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SEEDLING CHARACTERISTICS OF *ERYTHRINA SUBEROSA* ROXB.

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ABSTRACT

Seedling characteristics of *Erythrina suberosa* Roxb. are described. Its seeds were collected from a tree growing in the Campus of University of Karachi and germinated without any dormancy breaking treatment in pots filled with garden sandy loam soil maintained at 75% MWHC. Maximum germination was 50% achieved within a week. The seedlings were studied when they were 20-day (younger) and 50-day old (elder). The seedling was of Phanerocotylar – Epigeal Reserve type. The major allocation of biomass in 20-day seedlings was in leaves and in 50-day seedlings in leaves and hypocotylar stem. The major growth spur in seedlings during the 30-day period from 20^{th} to 50^{th} day was in hypocotylar and epicotylar stem and root. Tap root had profuse laterals. Numerous root nodules 3.5 - 5mm in diameter were present generally on the main root. Cotyledons were large, green fleshy –food laden, concave inside and convex outside with no visible venation. They were wholly consumed within 50 days after germination. Internode size reduced from base to apex regularly. The hypocotyl was green, shining and provided with little prickles. Epicotyl is hairy. The primary leaves were simple opposite, erect, glaucous dorsally and pubescent ventrally. The subsequent leaves were pinnately trifoliate (ternate) with three leaflets. Each leaf had small, green and linear-lanceolate stipules. Glanduliform stiples present. Epicotyl was longer than hypocotyl. The total leaf area of elder seedlings was (209.08 ± 15.71 cm^2) -1.6 times to that in the younger seedlings. The leaf venation was pinnate camptodromous (festooned brachidodromous) type. Vein-endings were straight or curved and unbranched. Two types of trichomes were seen – branched trichomes and capitate glandular trichomes. The leaves were hypo-amphistomatic – paucistomatic dorsally and multistomatic ventrally. The cotyledonary stomata were of paracytic type but on ventral surface of leaf five types of stomata (*sensu* Prabhakar, 2004) - paracytic, anisocytic, anisotricytic, anomocytic and staurocytic were present; paracytic being the most abundant and staurocytic the least. Stomata on dorsal side of leaf were rare and of paracytic type only along the main vein. Both surfaces of leaf had capitate glandular trichomes (6.16 per mm^2 on ventral surface and much infrequent on the dorsal side). The number of stomata on ventral surface of the leaf tended to be normally-distributed amongst the 100 sampling fields of the microscope vision (each of 0.10174 mm^2) at 45 x 10 X magnification. The mean density of stomata per mm^2 was 110.28 ± 2.07 (68.80 – 157.3; CV: 18.73%).

Key Words: *Erythrina suberosa* Roxb., seedling characteristics, stomatal types; Stomatal density. Glandular Trichomes, Seedling leaf area.

INTRODUCTION

The genus *Erythrina* has 112 species, 70 Neotropical, 31 African and 12 Asian (Kass, 1998). da Silva *et al.*, 2013) have reported 120 species in genus *Erythrina*. In Pakistan, genus *Erythrina* is reported to be represented by three species – *E. suberosa* Roxb., *E. glabrescens* (Prain) Parker, and *E. herbacea* L. (Ali, 1977). *E. suberosa* has been introduced in Pakistan. *E. glabrescens*, however, according to the Annonated Checklist of the Flowering Plants of Nepal (www.efloras.org) and ILDIS (International Legume Database Information – www.ildis.org/Legumeweb?genus =Erythrina &species=glabrescens) is accepted as the synonym of *E. suberosa* Roxb. [www.efloras.org/florataxon .aspx?flora_id=110&taxon_id=242422319; www.theplantlist.org/tpl/record/ild-46920; www.legume-online.net /ildis/aweb/td_15999.htm]. *E. suberosa* is well-known for its multiple uses and alkaloids (Soto-Hernandez *et al.*, 2012). The base chromosome number of Erythrina is 21 which is not found in other legumes (Kass, 1998). The morphological characterization of seed and seedlings provide subsidies for not only species differentiation but also species recognition (Matheus and Lopes, 2007) particularly at juvenile stage in the field. Seedling morphology in dicotyledons has been comprehensively treated in Vogel (1980). Deb and Paria (1986) have published an account on seedling morphology of some economic species of India which is valuable document to identify them in juvenile form. Das and Paria (1999) have described seedling structure of nine *Bauhinia* species from India. Nenggan (1983-84) and Sinjushin and Akopian (2011) are useful publications on the seedling structure of legumes. ElKhalifa and Aref (2004) have studied seedlings of 14 Acacias of Saudi Arabia. Miller and Miller (2011) have investigated seedling development in 287 spp. of genus *Acacia* and described two seedling form in *Acacia* – pinnate: bipinnate and pinnate: pinnate forms based on primary and secondary leaves. Reddy and Shah (1979) investigated cotyledonary and hypocotylar stomata and trichomes in some Caesalpiniaceae. Matheus and Lopes (2007) have studied *E. variegata* seedlings. Abubakar and Yunusa (1998) studied epidermal structure and stomatal ontogeny in Acacias. Wright *et al.* (2000) described seedling traits based on cotyledonary, hypocotylar, first leaf and colour of mature embryo across 1744 species of Australian dicotyledons and concluded that all seedling traits studies were evolutionarily malleable. They have assorted more or less independently of each other

and provided no evidence of being functional groups. Biradar *et al.,* 2013) performed pharmacognostic studies in *E. suberosa* Roxb. and *E. variegata* L. adult plants on the basis of several epidermal and anatomical characteristics and other properties like colour, odour and taste etc. Considering the size of genus E*rythrina*, our knowledge of seedling morphology of this genus is scarce. We have undertaken to describe here the seedling characteristics of *E. suberosa* Roxb. (Coral tree), one of the introduced ornamentals in Karachi and an economically useful tree. Such studies are essential in constructing modes of biodiversity management (Amritphale *et al.,* 2008).

MATERIALS AND METHODS

The seeds of *E. suberosa* were collected from its tree growing in the Campus of University of Karachi and germinated without any dormancy-breaking treatment in pots filled with garden loam soil maintained at 75% water holding capacity. Maximum germination was 50% achieved within a week. The seedlings were studied, when they were 20 and 50-day old for their morphological characters including stomatal types and biomass allocation into various seedling components. Wherever necessary, 6-month old saplings were also studied for comparison. Seedlings morphology was described and seedling type was described according to 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 ascertain stomatal types. This nomenclature does not recognize actinocytic and stephanocytic stomata and categorize them as anomocytic type. As a basic criterion, all the cells abutting the guard cells are considered distinct by Prabhakar (2004) from the other epidermal cells by virtue of their position (i.e. abutting nature to the guard cells) hence he prefers to call them subsidiaries. The abundance of stomata and trichomes was determined by counting them in 100 fields of vision (called frames) at 45 x 10 X magnification. Each frame at this magnification was around 0.10174 mm^2 in area. The density of stomata and trichomes was expressed per mm^2. The sampling of stomatal abundance was random in the laminar region of the leaf. For biomass determination, various components of seedlings were dried in oven at 60^oC for 24 h. The data was analyzed statistically (Zar, 2010). Aereolation was studied as given in Misra *et al.* (2010).

