14000X.

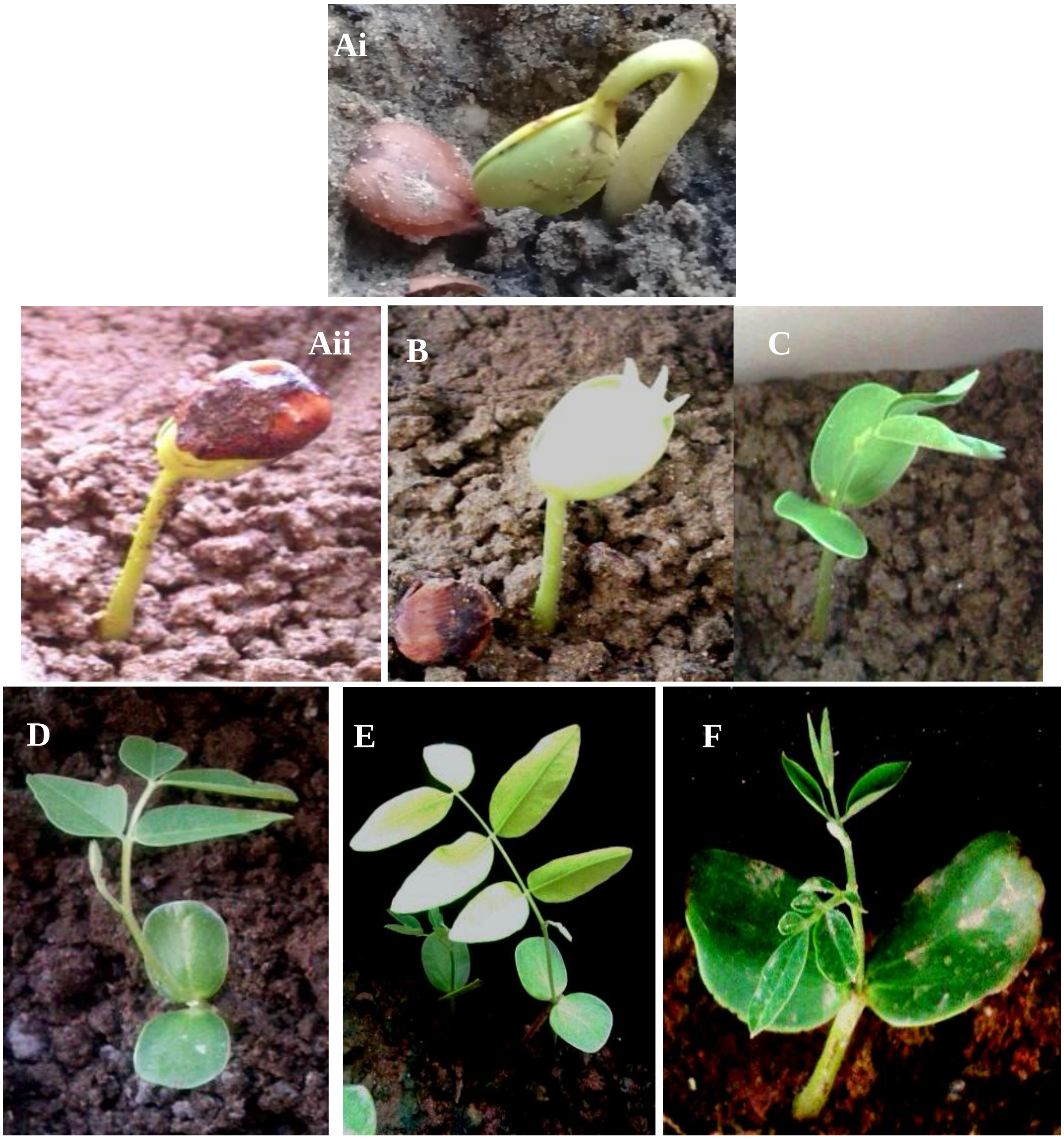


Fig. 1. Germination stages of *Cassia fistula* seed (A-C). Note that primary leaf is a paripinnate (D, F and F). Also note the fact that leaf is already quite developed before the cotyledons spread (B). The number of leaflets may vary from 4 to 6.

RESULTS AND DISCUSSION

Seedling: Germination in *C fistula* is epigeal, phanerocotylar type. Several legumes (*Albizzia lebback, Cassia auriculata, C. occidentalis, Delonix regia, Leucaena leucocephala, Pelltophorum pterocarpum*) are reported to show epigeal germination (Nakar and Jadeja, 2016). When *C. fistula* seed germinates hypocotylar growth brings the cotyledons enclosed in testa above ground. The hypocotyl is stout and bent but becomes straight and cotyledons still enclosed inside testa. With time they come out of seed coat. Hypocotyl is fast growing. It turns green on exposure to sun. The primary leaf, unlike many of the legumes, is unipinnate - paripinnate and not simple. After 72h of emergence seedling is well-formed but leaflets of the primary leaf still folded. They are full open after 84h. In most of the germinating seeds, leaflets of the primary leaf emerge out of the cotyledons even prior of the spreading of the cotyledons. The cotyledons are yellowish but gradually turn green and become photosynthesizing. They are more or less round in shape, thick (c 1.0mm) and succulent (Fig. 1). The seedlings in which cotyledons fail to come out of the testa generally die soon. This is similar to that in *Sesbania bispinosa* (D. Khan, unpublished data).

Cotyledons exhibited expansion during early days. Cotyledons of 1-day old seedlings were 74 to 94 mm^2 in size which increased to 255 to 267 mm^2 in size in 5-day old seedlings. There was apparently no further increase in cotyledonary size in older seedlings. Cotyledons were c 242 mm^2 in size in 40-day old seedling presumably being consumed with age when epicotyl develops. Nagesh *et al*. (2003) reported tricotyledony in *C. fistula*. We, however, couldn't observe any tricotyl seedling in this species. Seedling type as per Garwood's (1996) scheme may be referred to as "Phanerocotylar-Epigeal-Reserve type" (≈ PER) with some reserves of food in cotyledons sufficient for growth for quite some time. It is similar to *Acacia nilotica* (Amritphale and Sharma (2008). Seedling characters are in general agreement to Sanyal and Paria, 2014).

Root: Tap root strong. It was 9.5 cm long in 5-day old single-leaf-seedling - strongly elongating with profuse lateral roots.

Hypocotyl: Strong, basally brown. In 5-day old single-leaf-seedling, hypocotyl was straight and measured around 4 cm long. Root: shoot ratio in single leaf seedling was c 2.4. Straight or curved unicellular trichomes were present on the hypocotyl.

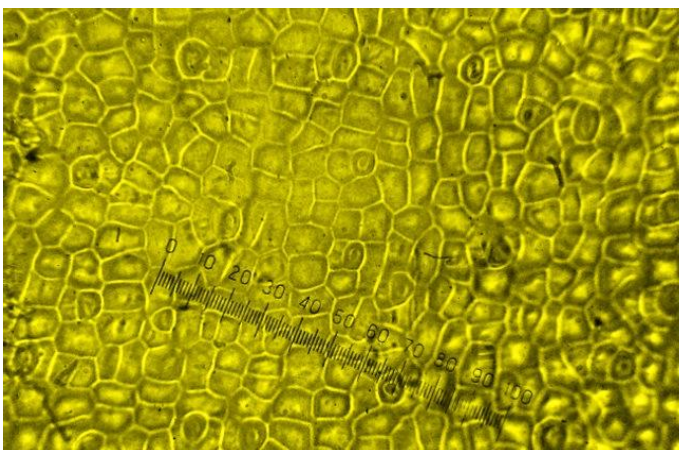


Fig. 2. Meristemoids and stomata scattered over upper or dorsal surface of cotyledon just emerging out of the seed coat of the germinating seed of *C. fistula*. Stomata anomocytic and tetracytic.

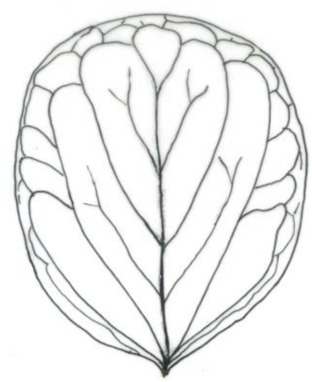


Fig. 3. Cotyledonary venation.

Cotyledons: Cotyledons in *C. fistula* are isocotylar, opposite, flat leathery, sessile to sub-sessile, coriaceous and oblong-obovate in shape. Both apex and basal angles are obtuse. Seven primary veins (5 very prominent, two relatively weak outer veins running closely along lateral margins) arose from the base. Four pairs of secondary veins arose from the main vein in opposite manner and joined forming loops indicating a complex venation – mixture of basal actinodromous and brochidodromous venations. Roy and Datta (2014) have reported actinodromous venation in *C. fistula* leaf. It needs to be further studied in detail. Cotyledons, in our studies, remained with seedlings for around 50 days. They have been considered to be foliaceous (Roy and Datta, 2014) but we found that cotyledons are substantially thick (c 1mm) and laden with sufficient nutrients to support growth of foliar leaves as the cotyledons are green and photosynthesizing also. They have been described as paracotyledons (Vögel, 1980). Of course, they are exposed, green, functionally leaf-like (photosynthesizing) but they are not as thin as leaf in general. They are succulent, leathery and moderately food-laden – much thicker than leaves. They should be considered "moderately reserve type"- efficiently photosynthesizing but not the "foliaceous type" as seldom considered (Roy and Datta, 2014). They support early hypocotylar growth before coming out of testa and later they support the epicotylar growth. Phanerocotylar epigeal foliaceous (≈ PEF) type cotyledons use light as energy source and are found associated with open vegetation whereas hypogeal reserve type cotyledons (≈ CHR) provide nutrients for quite longer periods as they generally occur in low light environments of closed vegetation. The relationship of phanerocotylar-epigeal-Reserve (≈ PER) type cotyledons is not clearly understood. This functional type may, however, presumably be useful for species that grow in diverse ecosystems.

C. fistula is tropical species of vast distribution growing in open as well as closed vegetation environments. It occurs in monsoon forests throughout greater parts of India, ascending 1300m in outer Himalaya. It occurs in Maharashtra throughout Deccan and Konkan (Gupta, 2010). It occurs in Changa Manga, Pakistan (Ali, 1973).The plant is seen throughout India, Pakistan and many parts of the World – South East Asia, South and South East China, Taiwan, Nepal, Guyana, Surinam, French Guiana (Champion *et al.*, 1965; Ali, 1973; Khare, 2007; Boggan *et al.*, 1997; Ecocrop, 2017; eFlora of Pakistan). It is now pantropical. Schmelzer (2008) described it as a vegetative element from tropical thorn to moist through subtropical thorn to moist forest zones and can tolerate moderate shade and drought but not frost.

Trichomes: Trichomes are present on lower hypocotyl, epicotyl, stipules and leaf (Fig. 4A, 7, 8B, 9 A & B, and 10). They are unicellular, unbranched, generally short, sometimes long, non-glandular, straight or curved (sword-like), hooked and with round basal cell, on veins as well as lamina. Banerjee *et al.* (2004) have described similar trichomes from *C. fistula*. They may be as large as 150 µm (Fig. 9 and 10) or even as large as 184µm. The epidermal pavement around a trichome is formed by radiating cells with sinuous anticlinal walls (Fig. 20). There are no glandular trichomes in *C. fistula.* Glandular trichomes are reported from *Cassia occidentalis* (Rani and Satish, 2014). Hypocotylar trichomes averaged to 51.04 ± 1.37µm (N = 40, 32.0 – 75.2 µm, CV = 16.96%). The trichome density on young leaflet (1.2 cm^2 in area) was found to be 85.12 ±2.55 trichomes per mm^2 varying from 49.12 - 127.88 (CV: 21.20%). The distribution of trichome density tended to be normal (KS-z: 0.983, $p < 0.278$).

