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Anatomical characters of taxonomic significance in *Syzygium caryophyllatum* (L.) alston and *Syzygium zeylanicum* (L.) DC

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Abstract
A study on the leaf and stem anatomy of *S. caryophyllatum* and *S. zeylanicum* was carried out. Fresh leaves and stem were fixed in alcohol: acetone (3:1) for 24 hours and carnoy's B solution for 24 hours and then subjected to infiltration and embedding. The measurements such as lamina, palisade, spongy layer thickness and midrib thickness, oil gland size, length and breadth of the epidermal cells from the adaxial and abaxial surface were taken following micrometry. The two species showed remarkable difference in all the parameters studied. Crystals were found as druses in *S.caryophyllatum* and prismatic crystals in *S. zeylanicum*. Oil glands were abundantly present. Two stomatal types have been recognised in *S. caryophyllatum* i.e. anisocytic and paracytic. Anomocytic stomata were recognised in *S.zeylanicum*. This information can be of use in refining the species systematic position.

Keywords: Anisocytic, anomocytic, druses, stone cells, *Syzygium*

Introduction

Syzygium is one of the genera which shares some of the diagnostic features such as glabrous, opposite, oil - dotted leaves with prominent intramarginal veins, flowers with 4 – 5 petals, round, concave, usually falling off as a calyptrate lid, numerous stamens that are bent inwards at the middle when in bud and the fruit is single or few- seeded berry. Even though the genera shares such unique features, the taxonomy of *Syzygium* and its generic allies in the old world has been confusing and complex (Craven, 2001) [1]. For a long time, anatomical characters are being used as an index in taxonomical studies (Radford *et al*., 1974) [2]. Many anatomical studies have been carried out for various genera in Myrtaceae and these features often compliment taxonomic problems where morphological differentiation was not clear. The genus *Eugenia* in the Myrtaceae family was one of the controversial genera to be defined (Amshoff, 1958) [3]. The classification problems have been extensively discussed (Kausel, 1956; Merrill & Perry, 1939) [4, 5]. Many species from both the old and new world were assigned to *Eugenia.* Airy Shaw (1949); Niedenzu (1893) [6, 7] confined *Eugenia* to American species and favoured the separation of most of the old-world species into a second large genus *Syzygium.* Based on the seed characters, Merrill & Perry (1939) [5] favoured the segregation of *Syzygium* from *Eugenia*. According to them, *Syzygium* contained naked embryo with two distinct cotyledons with loosely attached seed coat and the embryo in *Eugenia* was enveloped with a distinct seed coat. According to the studies of Henderson (1949); Wilson (1957) [8, 9] the extent of attachment of the testa to the pericarp and the degree of fusion of cotyledons was quite variable. On the other hand, the *Eugenia* and *Syzygium* floral anatomy such as the amount of schlerenchyma and tannins in the flower, ovarian tissue texture, vascular system architecture, large bundles of the floral tube etc were studied by Schmid (1970) [10] and provided strong evidence to segregate *Eugenia* from *Syzygium*. The anatomical features of *Eugenia* leaf studied by Hussin *et al*. (1992) [11] was also found to be useful in species identification: stomatal type, shape of midrib vascular bundle, cutinization of the outer wall, sclerenchyma sheath, shape of vascular strand, presence or absence of sclereids, idioblast, hypodermis, columnar epidermal cells, crystals, number of palisade layers and sclerenchyma sheath. Hence anatomy can be considered as an additional tool for taxonomic studies.

The present study aims to introduce the internal description of the leaves and stem of *S.caryophyllatum* and *S.zeylanicum.*

Materials and methods

A: The fresh materials of leaf and stem were fixed in alcohol: acetone (3:1) for 24 hours. The materials were then preserved in 70 % alcohol, followed by dehydration in ethyl alcohol and butanol series.

B: The fresh materials of leaf and stem were fixed in carnoy's B solution (chloroform: Alcohol: Glacial acetic acid) in the ratio 3:6:1 for 24 hours. The materials were then preserved in 70 % alcohol, followed by dehydration in ethyl alcohol and butanol series.