RESULTS AND DISCUSSION

Seedling type, Seedling growth and Biomass Apportionment

"Seedling" is considered to be the final stage of the regenerative process of a plant from a seed. The use of this term is quite liberal. We have used this term as ecologists employ i.e. stage up to which the cotyledons are attached with the juvenile. We studied *E. suberosa* seedlings at two stages i.e. when they were 20-day old (younger seedlings) and 50-day old (elder seedlings - when cotyledons have generally exhausted and abscized). The germination of seeds in this species was around 50% when untreated seeds were sown in garden soil. The seeds of *E. variegata* have also been reported not to require any pre-germination treatment (Matheus and Lopes. 2007). The comparative morphometric data of 20- and 50-day old seedlings is prented in Table 1.

Table 1. Age related morphometric data of *E. suberosa* seedlings.

S. No.	Morphometric Parameters	20-day old seedlings	50-day old seedlings
1.	Root Length (cm)	14.73 ± 1.47 (12.5 – 17.5)	16.56 ± 1.76
2.	Shoot Length (cm)	17.00 ± 0.76 (16.4 – 18.5)	19.83 ± 1.19
3.	Hypocotyl (cm)	6.50 ± 0.57 (5.5 -7.5)	7.27 ± 0.37
4.	Epicotyl (cm)	10.66 ± 1.16 (9.-12)	11.70 ± 1.23
5.	Number of Leaves	3.67 ± 0.33	5.67 ± 0.44
6.	Number of Internodes	3.0	4.0
7.	Root dry Wt. (mg)	142.63 ± 2.08	282.87 ± 51.40
8.	Hypocotyl dry Wt. (mg)	138.2 ± 0.002	458.17 ± 59.61
9.	Cotyledons dry weight (mg)	150.30 ± 6.30	Abscised
10.	Epicotyl dry Wt. (mg)* (Stem)	98.8 ± 17.08	296.63 ± 12.50
11.	Leaves dry Wt. (mg)	299.83 ± 25.30	507.93 ± 63.51
12.	Shoot Wt. (mg) **	687.40 ± 36.35	1262.73 ± 116.94
13.	Seedling dry Wt. (mg)	830.04 ± 56.6	1545.60 ± 75.37
14.	Number of Nodules	38.00 ± 4.58	44.0 ± 2.31
15.	Area of Primary Leaves (cm^2)	102.86 ± 5.24 (92.85- 105.18)	93.55 ± 13.67 (69.28- 116.57)
16.	Area of imparipinnate Leaves	31.96 ± 8.53 (15.10- 42.59)	115.53 ± 2.35 (113.1 – 120.24)
17	Total Leaf area (cm^2)	134.82 ± 8.22 (120.28- 148.75)	209.08 ± 15.71 (182.41- 236.81)

*, Sans cotyledonary weight; **, inclusive cotyledonary weight.

Fig, 1. Juvenile individuals of *E. suberosa*. 5-day old individual with foliage leaves still entangled together (A) Note the thick food-laden cotyledons and relatively longer hypocotyl; 8-day old individual (B and C). The primary opposite leaves are glaucous and shiny dorsally (B) but light green pubescent ventrally (C). Epicotyl and petiole are also pubescent.

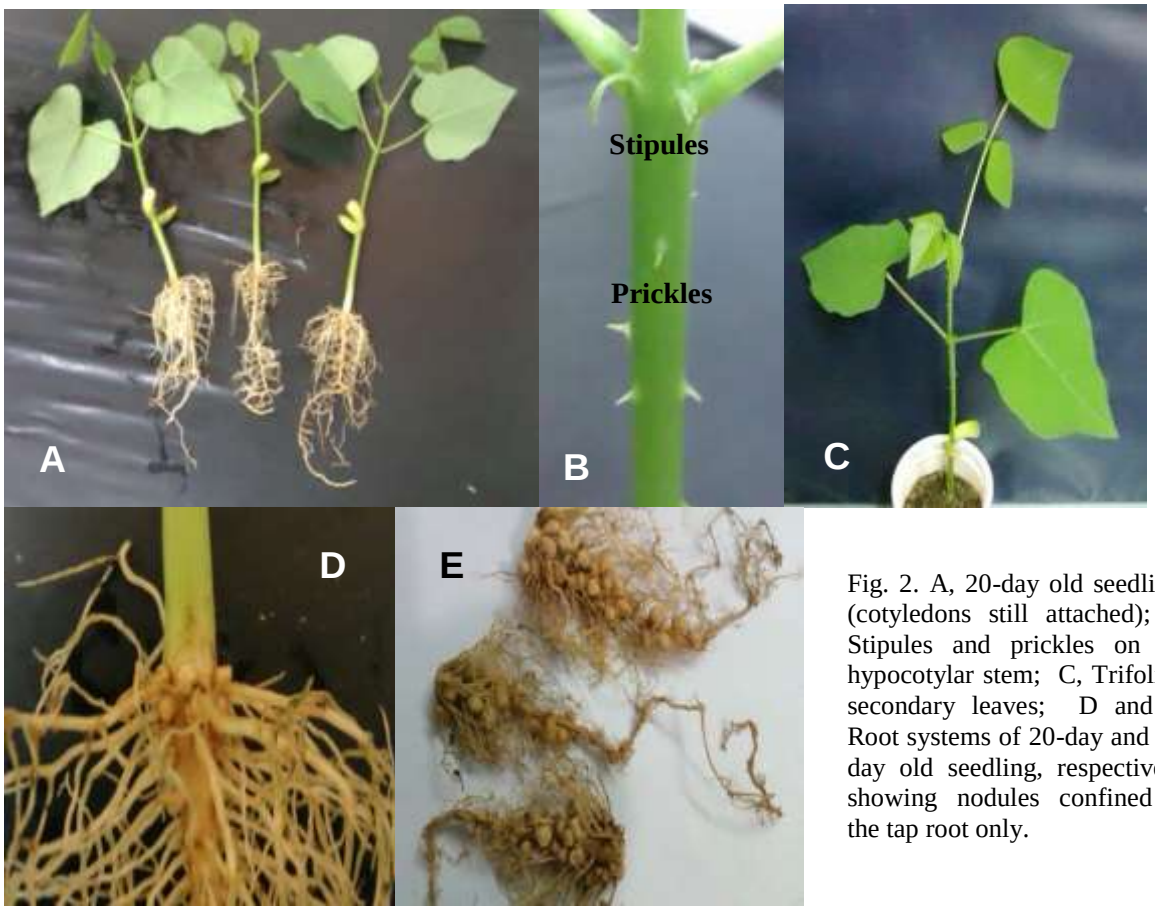

Fig. 2. A, 20-day old seedlings (cotyledons still attached); B, Stipules and prickles on the hypocotylar stem; C, Trifoliate secondary leaves; D and E, Root systems of 20-day and 50-day old seedling, respectively, showing nodules confined to the tap root only.