Epicotyl: In a week-old seedling bearing two leaves, epicotyl was 3.2 cm long. Two types of trichomes were present - short and exceptionally long. Short trichomes were curved. Internodes densely hairy.

Leaves (Eophylls): Leaves alternate, petiolate, stipulate (two deltoid stipules very small caduceus), paripinnate, thin, the number of leaflets in primary leaf of a single-leaf-seedlings varied from 4 to 6 (mean: 4.4 ± 0.27, N = 10), dorsiventral. Leaflets opposite, apex acute, base generally, symmetrical, round (obtuse). Petiolule 1 to 2 mm long. Leaflet size in a leaf variable. Leaflet blade in primary paripinnate leaf of a 40-day old seedling ranged in size from 245 to 400 mm^2 in one-sided surface area (mean = 308.83 ± 26.62mm^2). Leaflet margins entire. Unicellular non-glandular trichomes were present on both surfaces of the leaflets - their margins and apecies, rachii, petioles and stipules. In leaflet primary vein is one, secondaries arranged in probable semicraspedodromous manner. Midrib more prominent on lower side and terminating into a terminal appendage. There are several secondary veins arising from the main vein. Veinlets are very fine. Intercostately reticulate. Very young leaves are heavily trichomatous.

Table 1. Stomatal types observed in *C. fistula* seedlings.

Organ	Stomatal types observed
Hypocotyl	Anomocytic, occasionally numerous subsidiaries (11 subsidiaries surrounding a stoma may be seen in Fig. 4B, 18).
Cotyledon (Upper)	Numerous meristemoids in young cotyledons. In mature cotyledons stomata anomocytic staurocytic, tetracytic, anisocytic and abnormal (Fig.2, 5).
Cotyledon (Lower)	Anomocytic, staurocytic, tetracytic, anisocytic, paracytic , and sometimes adjacent with common subsidiaries between the two. (Fig.6)
Epicotyl (axis)	Paracytic, anisocytic (Fig. 13)
Leaflet (dorsal)	Paracytic, Tetracytic, 1 ½ - cyclic tetracytic, Anisocytic, anomocytic -generally near vascular nerves. Sometimes adjacent with common subsidiaries (Fig. 14, 15, 16).
Leaflet (Ventral)	Paracytic, anisocytic, tetracytic, anomocytic, staurocytic, and contiguous stomata (Fig. 17, 18, 19, 20). Sometime linearly arranged in a group.

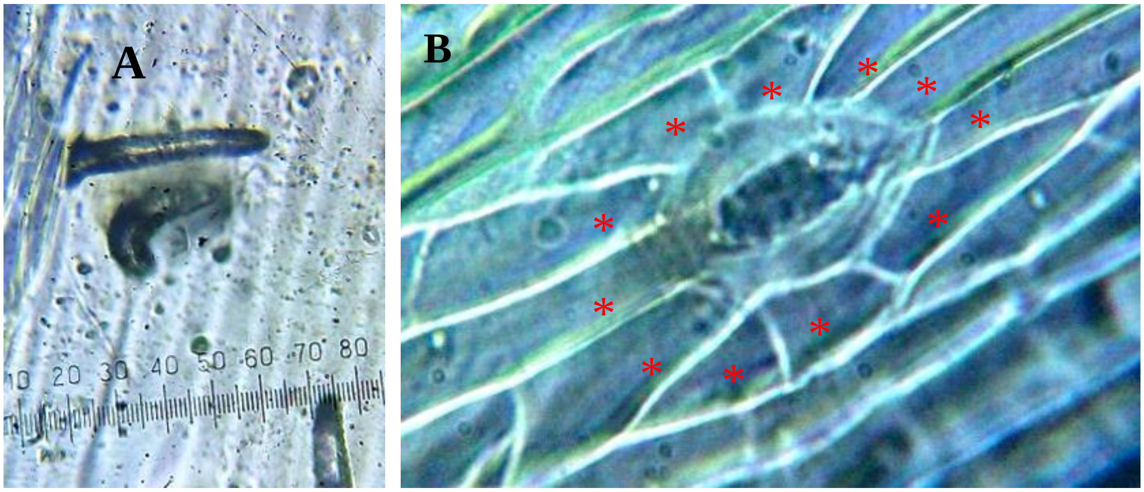


Fig. 4. Trichomes (A) and a stoma (B) on the surface of hypocotyl as seen in nail polish imprint. Note an anomocytic stoma surrounded by 11 subsidiaries as indicated by the asterisks.

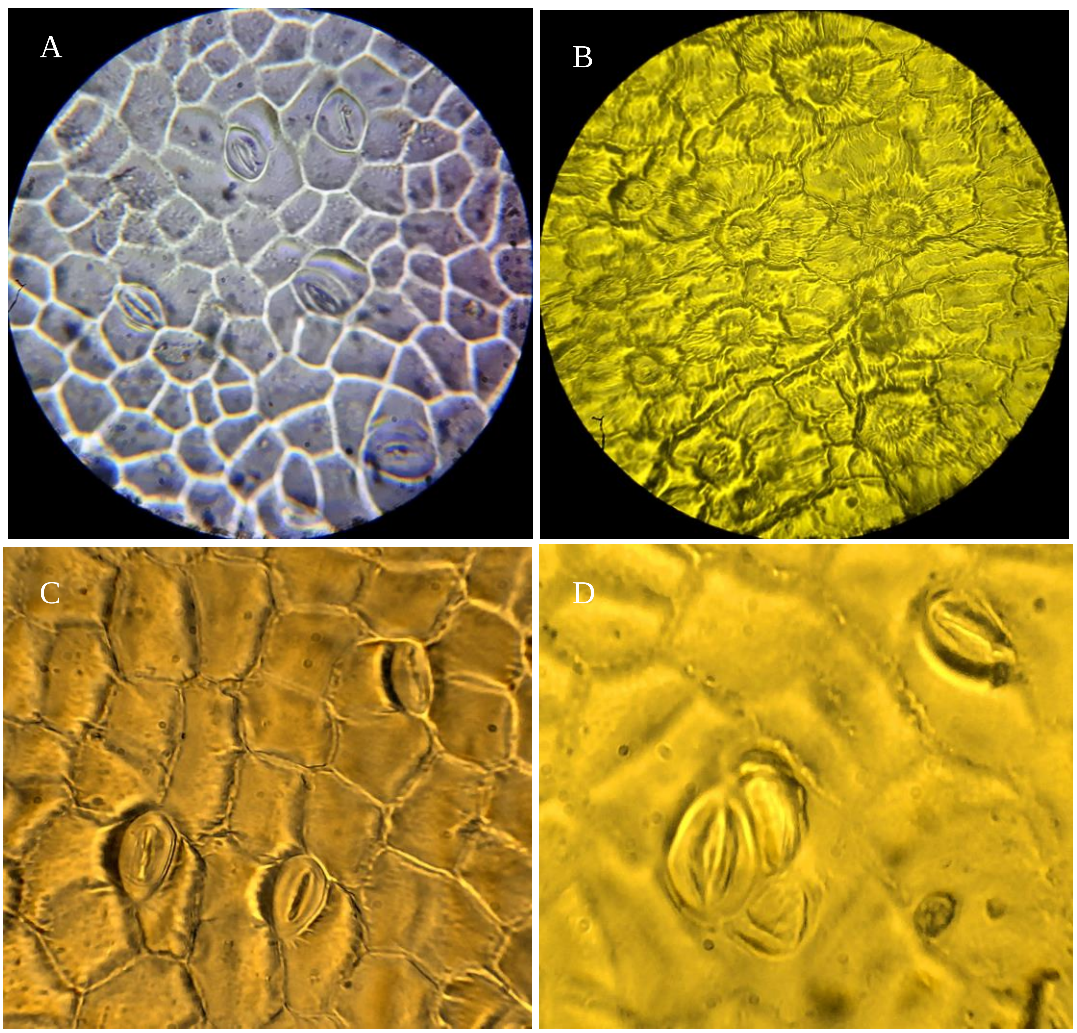


Fig.5. Stomatal types on Upper or dorsal surface of mature Cotyledons. A, staurocytic and tetracytic; B, anisocytic; C, anomocytic, and D, contiguous stomata and thick cuticle.

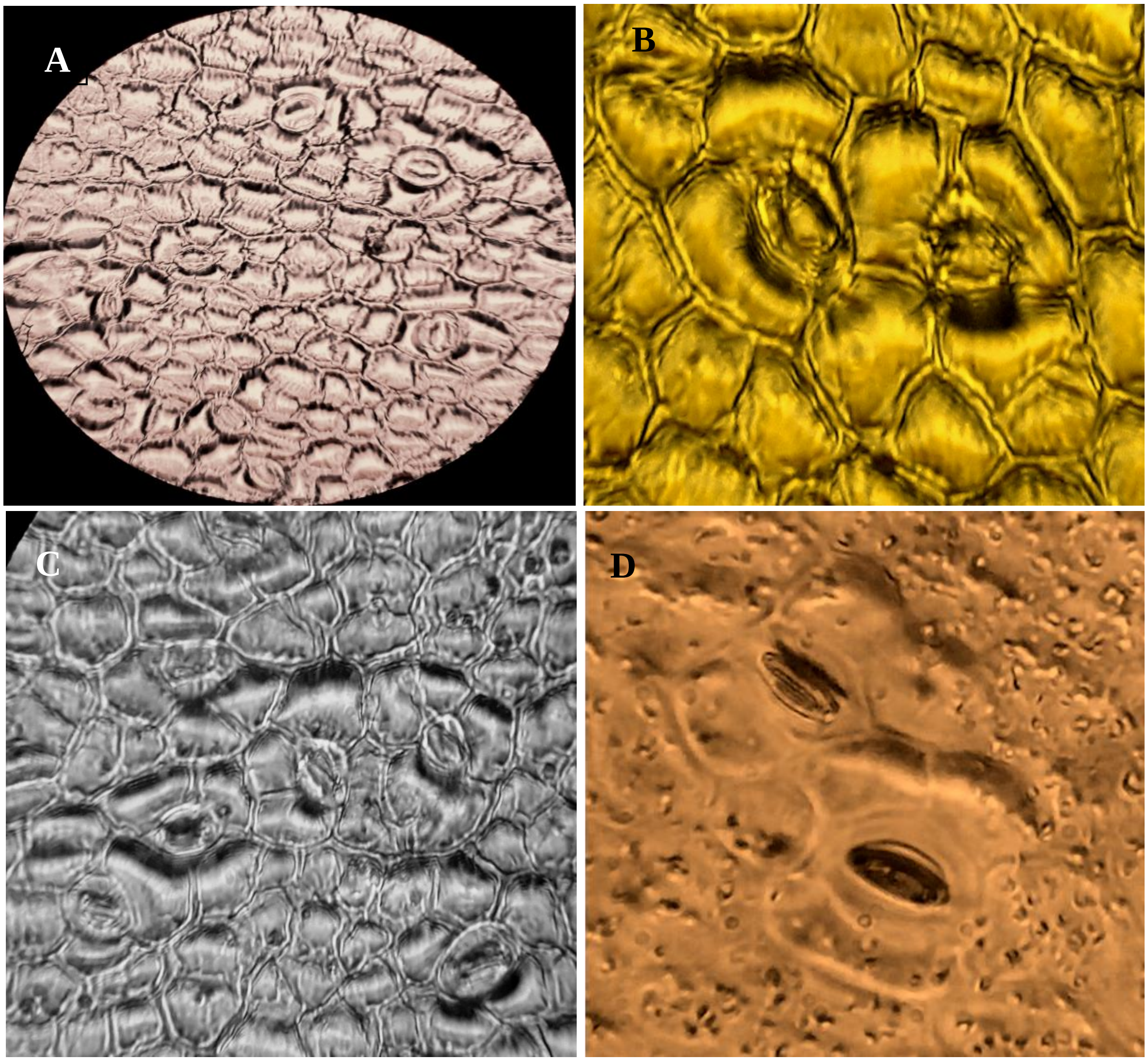


Fig. 6. Ventral (lower) surface of cotyledon. A, anomocytic stomata; B, Two stomata with four subsidiaries (staurocytic and tetracytic) showing two subsidiaries common between the two stomata; C, several stomata arranged in a group; D, Heavy cuticular encrustation – two anomocytic stomata visible. Subsidiaries are raised from the ground.