Infiltration and embedding

The dehydrated material was treated with a mixture of paraffin wax (melting point of 58° - 60°) and bee wax, with a small amount of butanol. The materials were kept at 60° C in an oven. After 24 hours, the molten paraffin was replaced with fresh molten paraffin wax. This process was continued for 7 days to remove last traces of butanol. The material was subsequently embedded in paraffin wax employing paper boat technique. The sections were cut on a rotary microtome (Lieca RM 2145). Sections were of 12 -14 µm thickness. The sections were stained with 2 % saffranin and mounted using DPX mountant (Sass, 1940) [12].

The epidermal peels were prepared by applying a layer of xylene and thermacol mixture. The leaf samples were kept overnight and then peeled off. The fresh sections of the stem were treated with iodine solution to confirm the presence of starch grains.

Results and discussion

1. Tranverse sections of leaf

The cuticle thickness is 2 µm and is found to be almost similar in both the species. Epidermal cells are square – shaped in *S.caryophyllatum* and square to rectangular in *S.zeylanicum.* Epidermis is uniseriate. The anticlinal walls of epidermal cells in *S.caryophyllatum* and *S.zeylanicum* are sinuate. In *S.zeylanicum*, the average length and breadth of epidermal cells in the abaxial surface ranges between 6 – 12 µm and 8 -18 µm respectively. The average length and breadth of epidermal cells in the adaxial surface ranges between 7 – 14 µm and 8 - 30 µm respectively. In *S.caryophyllatum*, the average length and breadth of epidermal cells in the adaxial surface ranges between 14 – 20 µm and 16 – 20 µm respectively. The average length and breadth of epidermal cells in the abaxial surface ranges between 6 -10 µm and 8 – 18 µm respectively. The lamina thickness ranges between 130 - 190 µm in *S.zeylanicum* and 290 - 420 µm in *S.caryophyllatum* (Table 1). The leaf margin contains lignified cells. (Fig1a, b). The cells in the leaf margin are slightly downwards in *S.zeylanicum.*

The hypodermis is absent in both the species. It is absent in many *Syzygium* species and is known to be present in species such as *S. gustavioides, S. ripicola and S. papillosum.* In *S. cumini, S. diospyrifolium* and *S. malaccense* the hypodermis is infrequently present (Soh & Parnell, 2011) [13]. The mesophyll is dorsiventral in both the species with 1 – 2 layers of palisade in *S. zeylanicum* and single layered in *S.caryophyllatum* with several layers of loosely arranged Spongy parenchyma. The palisade thickness ranges between 14 – 40 µm and 26 – 56 µm in *S.zeylanicum* and *S.caryophyllatum* respectively. The spongy thickness ranges between 22 – 154 µm and 158 – 200 µm in *S.zeylanicum* and *S.caryophyllatum* respectively. The adaxial midrib outline is concave, abaxial midrib outline is convex. The midrib thickness ranges between 830 – 1000 µm and 230 – 280 µm in *S.caryophyllatum* and *S.zeylanicum* respectively (Table 1). The shape of the vascular bundle in *S.caryophyllatum* is arc with incurved margin and arc with straight margin in *S. zeylanicum.* The vascular bundle is surrounded by sclerenchymatous sheath. Two layers of stone cells are present surrounding the vascular bundle in the midrib portion of both *S. caryophyllatum* and *S. zeylanicum* (Fig 2a, 2b). Few stone cells are also present around the vascular bundles of lamina. The xylem is surrounded by phloem with partition in the adaxial phloem and a single layered pericycle.