As per scheme of seedling classification of Garwood (1996), *E. suberosa* seedling may be designated as "Phanerocotylar Epigeal Reserve Type". It is evident from the Fig. 3 and 4 that main activity of growth up till 50th day of growth, in terms of dry mass, is located in hypocotylar and epicotylar parts of seedling. The cotyledons remained associated with the seedlings for c 45-50 days. They were completely consumed within 50-days (Fig. 4). The growth rates in various seedling components during 30 days of growth from 2oth to 50th day were found to be as –

Hypocotylar stem > Epicotylar stem > Root > Leaves.

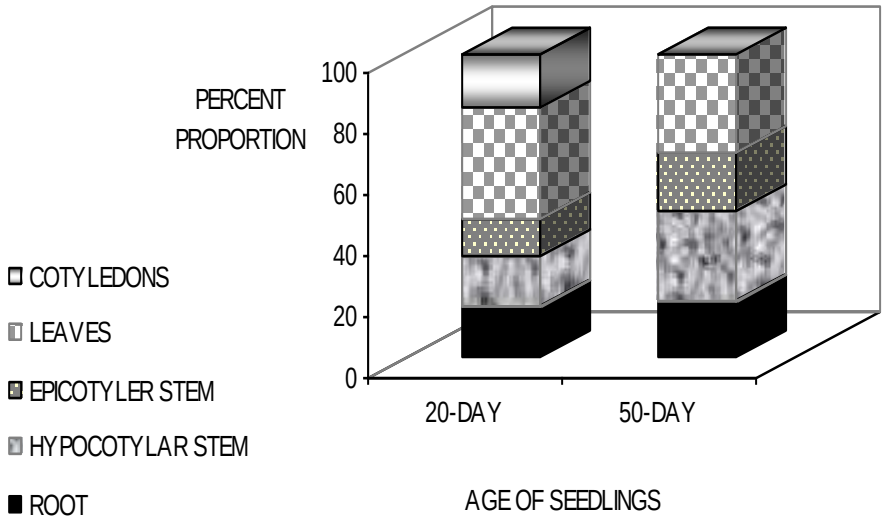

Fig. 3. Biomass apportionment among various components of seedlings.

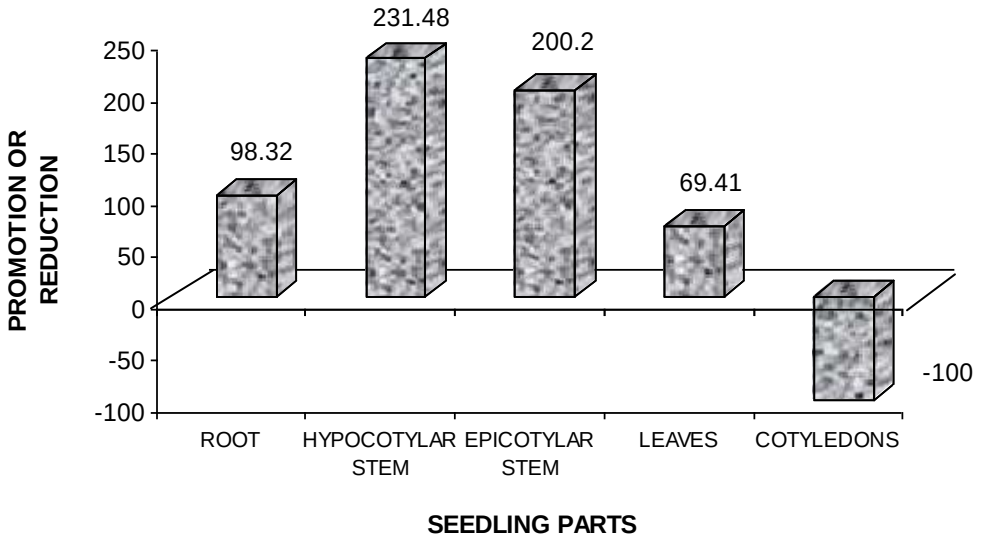

Fig. 4. Per cent promotion or reduction in growth (dry mass) of structural components of 50-day old seedlings over 20-day old seedlings. The cotyledons were completely consumed in elder seedlings.

Nanggan (1983-84) has described the seedling morphology of *Erythrina vespertilio* which deiffers from *E. suberosa* in many ways – 1) Cotyledons in *E. vespertilio* remain in the testa subterranean and thus seedling is Crypto-cotylar hypogeal Reserve type, 2) cotyledons are smaller, and 3) first through fourth leaves are opposite or nearly so otherwise alternate and fifth leaf is compound trifoliate. *E. suberosa* seedlings with simple opposite primary foliage leaves and trifoliate leaves produced subsequently with three leaflets and leaflets with no axillary bud resembled to the seedling of *Glycine max* (www.amnh.org) in this respect.

Stem

Green, hairy with conical prickles present in most of the seedlings but absent in few seedlings. The prickles are said to fall off after the third year (Ali, 1977).

Root

The tap root was light brown in colour with numerous light brown lateral roots (Fig.2 D and E). The nodules in younger as well elder seedlings were mostly confined to the tap root (Fig. 2 D and E). However, in 100-day old seedlings few lateral roots also harboured nodules. The nodules were large (3.5 to 5mm in size), ball-like quite

copious in number, pinkish brown in colour and densely clustered in 50-day old seedlings (Fig. 2 E). Allen (1981) has also described root nodules in *Erythrina* to be quite large, spherical and clustering on the central tap root system.

Cotyledons

The cotyledons were photosyntthetic storage type (c. 2.3 x 1.0 x 0.3 cm in size). They were thick, large and green on both sides with no visible venation. Their main function was to export reserves to the developing seedling. They remained attached with the seedlings up to around 50 days when they were completely exhausted. The fast growing hypocotyl raised the cotyledons quite high above the soil (Fig,. 1A). Single cotyledopn weighed 76.83 ± 6.39 mg of dry mass.