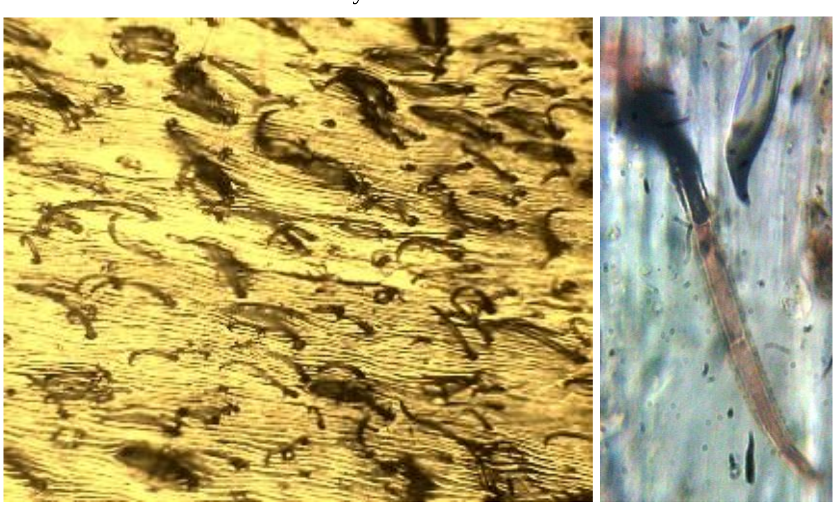


Fig. 7. Epicotylar surface showing sword shaped trichomes. The younger trichomes sometimes appear to be pink (unstained) with round black basal cell.

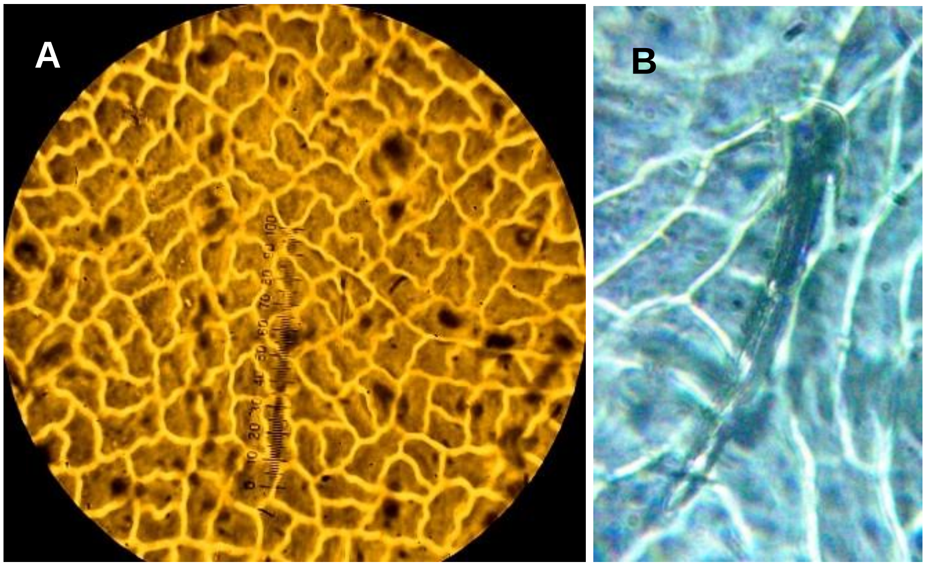


Fig. 8. General view (10 x10 X) of dorsal surface of leaf (A) and a trichome on the dorsal surface of mature leaf let (B) in nail polish imprint (45 x 15 X). The basal part of trichome is darker in colour.

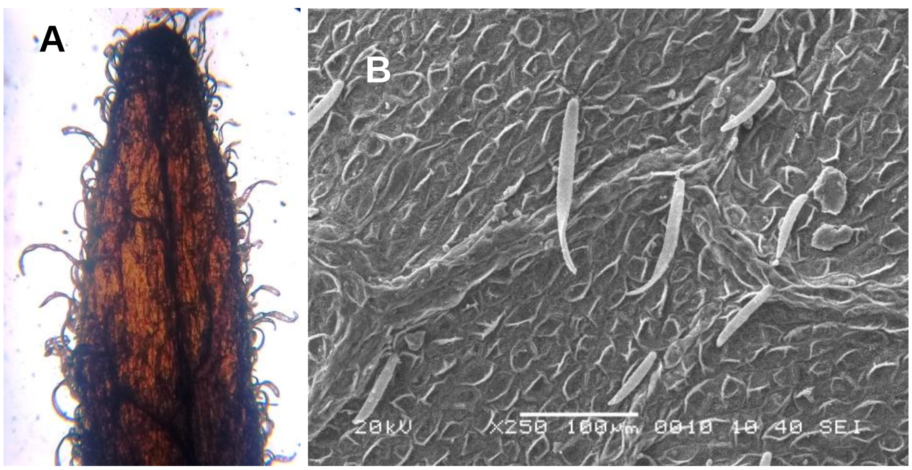


Fig. 9. Trichomes. A, Trichomes on the surface of young stipule; B, SEM view of trichomes (around 150µm in length, some twisted at the apex) and several stomata scattered over the ventral surface of leaf.

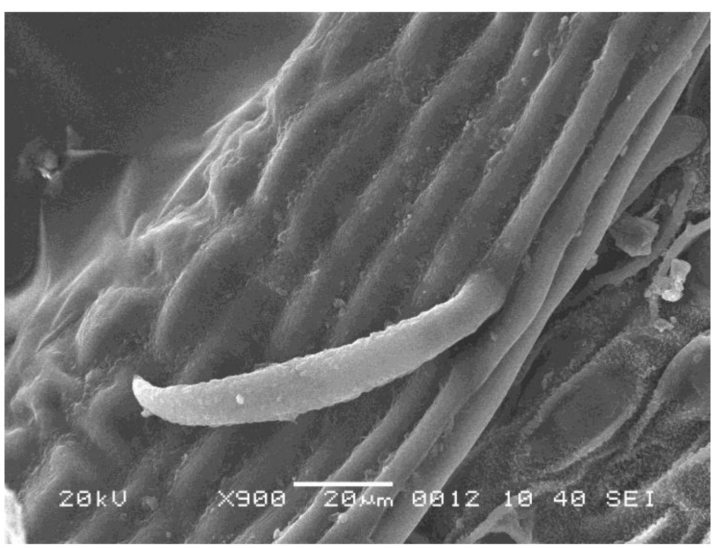
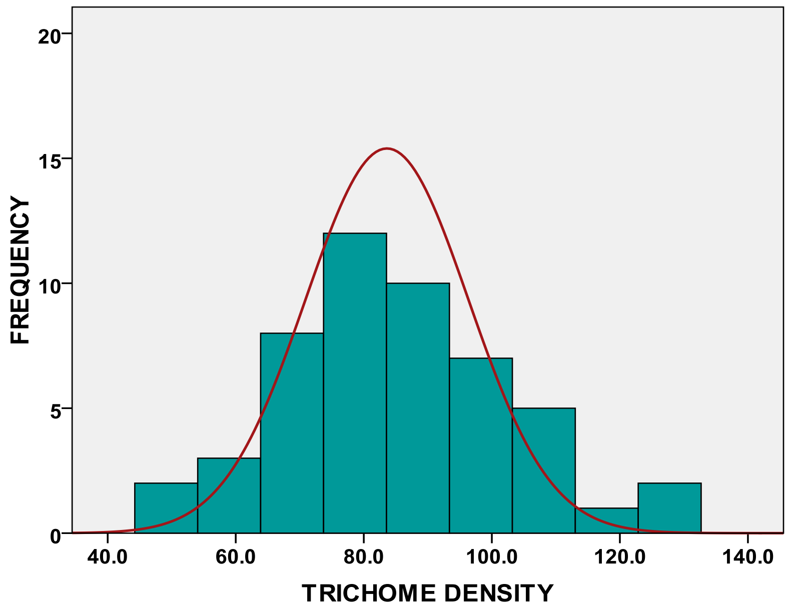


Fig. 10. SEM view of a unicellular trichome arising from the vascular region of ventral surface of leaf.



Trichome density per mm^2

N = 50
Mean = 85.12
SE = 2.5545
Median = 83.55
CV = 21.20%
G1 = 0.300
Sg1 = 0.337
G2 = 0.010
Sg2 = 0.662
Minimum = 49.14
Maximum = 127.78
KS-z = 0.983
P < 0.278

Fig. 11. Frequency distribution of trichome density per mm^2 of leaflet (c. 1.2 cm^2 one-sided surface area). KS-z, Kolmogorov-Smirnoff z; G1, skewness; g2, kurtosis, Sg1 and Sg2, Standard errors of skewness and kurtosis.

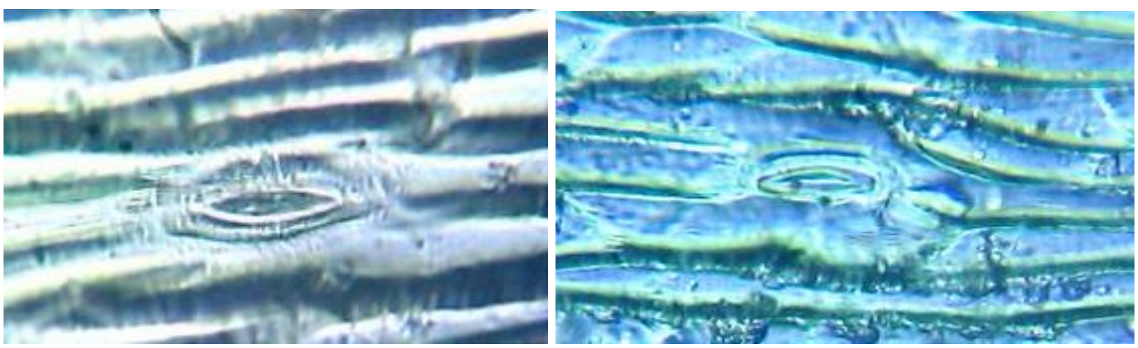


Fig. 12. Hypocotylar surface showing stomata (anomocytic). Magnification: 45 x 10 X).