Crystals are present in both the species. They are present as druses in *S.caryophyllatum* and prismatic crystals in *S. zeylanicum* (Fig 3, Fig 4). Druses are reported in of *S. ripicola, S. siamense, S. winitii, S. megacarpum, S. laetum* subsp. *jugorum, S. malaccense, S. diospyrifolium, S. cumini, S. aromaticum, S. cinereum and S. albiflorum.* Crystals are reported *in S. claviflorum.* Species such as *S. aqueum, S. formosum, S. jambos, S. samarangense, S. zimmermanii* are reported to contain both druses and crystals (Kantachot, 2007) [14]. Crystals are insoluble calcium salts formed in specialized cells called crystal idioblasts varying considerably in size, shape and number. The number and occurrence of crystal idioblast varies among taxa (Vincent, 1980) [15]. They are located in spongy and palisade layers (in enlarged idioblasts). Based on the crystal type, the species can be differentiated from each other as druses are predominant in subgenera *Syzygium*, while prismatic crystals are predominant in subgenera *Acmena*, *Perikion* and *Sequestratum* (Soh & Parnell, 2011) [13]. Crystals in some species are known to have defence mechanism against herbivores (Lucas *et al*., 2000) [16], to regulate calcium levels in tissues (Volk *et al*., 2002) [17] and also known to contribute in light distribution to the chloroplasts (Franceschi & Nakata, 2005) [18].

Oil glands are abundant and are present in the mesophyll usually located in the adaxial and abaxial epidermis in the palisade and spongy mesophyll layers and are globose to sub – globose in shape (Fig 5a, Fig 5b). They are lined with epithelial – like cells. The oil gland size differed in dorsal and ventral surface. The oil glands are 68 µm and 45.62 µm in the dorsal and ventral surface respectively (Table 1). The oil gland size of *Syzygium* species varied from 40 - 100 µm in diameter (Soh & Parnell, 2011) [13]. Kantachot *et al*. (2007) [14] reported globular oil cavities in the mesophyll region in all the 28 species of Myrtaceae.

The leaves are hypostomatic i.e. the stomata are present only on the lower surface. Stomata are rounded or elliptic – shaped. The guard cells are kidney - shaped. Two stomatal types are recognised in *S. caryophyllatum*: Anisocytic, where guard cells are surrounded by three cells that are not radially arranged. The subsidiary cells are unequal in size with one of the three cells smaller than the other two cells and Paracytic, where the guard cells are flanked by two subsidiary cells, either extending over the poles of guard cells or not, and of equal or unequal size (Fig 6). Anomocytic stomata are recognised in *S. zeylanicum* (Fig 7). The presence of different types of stomata on leaf surface is useful in classification and delimitation (Patel & Inamdar, 1969) [19]. The *Syzygium* species have been reported to possess different types of stomata on leaf surface such as anomocytic and anisocytic in *S. aksornae*, anisocytic and cyclo – staurocytic in *S. claviflorum.* Paracytic and Anisocytic stomata in *S. apodophyllum*, *S.malaccense*. Kantachot *et al*. (2007) [14] has also reported paracytic and anisocytic stomata in *S.cumini*, *S. laetum* subsp. *jugorum* and *S. ripicola. S.zeylanicum* is reported to possess anomocytic stomata, where the cells surrounding the guard cells do not form any recognisable and consistent pattern, and they are not differentiated from other epidermal cells. They also report the presence of anisocytic stomata infrequently (Soh & Parnell, 2011) [13].

2. Tranverse section of stem

The stem transection of *S.zeylanicum* is circular in outline. Epidermis is uniseriate, covered with a thin layer of cuticle. This is followed by a multilayered cortex. In the vascular cylinder, the cambium produces phloem toward the outside, and xylem, toward the inside, showing one - layered parenchymatous medullary rays. At the centre of the stem, xylem encircles the pith. The pith is parenchymatous. Starch grains are found in the pith region. The prismatic crystals are found in the cortex, phloem and pith. Stone cells are present in the pith region and also surrounding the pith region.

The stem transection of *S.caryophyllatum* is ovoid in outline. Epidermis is two - layered covered with a thin layer of cuticle. This is followed by a multilayered cortex. Cortex is bound internally by a continuous sclerenchymatous ring. The phellogen is installed in the first layers of the cortex, giving rise to phellem outside. Oil glands are distributed in the cortical region. They are filled with oil globules. In the

vascular cylinder, the cambium produces phloem toward the outside, and xylem, toward the inside, showing one - layered parenchymatic medullary rays. The vascular bundles are conjoint, bicollateral and endarch. The pith is large and made up of compactly arranged parenchyma cells. Starch grains are found in the pith region (Fig 8a, 8b). Stone cells are present in the pith region.