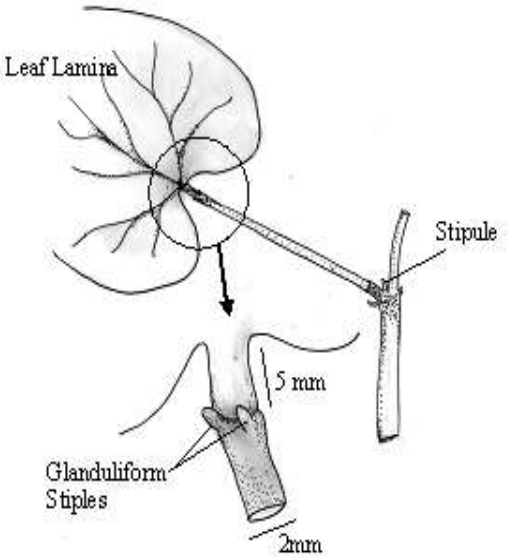

Table 2.Internodal lengths of the seedling.

Internode # Base to apex	20-Day	50-Day
I	7.03 ± 0.42	7.5 ± 0.40
II	2.96 ± 0.48	3.4 ± 0.38
III	0.50 ±	1.93 ± 0.34
IV	-	0.63 ± 0.05
Apex	0.55 ± 0.054	0.85 ± 0.11

Fig. 5. Glanduliform stiples of primary leaf of 50-day old seedling of *E. suberosa*. Note the basal embayment of lamina.

Hypocotyl, Epicotyl and the Internodal Elongation

Hypocotyl is smooth,and shining green provided with small prickles (aculeate) (Fig. 2B). Epicotyl is hairy and larger than hypocotyl in both younger and elder seedlings (Fig. 1 and 2). Length of internodes reduces from base to apex (Table 2).

Leaf Architecture

Leaf architecture denotes the placement and form of those elements constituting the venation pattern, marginal configuration, leaf shape and gland postion (Hickey, 1973). Hickey (1973, 1979) and LWG (1999) were important references to follow in this respect. Data on leaf architecture of *E. suberosa* leaves is given in Table 3-5 and Fig. 6, 7a and 7b. In early seedling of 5-day age, the leaves were densely pubescent on ventral surface but glaucous dorsally (Fig. 1b and C). In seedlings of 20 days also the leaves were more or less glabrous dorsally. The primary leaves were simple, opposite (Fig. 2A) and stipulate (stipules green, linear-lanceolate, caducous). The brown patches develop on lamina along the ribs during drying between the paper sheets.

The petiole length of primary leaf was more or less equal in 20-day and 50-day old seedlings (Table 5). The petiolule of terminal leaflet (1.4- 2.7 cm in length) was substantially larger than the petiolules of the laterals leaflet (< 0.5cm). The petiole of each leaf is provided with acropetiolular glandular stiples in form of protuberances (Fig. 5). There were two glands at the base of petiolule of terminal leaflet and one gland each with that of the lateral leaflet. Petiole as well as petiolules enlarged in length greatly in mature trifoliate leaf of 6-month old saplings. In younger seedlings, the total area of trifoliate leaves (31.96 ± 0.53 cm^2) was lesser than that of the primary leaves (102. 86 ± 5.20 cm^2). In elder seedlings the area of trifoliate leaves increased substantially to 115.53 ± 2.35 cm^2 per leaf. The total leaf area of elder seedlings (209.08 ± 15.71 cm^2) was 1.6 times of that of the younger seedlings (134.82 ± 8.22 cm^2) (Table 1).

The secondary and the subsequent leaves were pinnately trifoliate with three leaflets. Leaf base rounded and symmetrical and obtuse; leaf apex acute; primary vein straight, spacing between secondaries irregular (increasing towards base); leaf margin entire; pinnately veined captodromous (fastooned brochidodromus) venation. (Fig. 6). Brachidodromous venation (BD) is loop-veined venation in which main Secondaries emerging from the midrib at more or less at regular interval turns upwardly to the apex at or near margin and loops to join the next vein upwards

to form prominent arches. Besides arches formed due to subsidiaries, additional sets of loops outside the main brachidodromous loop were also observed. Tertiaries, percurrent, faint and weak. BD pattern of venation has also been reported in woody perennials of Family Cunoniaceae (Dickson, 1975), Family Rubiaceae (*Coffea arabica*) (Misra *et al.*, 2010), Family Bignoniaceae (Jain, 1978) and *Cinnamomum* spp. (Ravindran *et al.*, 2003). In arborescent flora, the brachidodromous pattern prevails in tropical floras whereas non-brachidodromous patterns prevail in Northern temperate floras (Bailey and Sinnot, 1916).

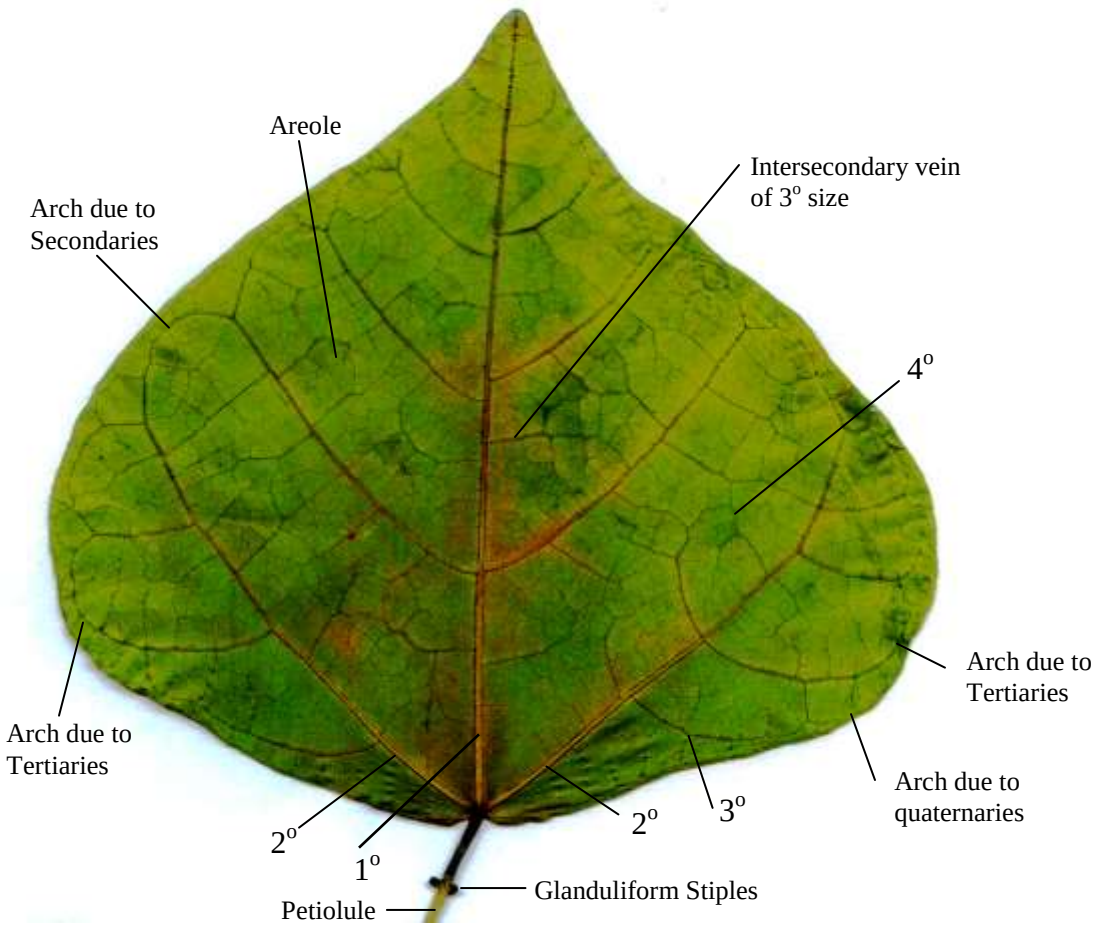

Fig. 6. Camptodromous (Brochidodromus) venation of terminal leaflet of six months old sapling of *E. suberosa*. Note that the leaf turns to copper red during drying between the paper sheets.