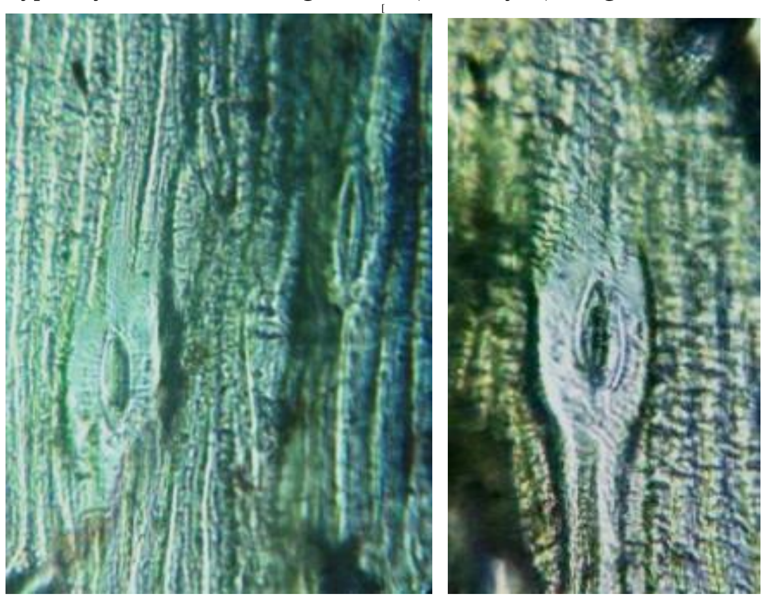


Fig. 13. Stomata on the epicotylar stem surface (paracytic, anisocytic). Magnification: 45 x 10 X.

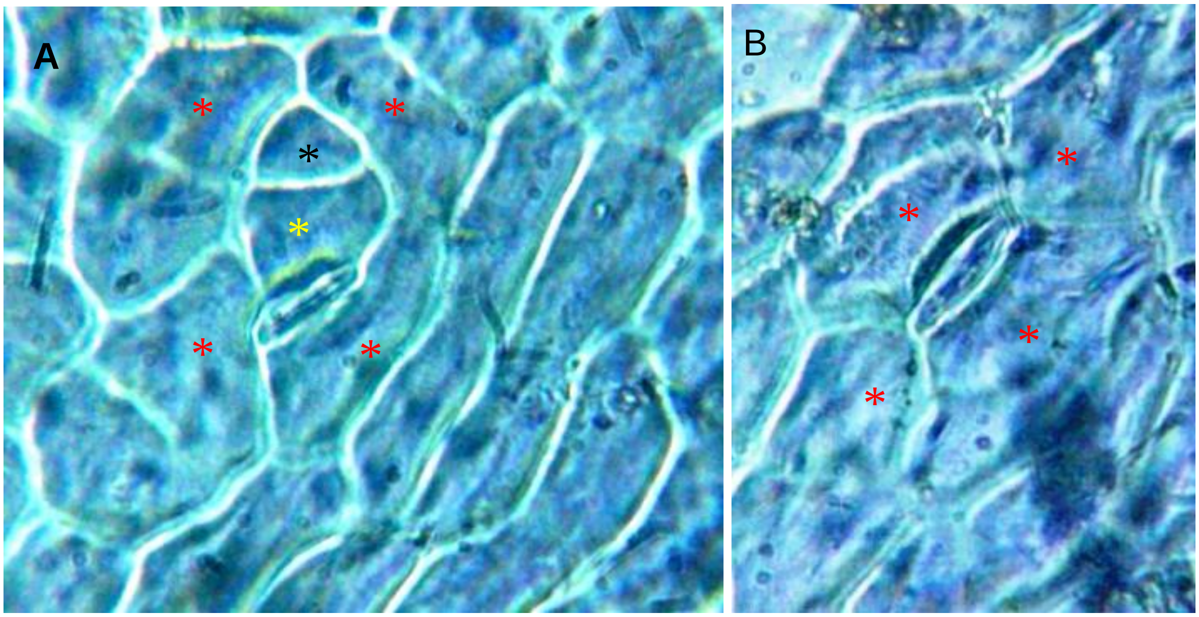


Fig. 14. A) a meristemoid (shown by a black asterisk) abutting an anisocytic stoma on dorsal surface of leaf (subsidiaries shown by red asterisks) – common subsidiary shown with a yellow asterisk. The meristemoid should have developed into an anisocytic stoma. B) A tetracytic stoma. Magnification: 45 x 15 X, zoomed)

Epidermis

Epidermal cells polygonal in shape. Anticlinal walls of the epidermal cells straight, arcuate or sinuous.

Stomata

Stomatal types occurring on various organs of *C. fistula* seedlings are described in Table 1. *C. fistula* leaflets were amphihypostomatic, contrary to Brigida *et al.* (2015) who reported *C. fistula* to be hypostomatic. In the 19 spp. of genus *Cassia* studied both hypostomatic and amphistomatic leaves have been reported. *Cassia javanica, C. siamea, C. surattensis, C. suffruticosa, C. spectabilis, and C. fistula* were reported to be hypostomatic and *C. absus, C. tora, C. occidentalis*, *C italica, C. splendida, C. alata, C. senna, C. sophera, C. hirsuta, C. sericea, C. auriculata, C. kleinii* and *C. mimosoides* were amphistomatic (Kotresha and Seetharan, 2000). However, we found stomata also on dorsal surface of *C. fistula* leaf that were generally confined to vascular or in near vascular region of leaf where anticlinal walls of the cells were almost straight (Fig. 14 & 15) whereas in the laminar region the epidermal cells were straight to arcuate (Fig. 16). Tripathi and Mondal (2012) reported *C. fistula* leaves to be amphistomatic similar to *C. alata, C. occidentalis, C. sophora* and *C. tora* (see Table 2). The stomata were oriented in various directions. Cuticular outer ledges of stomata developed in a protective membranous rim around the stomatal pore.

Taken together all of the organs (hypocotyl, cotyledons, epicotyl and leaflets) paracytic, anisocytic, tetracytic, 1½ cyclic tetracytic, staurocytic, anomocytic and abnormal contiguous stomatal type were seen in *C. fistula* seedlings. They were elliptical and oriented in various directions. Stomatal types in *C. fistula* have variously been reported. Tripathi and Mondal (2012) and Brigida *et al.* (2015) reported paracytic arrangement of subsidiaries. Khan, F. *et al.* (2014) have reported it to be of anisocytic type whereas Pandya *et al.* (2012) reported it to be paracytic and anomocytic. Metcalfe and Chalk (1950) reported only two types of stomata in genus Cassia – paracytic and anisocytic. The Stomata diversity (size, shapes, types and orientation) in the foliar epidermis has great value in plant systematic studies. A comparative micro-morphological study of stomata of 45 leguminous species (Tripathi and Mondal, 2012b) of diverse habits and habitats (terrestrial, marshy, climbers and shrubs and trees) showed that 31 species were amphistomatic and 14 hypostomatic in nature. The most diversified stomata were observed in trees species. The maximum numbers of tree species were hypostomatic which reflected that the habits and stomata appearance on foliar epidermis might be greatly co-related. Three types of stomata were observed viz. paracytic (64.1%), anisocytic (46.6%) and anomocytic (33.3%). Among these 3 types of stomata, the paracytic type of stomata was very common. In genus *Cassia* L., four types of stomata have, therefore, been reported – paracytic, anisocytic, tetracytic and anomocytic (Kotresha and Seetharan, 2000). We found that all the four types of stomata were present in *C. fistula* at seedling stage. The subsidiaries associated with a stoma, may be unequal in size and their number varies. Kotresha and Seetharan (2000) reported that the number of subsidiaries in a stoma varied from

2-8. In our studies, we found an anomocytic stoma on the surface of hypocotyl surrounded by 11 subsidiaries (Fig. 4B).

Table 2. Stomatal types reported in some species of genus *Cassia*.

Species	STOMATAL TYPES		
	Adaxial surface	Abaxial surface	References
C. abasus	Paracytic	Paracytic	Kotresha & Seetharan (2000)
C. alata	Anisocytic	Anisocytic, paracytic	Tripathi and Mondal (2012a)
C. alata	Anisocytic, Paracytic	Anisocytic, Paracytic	Kotresha & Seetharan (2000)
C. auriculata	Paracytic, tetracytic	Anisocytic, paracytic, tetracytic	Kotresha & Seetharan (2000)
C. bakeriana	Paracytic	Paracytic	Sihanat *et al.* (2015)
C. fistula	Paracytic	Paracytic	Tripathi and Mondal (2012a)
C. fistula	Paracytic	Paracytic	Rani and Satish (2014)
C. fistula	-	Paracytic, anomocytic	Pandeya *et al.* (2012)
C. fistula	-	Paracytic	Saheed and Illoh (2010)
C. fistula	-	Paracytic, Anomocytic	Banerjee *et al.* (2004)
C. fistula	-	Paracytic, tetracytic	Kotresha & Seetharan (2000)
C. fistula	-	Paracytic, anomocytic	Pandya *et al.* (2012)
C. fistula	-	Paracytic	Sihanat *et al.* (2015)
C. fistula	-	Anisocytic	Khan, F. *et al.* (2014)
C. grandis	-	Paracytic	Sihanat *et al.* (2015)
C. hirsuta	Paracytic	Paracytic, anisocytic	Kotresha & Seetharan (2000)
C. italica	Anisocytic, paracytic, tetracytic	Anisocytic, paracytic, tetracytic	Kotresha & Seetharan (2000)
C. javanica	-	Anomocytic, paracytic, tetracytic	Kotresha & Seetharan (2000)
C. javanica	-	Paracytic	Sihanat *et al.* (2015)
C. kleinii	Anisocytic, paracytic	Paracytic	Kotresha & Seetharan (2000)
C. mimosoides	Paracytic	Paracytic	Kotresha & Seetharan (2000)
C. occidentalis	Paracytic	Anisocytic, paracytic	Tripathi and Mondal (2012a)
C. occidentalis	Paracytic	Paracytic	Kotresha & Seetharan (2000)
C. occidentalis	Paracytic	Paracytic, anisocytic	Rani and Satish (2014)
C. siamea	No stomata	Anomocytic, paracytic	Tripathi and Mondal (2012a)
C. sophora	Paracytic	Anisocytic, paracytic	Tripathi and Mondal (2012a)
C. spectabilis	-	Paracytic, tetracytic	Kotresha & Seetharan (2000)
C. splendida	Anisocytic, paracytic	Anisocytic, paracytic, tetracytic	Kotresha & Seetharan (2000)
C. suffruticosa	-	Anisocytic	Kotresha & Seetharan (2000)
C. surettensis	-	Anisocytic, tetracytic	Kotresha & Seetharan (2000)
C. tora	Paracytic	Anisocytic, paracytic.	Tripathi and Mondal (2012a)
C. tora	Paracytic	Paracytic. anisocytic	Rani and Satish (2014)

Fifteen basic and 25 combinations foliar stomatal distribution patterns have been recorded in 95 legumes studied by Leelavathi *et al*. (1980) from India. Gill *et al.* (1982) have reported diacytic stomata to be by far the most common in Nigerian legumes (74 species studied). Stomatal diversity was more in Caesalpinoideae and papilionoideae than in the Mimosoideae. Some workers, however, appeared to have mistaken in identifying stomatal types in Nigerian legumes and some have ignored the existence of diacytic stomata in legumes. Edeoga *et al. (*2008) appeared to have misidentified paracytic stomata as diacytic from species of *Mimosa* (*M. pudica*, *M. pigra* and *M. invisa*) and Idu *et al. (*2000) failed to notice the existence of diacytic stomata in *Albizia zygia*, and some other species. They identified paracytic, anisocytic and tetracytic stomata in Nigerian legumes but overlooked desmocytic stomata in *Amphimas pterocarpoides.* Some species of *Trifolium* and *Ononis* from Egypt have been reported to bear diacytic stomata besides paracytic, brachyparacytic and anomocytic stomata (Taia, 2004). The species of genus *Cassia* has not been reported to bear diacytic stomata.