Conclusion

The anatomical characters such as oil gland, prismatic crystals, druses, types of stomata, lamina, palisade, spongy and midrib thickness, the height and width of epidermal cells are highlighted in this study. The prismatic crystals were observed in *S.zeylanicum* and druses were observed in *S. caryophyllatum* and two types of stomata i.e. paracytic and anisocytic were present in *S. caryophyllatum* and anomocytic stomata in *S.zeylanicum.* The druses, paracytic and anisocytic stomata are the characteristic features of Subgenera *Syzygium* and prismatic crystals and anomocytic stomata are the characteristic features of Subgenera *Sequestratum*. Hence *S.caryophyllatum* and *S.zeylanicum* can be classified under two different subgenera i.e. *Syzygium* and *Sequestratum* respectively. The leaf and stem anatomical characteristic alone are inadequate for the delimitation of some generic groups. But these features provide additional evidence in identification and delimitation of species.

Table 1: Various attributes showing differences between *S.caryophyllatum* and *S.zeylanicum*

Attributes	*S.caryophyllatum*		*S.zeylanicum*	
Lamina thickness	357.3 ± 2.91		166 ± 1.24	
Palisade thickness	48 ± 4.84		28.26 ± 4.54	
Spongy thickness	184.5 ± 14.25		94.81 ± 30.89	
Oil gland	Dorsal 67.2 ± 7.67	Ventral 45.63 ± 7.20	-	
Midrib thickness	92.6 ±5.66		25.22 ± 1.39	
Epidermal cells (Abaxial)	Ht 8 ± 1.41	Wt 11.71 ±3.90	Ht 9.8 ± 2.2	Wt 12.2 ± 4.04
Epidermal cells (Adaxial)	Ht 16.25 ± 1.98	Wt 18.33 ± 1.96	Ht 9.7 ± 2.11	Wt 18.2 ± 6.76

Note: Each value is expressed as mean ± standard error (n = 20).