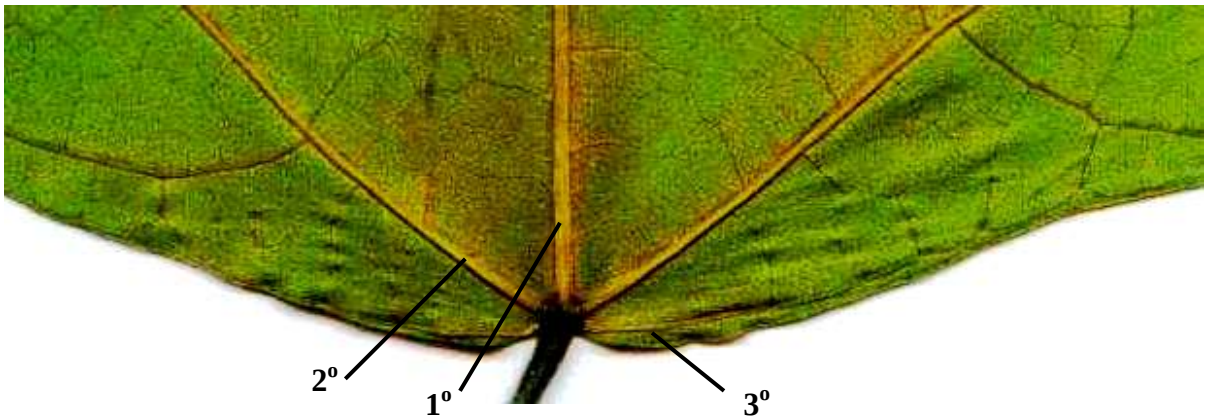

Fig.7a. Enlarged view of the umbo region of the terminal leaflet of a mature trifoliate leaf of six-month old plant of *E. suberosa* showing a primary, two Secondary and two Tertiaries veins as given by their width.

In the umbo region of terminal leaflet, there are five veins (one main mid-vein, 2 secondaries and two tertiaries as designated by their relative size) (Fig. 7a). In lateral leaflets of trifoliate, there are, however, only three veins arising from the umbo - one 1^o and two 2^o (Fig. 7b). Copper brown patches develop on lamina especially in the basal part on drying between paper sheets. Interseconadary veins are conspicuously present. Narrow inter-Secondaries reticulate i.e. inter-secondaries join with tertiaries or quaternaries to form reticulum. Areoles are polygonal in shape and vein-ending linear or curved (Fig. 8) not branched. The angle of divergence (AOD) measured between the branch and the continuation of its source vein above the point of branching was moderate ($52\text{-}60^o$) between 1^o and 2^o veins and wide ($72^o\text{-}90^o$) between 2^o and 3^o veins. In *E. suberosa* – the leaf base of primary leaf lamina is embayed in a sinus with straight or convex sides (leaf base extension is > zero).

Table 3. Architectural parameters of primary leaves of 20- and 50-day old seedlings.

Leaf Architectural Parameters	Primary Leaves
20-DAY OLD SEEDLINGS	
Mid Vein Length (Lm) cm	8.57 ± 0.15
Basal Leaf Extension (Lb) cm	1.53 ± 0.0995
Lamina Length, L= (Lm + Lb) cm	10.10 ± 0.215
Apex Angle (^{o})	79 ± 1.79 (Apex acute)
Basal Angle (^{o})	261.2 ± 7.40 (Base wide obtuse)
Lamina Width (W) cm	8.45 ± 0.161
Aspect ratio = W /L *	0.837 ± 0.122
Shape of lamina base**	Cordate
Leaf area (cm^2) -I	51.38 ± 2.73
Leaf area (cm2)- II	51.48 ± 2.50
50-DAY OLD SEEDLINGS	
Mid Vein Length (Lm) cm	7.78 ± 0.605
Basal Leaf Extension (Lb) cm	1.483 ± 0.127
Lamina Length, L= (Lm + Lb) cm	9.266 ± 0.683
Apex Angle (^{o})	77.5 ± 2.63 (Apex acute)
Basal Angle (^{o})	248.33 ± 8.046 (Base wide obtuse)
Lamina Width (W) cm	7.75 ± 0.2094
Aspect ratio = W /L	0.853 ± 0.0463
Shape of lamina base	cordate
Leaf area (cm^2) -I	49.28 ± 4.75
Leaf area (cm2)- II	44.96 ± 8.96

Fig. 7b. Two opposite Lateral leaflets of a mature trifoliate leaf of E, *suberba*. Note there are three veins arising from the umbo and not five as in case of terminal leaflet.

Foot Note to the Table 3: Apex angle is the angle from the apical termination of the mid-vein to the pair of points where a line perpendicular to the mid-vein and 0.75Lm from the base intersects the margin. Base angle sensu LWG (1999) is the angle from the vertex (vertex lies in the centre of the petiole at the point where the basal most laminar tissue touches the point) to the point where a line perpendicular to the mid-vein at 0.25 Lm from the base. Base extension length (Lb) is the distance on a perpendicular from the proximal most point of the mid-vein to the proximal most extension of leaf tissue. It can be equal to zero. *, after Lu *et al.* (2012). Apex acute = Apex angle below 90^o. Base is wide obtuse if base angle > 180^o. **, leaf base is cordate i.e. the leaf base at umbo is significantly embayed in a sinus with straight or slightly curved sides and Lb > zero.