In view of our studies in *C. fistula* seedlings, the basic type of stomata appeared to be 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 in Fig. 21. Such a structure by further development of cell walls (Fig. 22) may change to anomocytic type. Our results are in confirmation to the speculation made by Stace (1966). He 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."*

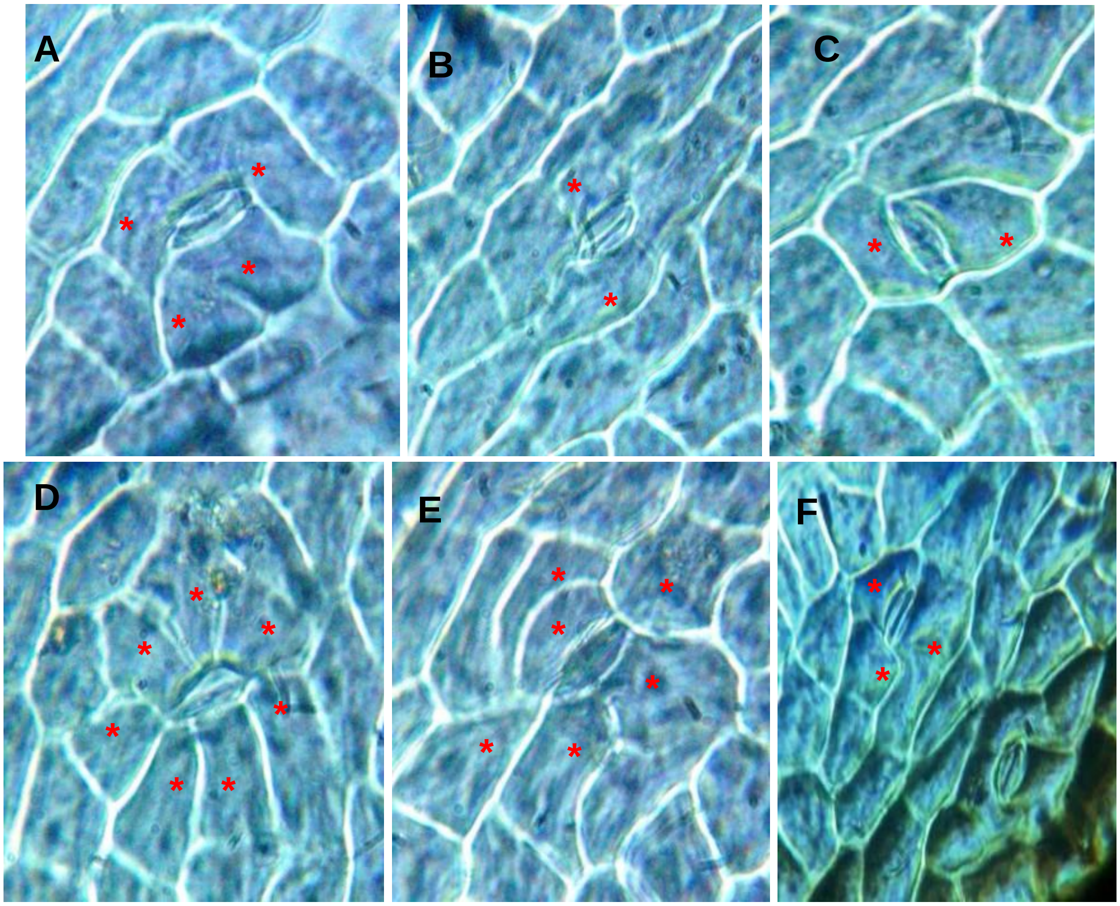


Fig. 15. Some stomatal types on the dorsal surface of young leaflet. A) Developing anomocytic stoma, B and C) paracytic, D) Anomocytic, E, 1 ½ cyclic tetracytic and F) An anisocytic stoma.

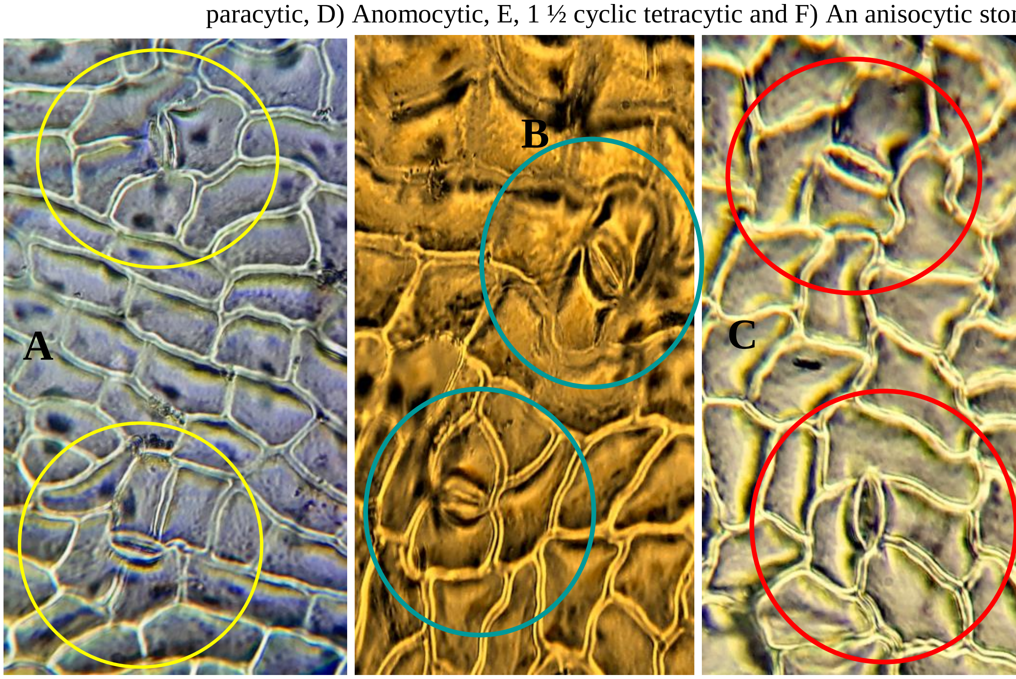


Fig. 16. Dorsal surface of a leaflet of *C. fistula* seedling. A, Two stomata (each with four subsidiaries, staurocytic and tetracytic), on either side of the vascular nerve; B, two paracytic stomata; C, anisocytic and tetracytic stomata. All these stomata were on the vascular nerve or near vascular region.

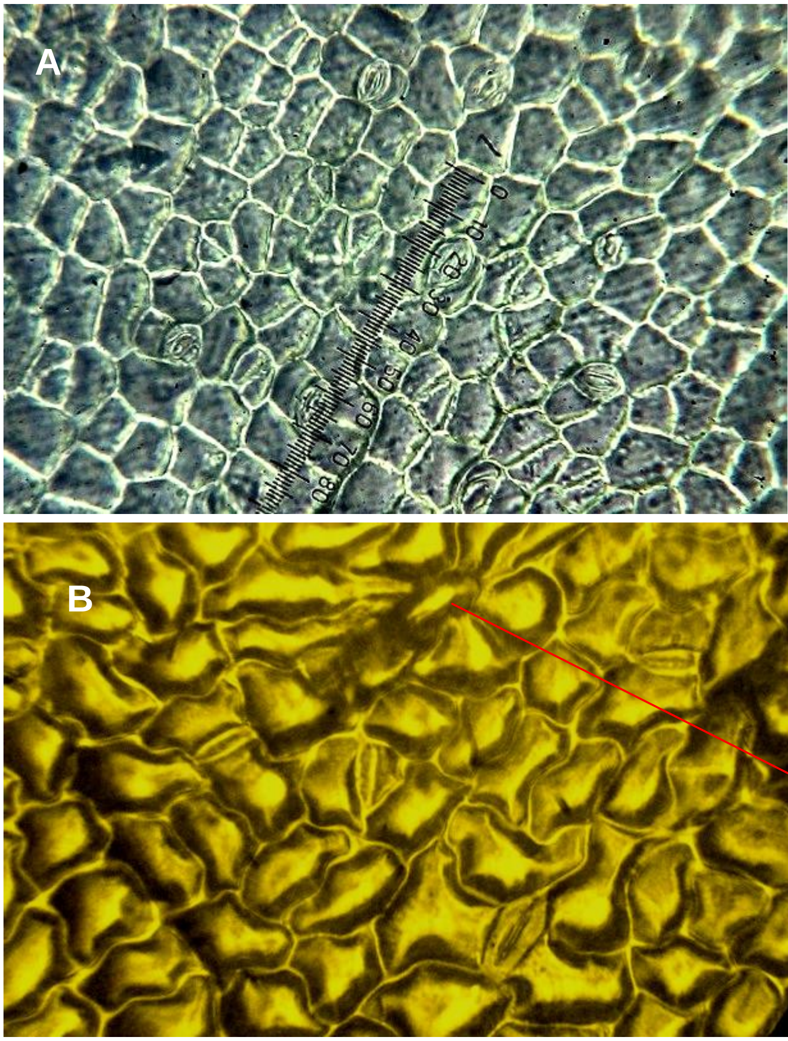


Fig. 17 (A & B). Nail polish imprint of ventral surface of leaf showing various types of stomata (Paracytic, anisocytic and anomocytic and stomata with common subsidiary). (Magnification: A, 15 x10 X) and B, 45 x 10 X.

Moreover, the SEM image of leaf surface disclosed that stomata in *C. fistula* are provided with raised labium like structure forming an elliptical rim (Fig. 21 & 23) probably due to the strong growth of outer ledge. Metcalfe and Chalk (1979) have reported stomatal rim in form of a funnel-like structure in *Coriaria nepalensis* with long aperture. Stomatal size in this image of stomata appeared to be 7.57 x 3.30 µm.

Epicotylar stomatal size and density

Epicotylar stomata, in our studies, generally admeasured 44.8 – 48-micron x 17.6 – 24.0 µ. The pore length measured 36.8 x 32µ. Stomatal density was found to be low (78.06 ± 2.89 stomata per mm^2).

Cassia fistula was reported by Tripathi and Mondal (2012b) to exhibit epicotylar stomatal density of 87.34 per mm^2 and size of the stomatal complex (pore + guard cells). Stomata were 13.74 (13.04-14.65) x 11.66 (11.32-12.59) µm in size on adaxial surface and 15.22 x 10.25 um abaxially which is much smaller *than* the size of stomata reported by Khan *et al.* (2014) in *Erythrina suberosa* (59.2 ± 31.9µm in length and 20.2 ± 11.3µm in width. Stomatal size in *C. fistula* was found to be smaller as seen in Fig. 23 – (7.57 x 3.30 um) which was smaller than several other species of genus *Cassia* (Tripathi and Mondal, 2012a). In another paper, they reported stomatal size of *C. fistula* to be 13.74 x 11.66 µm (Tripathi and Mondal, 2012 b). Kotresha and Seetharan (2000) reported stomatal size in genus *Cassia* be 16 x 16 to 32 x 22 µm on adaxial and 20 x12 to 32 x 24 µm on abaxial surface of leaves.