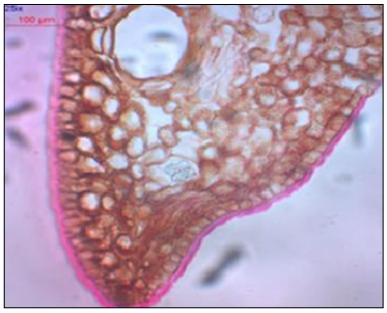

Fig 1a

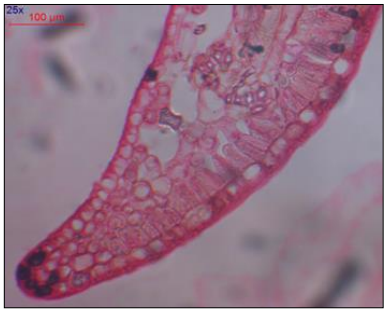

Fig 1b

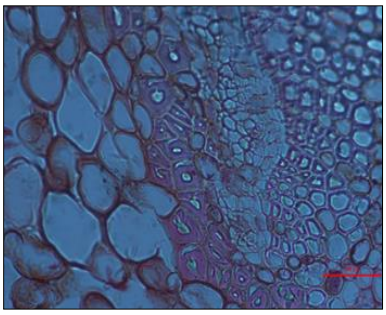

Fig 2a

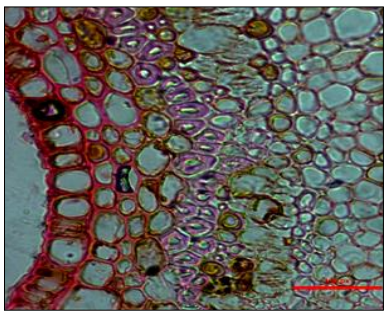

Fig 2b

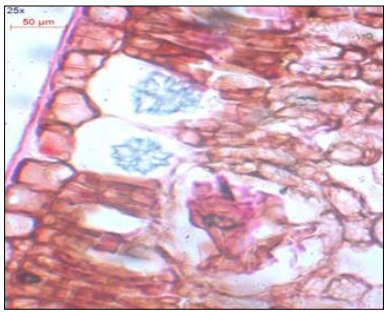

Fig 3

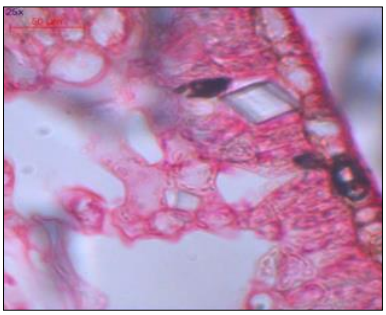

Fig 4

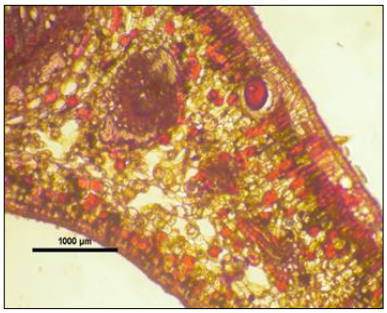

Fig 5a

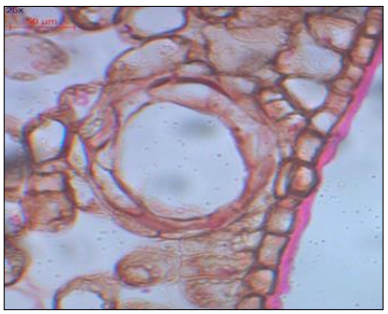

Fig 5b

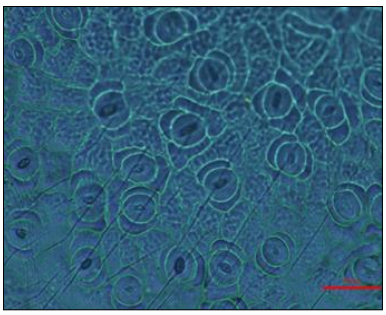

Fig 6

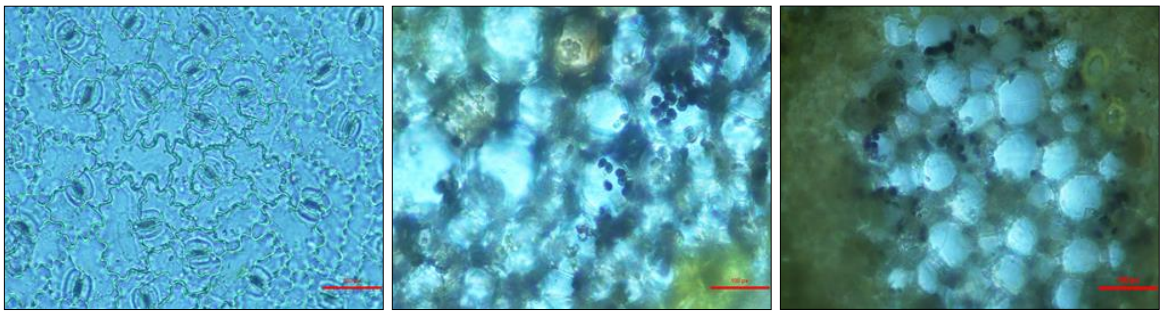

Fig 7 **Fig 8a** **Fig 8b**

Fig 1a: Lignified cells: *S.caryophyllatum*, **Fig 1b:** Lignified cells: *S. zeylanicum*, **Fig 2a:** *S.caryophyllatum*: Cells enlarged to show stone cells (SC), **Fig 2b:** *S.zeylanicum*: Cells enlarged to show stone cells (SC), **Fig 3:** Druses (D) present in the mesophyll region, **Fig 4:** Prismatic crystals (PC) present in the mesophyll region:, **Fig 5a:** Oil globule (Og) present in the oil gland, **Fig 5b:** Oil gland (OG), **Fig 6:** Lower epidermis with paracytic (P) and Anisocytic (An) stomata, **Fig 7:** Lower epidermis showing the presence of Anomocytic (A) stomata, **Fig 8:** Pith stained with iodine to confirm the presence of starch grains a) *S.caryophyllatum* b) *S. zeylanicum*

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