The ratio of single lateral leaflet area to that of the terminal leaflet area in trifoliate leaves of 20-day old seedlings was 0.4339 ± 0.02696 (0.3229- 0.5688). and in 50-day old seedlings 0.4470 ± 0.0195 (0.2269 – 0.5480). In pooled samples of younger and elder seedlings this ratio amounted to 0.4466 ± 0.0154 which was not significantly different from younger (N=10; t = 0.1468, NS) or elder seedling ratio (N = 20; t = 0.01799, NS). It follows that the area of the lateral leaflet was generally slightly lesser than the half or, at the most, roughly equal to the half of the terminal leaflet area. *E. suberosa* seedlings are provided with large leaves and so transpiring surfaces are larger (Kozlowski and Pallardy, 1997*).*

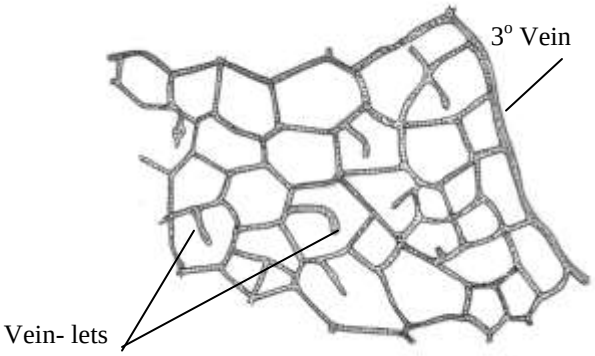

Fig. 8. Areolation in mature leaf of *E. suberosa*.

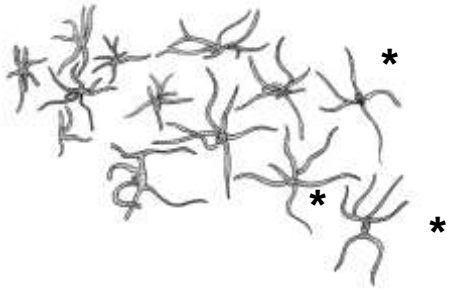

Fig. 9. Branched trichomes from petiole and ventral leaf surface of six months old sapling. Marked with asterisks are from the leaf surface. Such trichomes on dorsal side of leaf are infrequent – more on the basal part of the leaf than apical part.

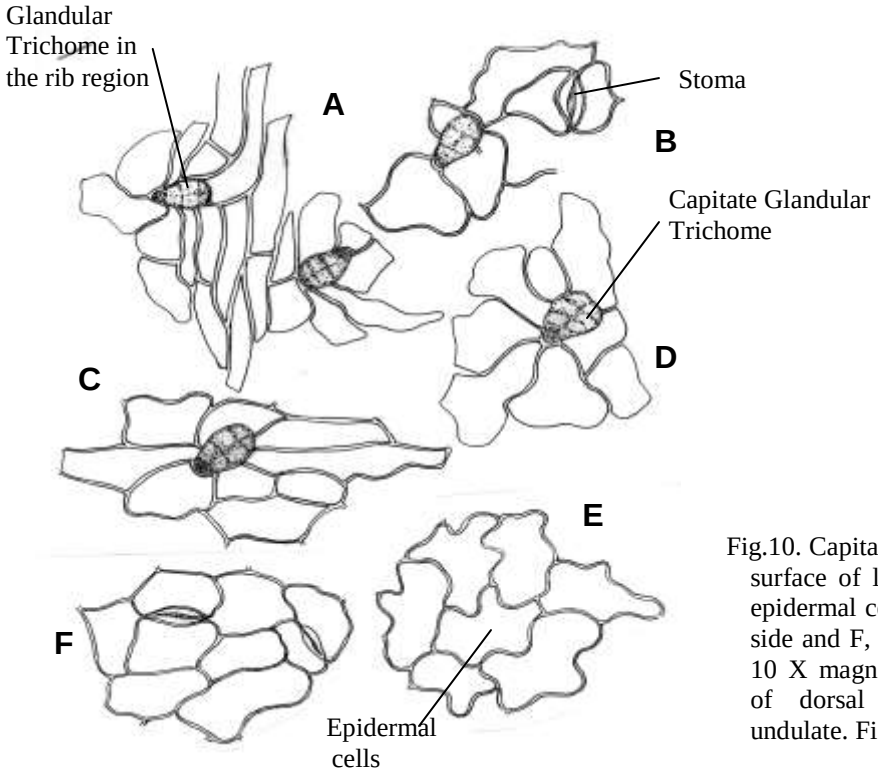

Fig.10. Capitate glandular trichomes of lower surface of leaf of *E. suberosa* (A-D) and epidermal cells of the leaf blade (E, dorsal side and F, ventral side) as seen with 45 x 10 X magnification. Periclinal cell walls of dorsal epidermal cells are more undulate. Figures, not drawn to scale.

Leaf Ornamentation

E. suberosa showed a great epidermal ornamentation in form of trichomes and various stomatal types.

1. Trichomes

Two types of trichomes were observed- branched trichomes and capitate glandular trichomes (Fig. 9 and 10). Branched trichomes are denser near the basal part of the leaf and the petiole than apical part of the mature leaf of 6-months old seedling. Being situated on a delicate stock cell, they are easily removed. The branched trichomes were composed of 5-8 arms arranged in varying patterns (Fig. 9).

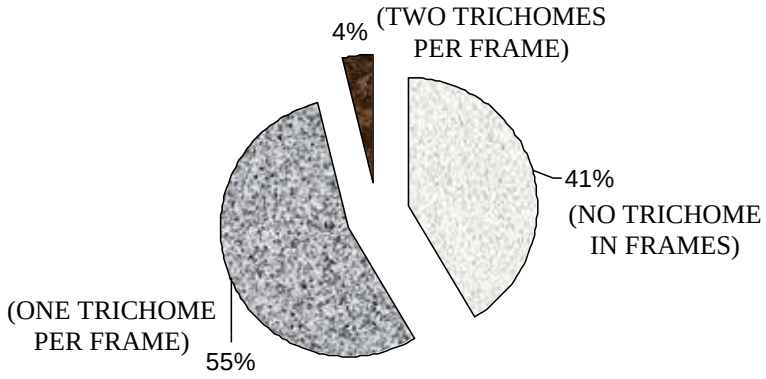

Fig. 11. Frequency of occurrence of capitate glandular trichome in the fields of vision (N = 75) of compound microscope at 45 x 10 X magnification of ventral surface of leaf of 20-day old *E. suberosa* seedling. Each field of vision occupied an area of 0.10174 mm^2.

Table 4. Architectural parameters of trifoliate leaves of 20- and 50-day old and six-month old seedlings of *E. suberosa*.