Cotyledonary stomatal density

Cotyledons of *C. fistula* are amphistomtatous. Young cotyledons showed higher stomatal density per mm^2 than mature cotyledons on upper as well as lower surfaces. The distribution of the stomatal density in both young and mature cotyledons tended to be normally-distributed as indicated by the insignificant value of KS-z (Table 3). The stomatal density was somewhat higher on upper surface (233.49 ± 4.085 stomata per mm^2 than lower surface

(212.85 ± 4.99 stomata per mm^2). The substantially lower density of stomata in case of mature cotyledon compared to the young one may presumably be attributed to the cotyledonary expansion with age of the seedling.

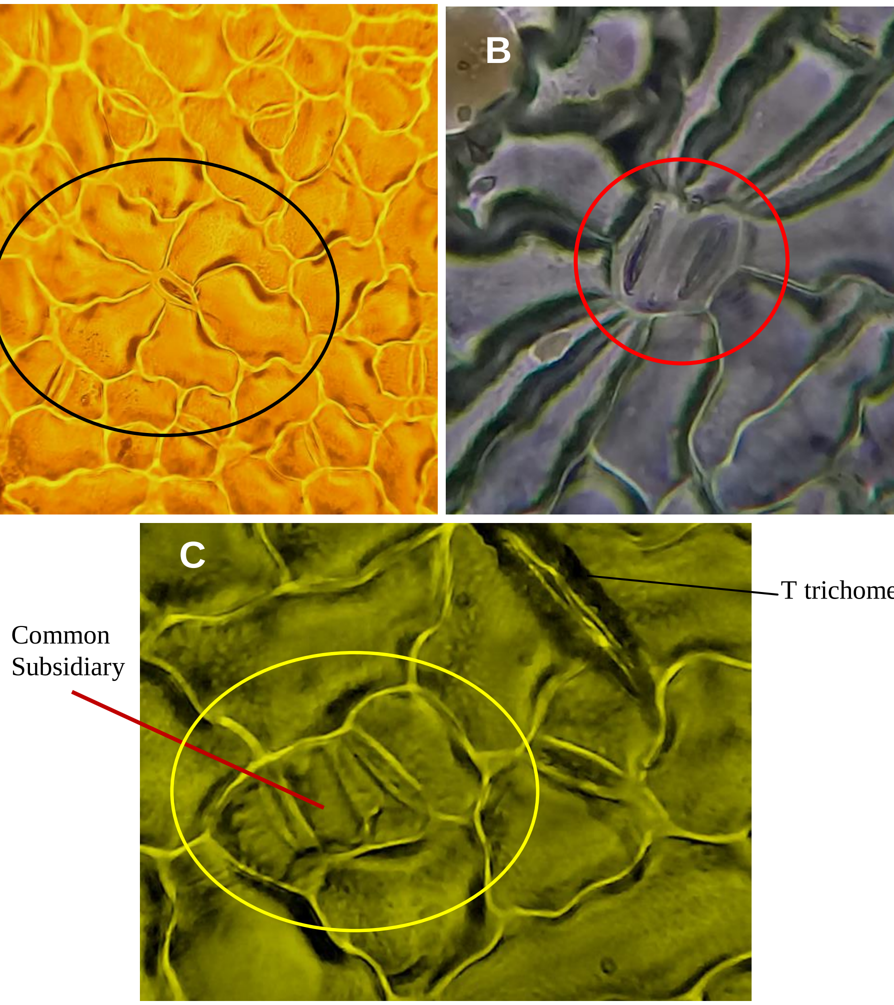


Fig. 18. Ventral surface of leaf – A, an anomocytic stoma; B, an abnormal stoma with two contiguous pores surrounded with 10 subsidiaries arranged in anomocytic manner; C, Two paracytic stomata with a common subsidiary. Note the radial walls of epidermis – arcuate to wavy. Magnification: 45 x 15X.

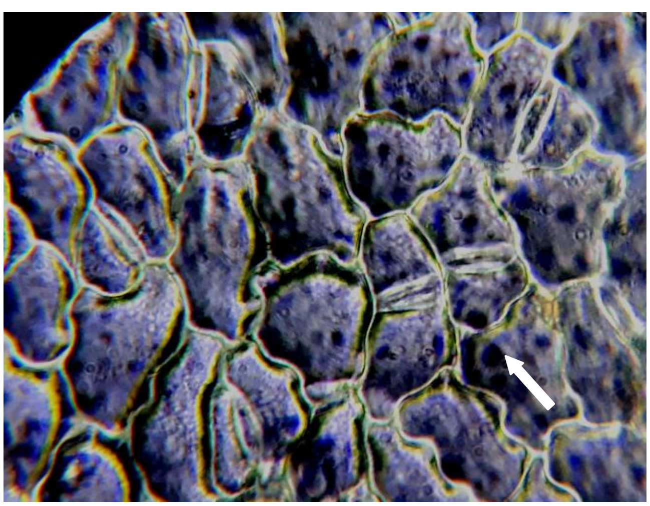


Fig. 19. Five Paracytic stomata linearly arranged in a group (white arrow) on the ventral surface of a terminal leaflet. Magnification: 45 x 15 X.

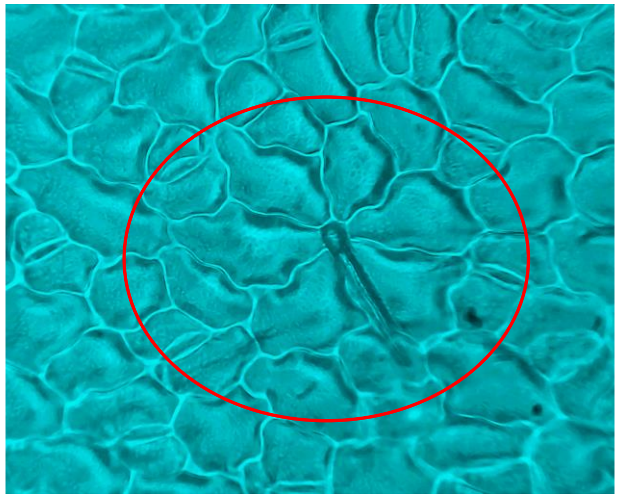


Fig. 20. Ventral surface of leaf showing some paracytic stomata and the radiating pavement cells surrounding the basal cell of a trichome (in a circle). Magnification: 45 x 10 X.

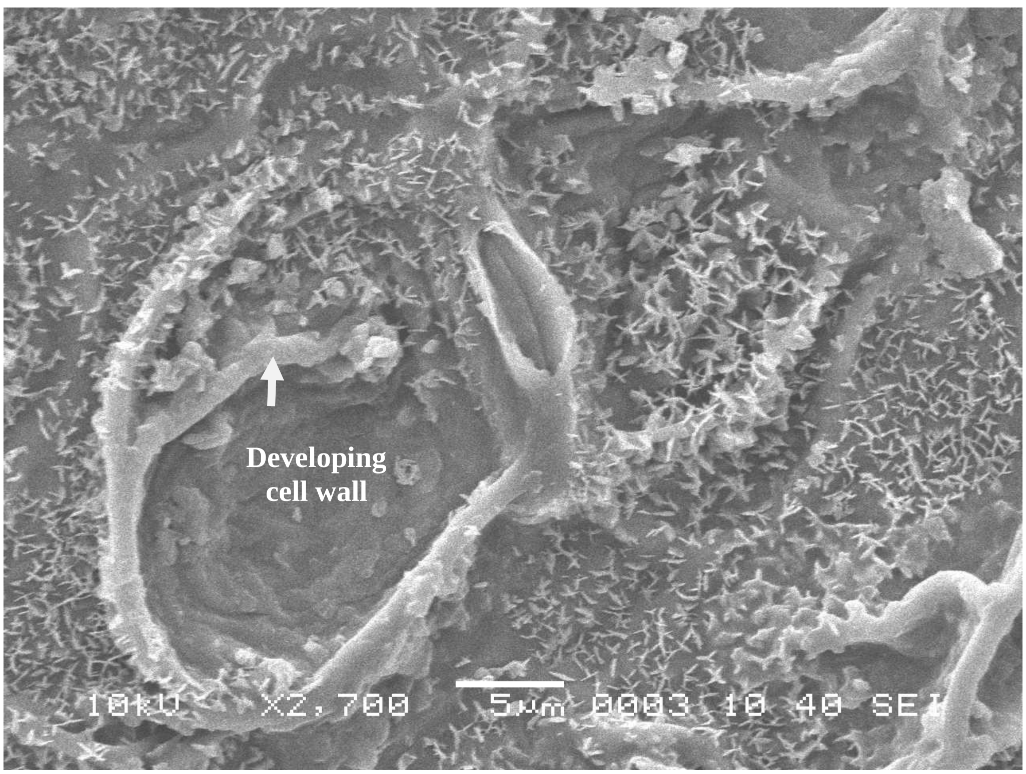


Fig. 21. SEM view of paracytic stoma developing into an anisocytic stoma by developing cell wall in a subsidiary cell on ventral surface of leaf. Magnification: 2700X.

Foliar stomatal density

Stomatal density on dorsal surface of the leaflets was not determined due to stomatal rarity. On ventral surface of terminal leaflets stomatal density varied considerably among leaflets (CV: 19.66 to 35.85%). It averaged to 222.6 ± 7.99, 146.52 ± 6.83, 139.59 ± 9.13 and 199.01 ± 10.58 stomata per mm^2 in four leaflets, respectively (Table 4). The pooled sample averaged to 176.94 ± 5.38 stomata per mm^2 varying aroundv 33.3%. In all samples the variable of stomatal density tended to follow normal distribution in indicated by the insignificant values of KS-z.

Sihanat *et al.* (2015) have reported stomatal density on abaxial surface of *C. fistula* leaf to be quite higher (320.8 ± 7.54 per mm^2; 320 to 342) substantially lower than that in case of *Cassia grandis* (431.47 ± 7.75, 420 to 454), *C. bakeriana* (426.80 ± 10.94, 40 to 442) and *C. javanica* (466.93 ± 12.04, 440 to 488).