Leaf Architectural Parameters	Secondary Leaf (Trifoliate)		
	Terminal leaflet	Lateral leaflet I	Lateral Leaflet II
20-DAY OLD SEEDLING			
Mid Vein Length (Lm) cm	4.26 ± 0.68 (2.75-5,6)	2.76 ± 0.31 (1.5-3.9)	2.61 ± 0.27 (1.9-3.7)
Basal Leaf Extension (Lb) cm	Zero*	Zero	Zero
Lamina Length, L= (Lm + Lb) cm	4.26 ± 0.68 (2.75-5,6)	2.76 ± 0.31 (1.5-3.9)	2.61 ± 0.27 (1.9-3.7)
Apex Angle (^{o})	80.60 ± 2.0 (75-88)	81.83 ± 5.60 (70-108)	78.16 ± 1.64 (70-80)
Basal Angle (^{o})	120.40 ± 4.55 (110-137)	89.14 ± 4.51 (78-108)	94.0 ± 5.53 (78-120)
Lamina Width (W) cm	4.18 ± 0.52 (2.1-5.8)	2.160 ± 0.056	1.923 ± 0.098
Aspect ratio =W /L *	0.9814 ± 0.052	0.76 ± 0.061 (0.59-.80)	0.74 ± 0.063(0.67-0.93)
Lamina shape	Rhomboidal	Ovate***	Ovate
Leaf area (cm^2)	10.63 ± 3.66	4.26 ± 1.18	4.21 ± 1.95
50-DAY OLD SEEDLING			
Mid Vein Length (Lm) cm	5.42 ± 0.46 (2.7-7.7)	4.44 ± 0.352 (3.5-6.1)	3.74 ± 0.48 (2.8-5.3)
Basal Leaf Extension (Lb) cm	Negligible*	-	-
Lamina Length, L= (Lm + Lb) cm	5.42 ± 0.46 (2.7-7.7)	4.44 ± 0.352 (3.5-6.1)	3.74 ± 0.48 (2.8-5.3)
Apex Angle (^{o})	78.36 ± 2.54 (65-95)	66.30 ± 2.42 (60-85)	68.20 ± 2.42 (62-88)
Basal Angle (^{o})	123.82 ± 1.71 (115-131)	105.9 ± 2.80 (101 -128)	106.50 ± 1.85(100-115)
Lamina Width (W) cm	4.47 (2.2-6.8)	3.0 (2.0-3.7)	2.50 ± 0 (1.00 -3.7)
Aspect ratio = W /L	0.8613 ± 0.0394	0.673 ± 0.026	0.663 ± 0.036
Lamina shape	Rhomboidal	Deltoid / Ovate	deltoid /Ovate
Leaf area (cm^2)	29.70 ± 6.46	13.41 ± 3.12	14.84 ± 3.64
SIX-MONTH OLD SEEDLINGS	Trifoliate Leaf		
Mid Vein Length (Lm) cm	9.250 ±	7.55 ±	7.50 ±
Basal Leaf Extension (Lb) cm	Zero*	Zero	Zero
Lamina Length, L= (Lm + Lb) cm	9.25±	7.55 ±	7.50 ±
Apex Angle (^{o})	88.4 ±	70.6 ±	70.3 ±
Basal Angle (^{o})	122.4 ±	110 .8±	112.6 ±
Lamina Width (W) cm	9.73 ±	5.52 ±	5.50 ±
Aspect ratio = W /L	1.0543	0.733	0.805
Lamina shape	Rhomboidal	Deltoid / Ovate	deltoid /Ovate

Apex acute = Apex angle below 90^o. *, leaf base at the umbo is very slightly embayed in a sinus (Lb is negligible); **, After Lu *et al.* (2012); ***, Lamina shape is ovate if the widest part of the leaf is on an axis in the basal 2/5 of the leaf (LWG, 1999).

Capitate glandular multicellular trichomes (Fig.10) were found to be distributed irregularly with a mean density of 6.16 ± 0.64 per mm^2 on the ventral surface of simple leaf of 20-day seedling (Table 6). These trichomes were present on the veins and also the leaf blade surrounded by the veins. These trichomes measured 47.25 ± 0.75 µm in length and 30.6 ± 0.50 µm in width at the widest. On dorsal surface such trichomes were very rare. Even on ventral surface of young leaf of 20-day old seedling, the frequency of not finding a trichome in a field of microscope vision (Frame) was as high as 41%. The frequency of finding one trichome per frame was 55% and two trichomes per frame merely 4% (Fig. 11). On terminal leaflet of a mature leaf the density of glandular trichomes was quite high (2.17 ± 0. 094 per frame of vision of 0.10174mm^2 corresponding to 21.3 ± 0.92 trichomes per mm^2) varying from zero to 5 but predominantly 1 to 3 per frame of vision; their distribution tended to significantly deviate from the normal distribution (Fig. 12). The distribution of glandular trichome on terminal leaflet of a younger trifoliate leaf of 4.2 cm^2 of six months old seedling tended to follow normal distribution (KS-z: 1.115, $p < 0.174$) and exhibited significantly larger density (9.47 ± 0.27 per frame of vision of 0.10174 mm^2 corresponding to 93.08 ± 8.65 per mm^2) (Fig. 13). The reason for such a variation of trichome frequency is not known. It may be somehow related with the age of the age of the leaf or due to their easy removal from the leaf surface.

It was noted that where a glandular trichome was present, the shape of the epidermal cells underneath was greatly different from the normal epidermal ground cells. The epidermal cells of the dorsal side of leaf exhibited comparatively lesser undulation of cell wall as compared to the lower epidermal cells (Fig. 10 E & F). Glandular trichomes have also been reported in *Erythrina velutina* by da Silva *et al.* (2013) on both adaxial and abaxial surfaces. Like *E. velutina, E. suberosa* had branched trichomes. Metcalfe and Chalk (1950) considered branched trichomes to be characteristic to genus *Erythrina*. *E. falcata* and *E. speciosa* have been reported to bear branched-trichomes (Almeida, 2010, 2011), however, epidermis in *E. cristagalli* presents no such adoration (Cratieri-Sosselsa, 2005). The trichome frequency is, however, reported to be environmentally-controlled (Metcalfe and Chalk, 1979).

Table 5. Petiole / petiolule lengths (cm) of primary (simple) and secondary (trifoliate) leaves of 20- and 50-day old seedlings.

Primary or Simple Leaves	Secondary or Trifoliate			
20-DAY OLD SEEDLINGS				
Petiole	Petiole	Terminal leaf let (Petiolule)	Lateral leaf let - - I (Petiolule)	Lateral leaf let - II (Petiolule)
N = 5 5.75 ± 0.112 (5.5-6.0)	N = 5 7.8 ± 0.73 (5-10)	N = 5 2.2 ± 0.32 (1.6-2.7)	N = 5 0.4 ± 0.10 0.3-0.5	N = 5 0.4 ± 0.10 0.3-0.5
50-DAY OLD SEEDLINGS				
N = 6 5.28 ± 0.13 (5-5.8)	N = 6 6.64 ± 0.41 5.2 -9.3	N = 6 2.46 ± 0.18 1.4 -2.6	N = 6 0.30 ± 0.10 0.25 – 0.45	N = 6 0.36 ± 0.03 0.25 – 0.45
6- MONTH OLD SAPLING				
---.	13.5	4.6	0.9	0.8

2. Stomata

Cotyledonary Stomata

Cotyledonary stomata are of paracytic type (Fig. 14, A and B). They are smaller than the foliar stomata.