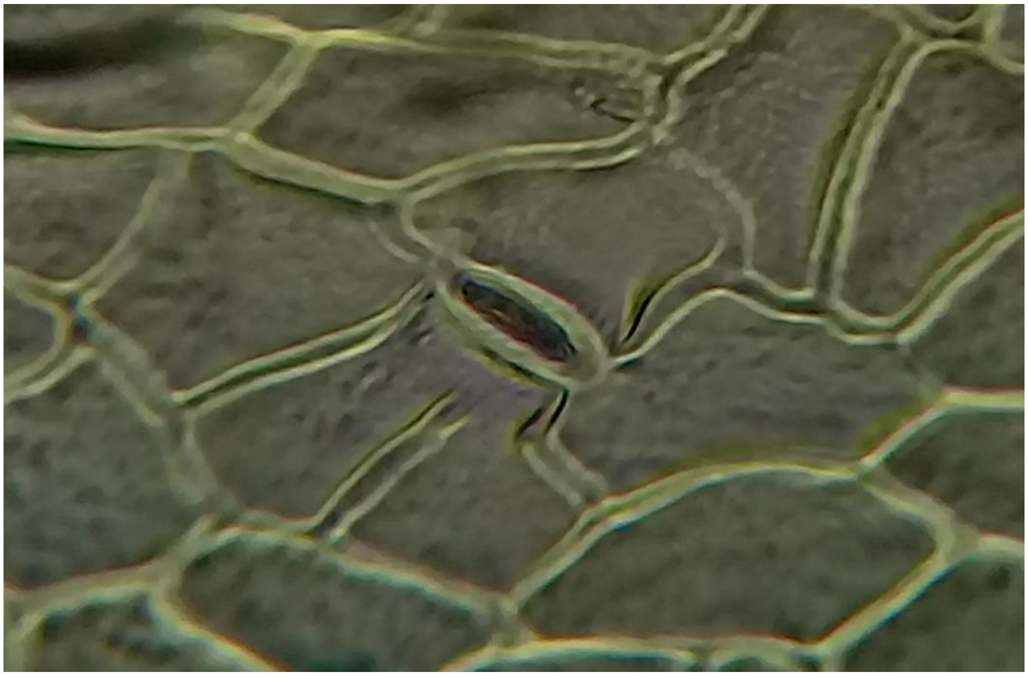


Fig. 22. Dorsal surface of leaf : a stoma under transformation into an anomocytic stoma by development of walls in the subsidiary cells. Magnification: 45 x 15 X, zoom 2X.

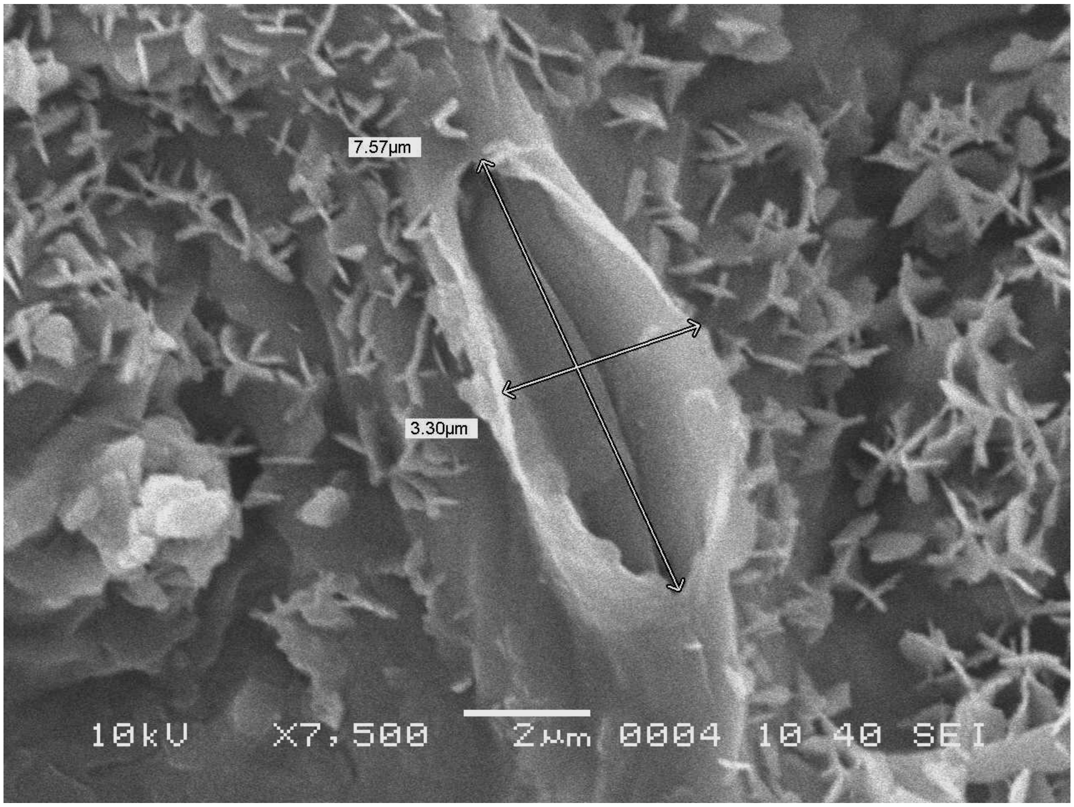


Fig. 23. SEM view of stoma and cuticular encrustation on ventral surface of leaf of *Cassia fistula* (magnification of 7500X). Stomatal outer ledges form a protective rim around the stomatal pore. The outer stomatal ledge aperture measured 7.57 um in length and 3.30 um in width and rose to little > 2 µm in height above the stomatal pore. Scattered epicuticular wax rosettes of platelets are obvious.

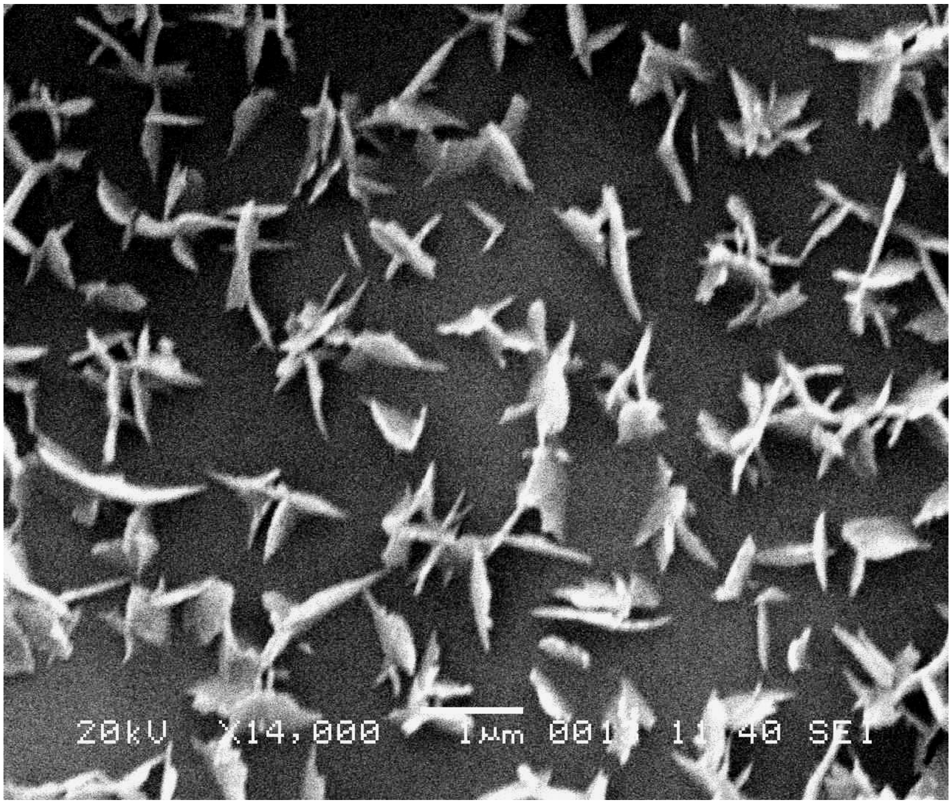


Fig. 24. SEM of Faballes or rosette type of epicuticular wax crystalloids (non-entire platelets) at magnification of 14000X.

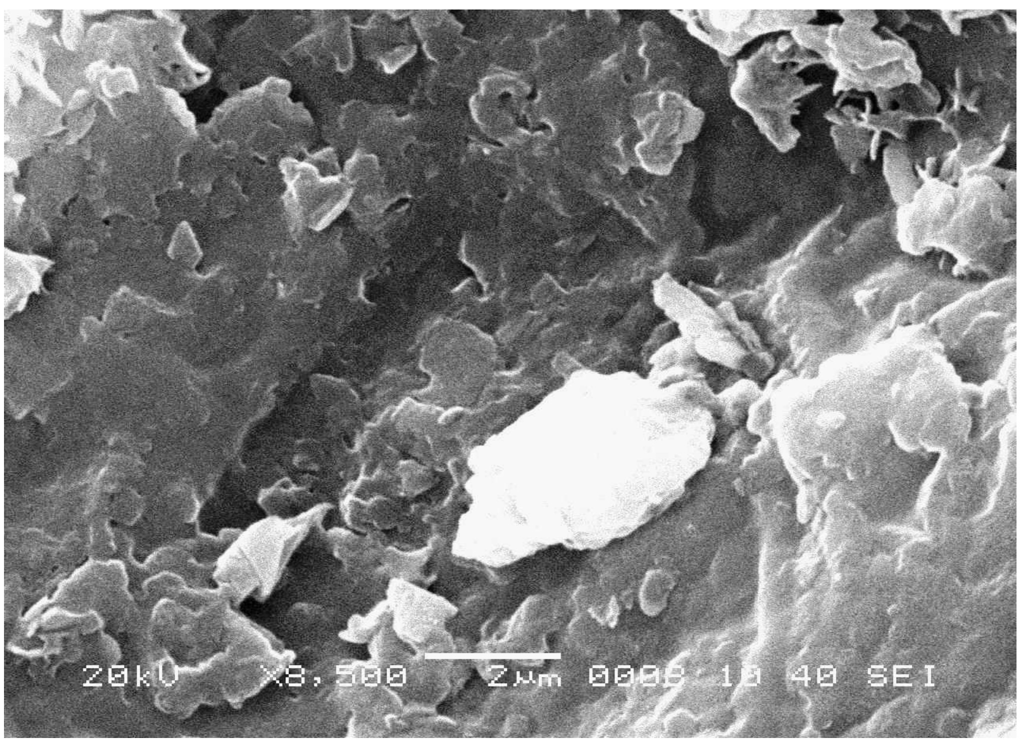


Fig. 25. SEM view of epicuticular wax in form of non-entire platelets and the lumps formed presumably due to fusion of rosettes of non-entire platelets in the vicinity of vein in the leaf base region on ventral surface. Magnification: 8500X.

Table 3. Cotyledonary stomatal density per mm^2 on upper (dorsal) and lower (ventral surface) in variously-aged cotyledons.

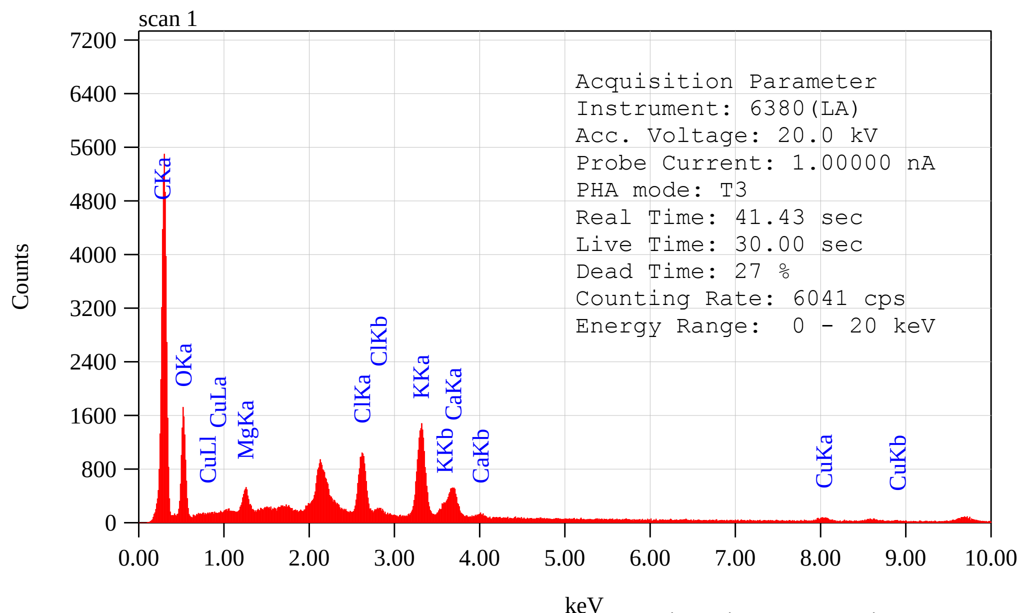
Parameters	Upper surface				Lower surface			
	Cotyledon (Mature)	Cotyledon (Adult)	Cotyledon (Adult)	Cotyledon (Young) *	Cotyledon (Mature)	Cotyledon (Adult)	Cotyledon (Adult) *	Cotyledon (Young)**
N	30	30	30	45	30	30	40	50
Mean	136.13	100.37	140.75	233.49	184.01	159.21	114.21	212.85
SE	5.549	4.0985	4.2599	4.849	5.214	7.157	4.669	4.999
Median	138.439	103.829	138.439	224.964	190.35	155.74	121.35	207.657
CV (%)	14.98	22.37	16.69	13.99	15.52	24.62	25.86	16.61
G1	-0.274	0.398	-0.346	0.378	0.439	0.078	0.472	0.518
Sg1	0.427	0.427	0.427	0.354	0.0427	0.427	0.374	0.337
G2	-1.042	-0.325	-0.632	-0.498	-0.723	-0.439	-0.267	-0.167
Sg2	0.833	0.833	0.833	0.695	0.833	0.833	0.733	0.662
Minimum	86.52	69.22	86.52	173.05	138.4	86.52	69.22	155.74
Maximum	190.35	155.35	173.05	311.49	242.47	242.27	190.35	311.49
KS-z	1.135	1.084	1.123	1.101	1.126	0.558	1.112	0.978
p	0.152	0.190	0.164	0.177	0.159	0.915	0.169	0.294

*, Cotyledonary area c. 1.5 cm^2; **, Cotyledonary area c. 1.0 cm^2 (just emerged out of testa). Adult and mature cotyledons were as large in size as around 1.76 cm^2). KS-z, Kolmogorov-Smirnoff z; G1, skewness; G2, kurtosis, Sg1 and Sg2, Standard errors of skewness and kurtosis.

Table 4. Foliar stomatal density per mm^2 on the ventral surface of terminal leaflets of *C. fistula*.

Parameters	TERMINAL LEAFLETS				
	A	B	C	D	Pooled sample
N	30	30	30	30	120
Mean	222.66	146.52	139.59	199.01	176.94
SE	7.9899	6.8285	9.1243	10.5849	5.3775
Median	207.66	155.74	129.79	207.66	173.05
CV (%)	19.66	25.53	35.85	29.13	33.29
G1	0.229	-0.191	0.113	-0.207	0.108
Sg1	0.427	0.427	0.427	0.427	0.221
G2	-0.981	-0.626	0.380	-0.822	-0.471
Sg2	0.833	0.833	0.833	0.833	0.438
Minimum	155.74	17.30	17.30	86.52	17.30
Maximum	294.18	259.57	259.57	294.18	294.18
KS-z	1.100	0.716	0.934	0.764	0.928
p	0.178	0.684	0.348	0.604	0.356

A, young and small; B and C, Adult and D, Adolescent. KS-z, Kolmogorov-Smirnoff z; G1, skewness; g2, kurtosis, Sg1 and Sg2, Standard errors of skewness and kurtosis. KS-z, Kolmogorov-Smirnoff z; G1, skewness; G2, kurtosis, Sg1 and Sg2, Standard errors of skewness and kurtosis.



ZAF Method Standard less Quantitative Analysis
Fitting Coefficient: 0.5229

Element	(keV)	mass%	Error%	At%	K
C K	0.277	52.32	0.26	63.66	43.3189
O K	0.525	33.99	1.13	31.05	27.1152
Mg K	1.253	1.28	0.34	0.77	1.5912
Cl K	2.621	3.13	0.26	1.29	7.4069
K K	3.312	5.73	0.36	2.14	13.1411
Ca K	3.690	2.05	0.43	0.75	4.7007
Cu K	8.040	1.51	2.05	0.35	2.7261
Total		100.00		100.00	

JED-2300 AnalysisStation JEOL

Fig. 26. Elemental concentration as determined by EDS for the field given in Fig. 24. Magnification 8500 X; Volt: 20kV; pixel: 1280 x 960.

Epicuticular wax crystalloids

Cuticular wax crystalloid forms seen on ventral surface of leaf are presented in Fig. 23, 24 and 25. In *Cassia fistula*, the wax crystalloids were composed of irregular platelets (rosettes of platelets) as per terminology of Barthlott *et al.* (1998). Platelets were of the approximate size 0.5 to 1.5 µm, variously shaped -like, oval, triangular, round, irregular or rhombic. The number of platelets per rosette averaged to 4.08 ± 0.45 (N = 35) and varied from 1 to 12. Rosette type of epicuticular waxes pattern has also been reported in *Bauhinia fornicate* by Lusa and Bona (2009). This type of pattern of epicuticular waxes is also reported by Barthlott *et al.* (1998) in leguminous *Calliandra haematoma* (Bert.) Benth. where the rosette consisted of 5-15 platelets, rarely 2-5 or more than 15 single platelets. The rosette-like pattern is also referred to as “Faballes-type” (Ditsch *et al.*, 1995). In *Cassia fistula*, wax crystalloid appeared to coalesce near base of leaf or by the sides of veins to forms lumps of non-entire platelets. Cuticular wax in *Trifolium pretense* and *T. hybridum* is also reported to be in form of platelets with irregular edges (Zoric *et al.*, 2009). Neves *et al.* (2016) have reported rosette type of epicuticular wax crystalloids from abaxial surface of *Dalbergia ecastaphyllum* leaf. Rosette pattern of wax crystalloids were also found in families Counaraceae, Malpighiaceae, Erythroxylaceae (Ditsch and Barthlott, 1997) and Asteraceae (Barthlott, 1998). The species of *Aristolochia* are known to bear rodlets (37 spp.) or irregular wax platelets (12 spp.) or mixed type (1 sp.) of epicuticular waxy crystalloids (Mahfoud *et al.*, 2018). Wax crystalloids of *Euphorbia cyparissias* leaf are quite similar in pattern (platelets crystalline) to that of *C. fistula* (Hemmers and Gülz, 1986). Thirty-two species of genus *Gethyllis* studied for epicuticular wax morphology yielded quite diverse results (Weiglin, 2001) – Non-entire platelets were observed in 12 species, entire platelets with transitions to granules in 7 spp., membranous platelets in 9 spp. and smooth layer in 8 species. Weiglin (2001) suggested that epicuticular characteristics may be employed for further division of the genus. Non-entire plates have also been reported in *Odosicyos* spec. (Cucurbitaceae). . Wax micromorphology bears close relationship with chemical composition and therefore significant meaning in the ecohydrtology of the plant (Baker, 1962; Meusel *et al.,* 1999). Plant cuticle appears to play an important role also in the processes ranging from development to interaction with the microbes (Yeats and Rose, 2013).

The cuticles of plants provide a multifunctional interface between plants and their environments. The cuticle with its associated waxes forms a protective layer that minimizes water loss by transpiration and provides several functions such as hydrophobicity, light reflection and adsorption of harmful radiations. Wax coating shows self-healing of voids on the surfaces (Koch *et al.*, 2009). Epicuticular waxes bring the "lotus effect" i.e. cleansing effect of leaves from debris (Barthlott and Neinhuis, 1997). Leaf epicuticular wax is considered to be an antixenotic factor in Brassicaceae for flea beetle (*Phyllotreta cruciferae*) rate and pattern of feeding (Badnaryk, 1992).

Wax composition of a species may vary for different parts of the same plant and also with season, locality and age of the plant (Tomaszewski and Zielińsky, 2014; Eglinton and Hamilton, 1967). The occurrence of well-formed epicuticular waxy pattern on leaflets of *C. fistula* seedlings pointed out to the fact that formation of waxy encrustation starts quite early in in the life of plants. This agrees with Tomaszewski and Zielińsky (2014).

Epicuticular waxes may differ in composition from intracuticular waxes (Buschhaus and Jetter (2011). Variation of form of foliar epicuticular waxy crystalloids is reported from *Phormium tenax* cultivars which may be useful as a diagnostic tool in cultivar identification (Carr *et al.*, 2009). They have, however, pointed out that it remains to be determined whether a given cultivar growing at different locations could retain the wax crystal morphology. We do not know the amount of the crystalloids on the leaf of *C. fistula*. Biswas *et al.* (2017) have reported that surface wax crystalloids amounts to 149 ± 0.21 mg / 100g fresh weight of leaves and 55 ± 0.21 mg per g fresh weight of pods of *Cassia alata*.

Determination of Elements

Elements detector system (EDS) based on Energy dispersive X-ray spectroscopy (EDS) attached to SEM was employed for element detection and quantitative elemental analysis. The result of scan for the specified foliar region is presented in Fig.26. In scan, the dominating element was carbon (52.32 ± 0.26%), followed by oxygen (33.99 ± 1.13%), Calcium (2.05 ± 0.43%), Potassium (5.73 ± 0.36%), Magnesium (1.28 ± 0.34%) and Chlorine (3.13 ± 0.26 %). In EDS analysis, since single shelled elements are not detected, we have no estimate of Hydrogen. However, the higher percentages of Carbon and Oxygen indicate the preponderance of organic molecules.

Table 5. Composition of mineral elements in *C. fistula.**

Elements	Leaf (mg / kg) (Raju *et al*., 2016) *	Whole plant (mg/kg) (Nadeem *et al.*, 2010) **
Na	117 – 319	537.0 ± 0.53 to 3691.9 ± 0.56
K	8501 – 21654	1490.62 ± 0.51 to 5098.75 ± 0.63
Ca	7520 – 9797	625.37 ± 0.52 to 1597.25 ± 0.74
Mg	2095 – 3700	177.3 ± 0.63 to 377.5 ± 0.14
Mn	190 – 974	109.36 ± 0.61 to 242.50 ± 0.75
Zn	17.9 – 22.3	12.62 ± 0.67 to 19.12 ± 0.61
Pb	-	2.12 ± 0.07 to 2.62 ± 0.06
Cu	-	5.25 ± 0.03 to 8.87 ± 0.08
Cr	-	2.01 ± 0.02 to 2.75 ± 0.04
Al	760 – 492	-
Fe	104 – 147	-
La	0.54 – 1.09	-
V	0.02 – 0.05	-
C	-	-
O	-	-
Cl	-	-

*, Mineral analysis performed on the basis of Instrumental Neutron Activation Analysis for leaf from three sites of Godavari, AP, India. Elemental composition varied with soil composition, location and climate. **, Analysis made on Flame photometry / Flame Atomic Absorption Spectroscopy. Data range for leaves, stem bark, gum and seeds.

Mineral or elemental elements from *C. fistula* are described in Table 5 on the basis of weight per unit plant mass from Godavari, Andhra Pradesh, India (Raju *et al.,* 2016) and Karachi, Pakistan (Nadeem *et al*., 2010).

Comparing the two data sets, there appeared a great disparity of mineral composition. K, Ca and Mg were very much higher in Indian sample based on leaf material only whereas Na was much higher in Karachi sample based on composite sample of all components of the plant (leaf, stem, bark, gum and seeds).

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