Foliar Stomata

The leaves of *E. suberosa* seedlings were hypo-amphistomatic. The upper surface was paucistomatic (stomata rare) and the lower surface multistomatic. There were five types of stomata on ventral side of leaf of *E. suberosa* (Fig. 14 and 15) – 1) paracytic (A stomatal complex in which one or more of the subsidiary cells that flank the stoma are parallel with the long axis of the guard cells), 2) anisocytic (a stoma completely surrounded by only three subsidiaries variable in position and shape but one of the subsidiaries is distinctly small 3) anisotricytic (A stoma completely surrounded by only three subsidiaries variable in position and shape but one of subsidiaries is distinctly large) 4) anomocytic (Stoma completely surrounded by four or more subsidiaries, variable in position, shape and size and 5) staurocytic (Stoma completely surrounded by only four subsidiaries variable in shape and size but two of their conjoint wall polar, while the other two are lateral to the guard cells (cf. Prabhakar, 2004). Staurocytic stomata are known to develop from anisocytic stomatal complexes of the seedling leaves in *Monocalyptus* (*Eucalyptus*, Myrtaceae) (Carr and Carr, 1990b).

The number of subsidiary cells associated with different types of stomata varied from two to seven. Regarding the number of subsidiaries associated with stomata, the studies by Car and Car (1990a), Obiremi and Oladale (2001) and Oyeleke *et al.* (2004) had confirmed that larger the number of subsidiaries cells surrounding the guard cells, the

faster the opening of the stomata i.e. more transpiration and CO_2 absorption. It is well known that most of the CO_2 used in stomata is absorbed by the stomata.

In *E. suberosa* seedlings, stomata were rarely present on dorsal side and they were of paracytic type mainly along the veins. Staurocytic stomata and other types of stomata were only seen on ventral surface (Fig. 6). This signifies the diversity of stomatal types even on the same surface of a leaf as also been reported by Saheed and Illoh (2010) and Aniesua and Silas (2012). Metcalfe and Chalk (1979) have reported several types of stomata in Papilionaceae – Anomocytic, Paracytic, and Parallelocytic restricted on adaxial surface or found on both surfaces singly or in groups. They have reported no anisocytic stomata in Papilionaceae. The thirteen species of the family Fabaceae (Genus *Ademsia, Galega, Lotus, Lupinus, Melilotus, Parkinsonia, Senna, Trifolium* and *Vicia*) were reported to be characterized with anisocytic, anomocytic stomata. Stomata are predominantly paracytic in leaves of *Citrus* spp. (Obiremi and Oladele, 2001) and many *Macaranga* spp. (Norfaizal *et al.,* 2012). Stomata are extremely variable even in the members of a tribe and even within a genus (Metcalfe and Chalk, 1950) and in a species as well. That is more than one type of stomata frequently occur on the same leaf surface.

Paracytic stomata are most frequent in several papilionaceous plants (*Alysicarpus bupleurifolius, A. monilifer, A. rugosus, Arachis hypogea, Cajanus cajan, Canavalvia gladiata, Clitoria terneata, Erythrina cristagalli, E. indica, Lathyrus sativus, Lens esculentus, Medicago sativa*, and *Tephrosia purpurea*. The genus *Senna* has been reported to have paracytic stomata (Freire *et al.,* 2005). There are, however, anisocytic stomata in *Glycine soja, Pisum sativum* and *Sesbania sesban* and anomocytic stomata in Sesbania *grandiflora* and *Trigonella foenum-graceum.* The stomata on leaf of *Alhagi maurorum* (Fabaceae) are paracytic and anisocytic types and on stem anomocytic type (Bokhari and Dasti, 1991). Thirty-six dicotyledonous species of 34 genera and 20 families of district Tank (Khyber Pukhtoonkhwah, Pakistan) were examined by Ahmad *et al.* (2009). Most of them were amphistomatic. Anisocytic type of stomata were the dominant type in 12 spp. Staurocytic and diacytic stomata were only present in seven and six species, respectively. In six species two or three types of stomata were present simultaneously. Staurocytic stomata are reported in *Erythrina subrosa* L. (? *E. suberosa* Roxb.) by Khan *et al.* (2011*).* Several species of genus *Erythrina* have been reported to have paracytic stomata for instance, *E. speciosa* and *E. falcata* (Almeida 2010, 2011); *E. velutina* (da Silva *et al.,* 2013), *E. variegata* (Matheus and Lopes, 2007), *E. suberosa* (Khan *et al,* 2011; Biradar, *et al.,* 2013), and *E. indica* (Tripathi and Mondal, 2012). Contrary to Khan et al. (2011) *E. suberosa* is found to be amphistomatic and not hypostomatic. Stomata on dorsal surface of leaf are very rare and but not absolutely absent. Of 45 species of order Leguminales, 31 species are reported to be amphistomatic and only 14 spp. hypostomatic by Tripathi and Mondal (2012). According to them, three stomata types of Leguminales were paracytic, anisocytic and anomocytic - found in various combinations. The most common stomata in legumes are of the paracytic type and paracytic and anomocytic types may although occur together in Caesalpiniaceae but never occur together in Fabaceae. Family Fabaceae is more diverse in stomata than Families Caesalpiniaceae and Mimosaceae (Tripathi and Mondal, 2012).

The stomatal frequency is known to vary on upper and lower surfaces of leaf (Ekenayake *et al.,* 1998). Greater stomatal density on ventral surface of leaf is common in species that occur in xeromorphic environments, a fact explained as a feature that minimizes water loss by ostiolar evapo-transpiration (Esau, 1974; Cutter, 1986). In *E. suberosa,* the number of stomata per frame of vision of microscope ranged from 7 to 16 with overall variation of 18.73% (mean = 11.22 ± 0.210 per frame. The mid-region classes of distribution (10-14 stomata per frame) occupied a large proportion of 69% of the total observations (N = 100). The distribution of number of stomata per frame of vision of microscope was normal in both younger and mature leaf (Fig. 16 and 17) as the KS-d and KS-z both were found to be insignificant in both cases. It indicated that spatial distribution of stomata on the leaf surface, irrespective of their kind, was heterogeneous. Stomata were comparatively denser in case of mature leaf as compared to the younger leaf. The mean density of stomata per mm^2 was 110.28 ± 2.07 [68.80 – 157.3; CV (%): 18.73] (Table 6). The pore size of the stomata in *E. suberosa* irrespective of the stomatal type ranged from 32 to 35.5 µm in length. The stomata of *E. suberosa* are of moderate size. The stomata of *E. variegata* are reported to range from 112.2 to 19.6 µm and 4.9 to 11.9 µm in length in flat and mountain areas of Philippines, respectively (Combalicer *et al.,* 2010). Our stomatal measurements were comparable to the adaxial stomatal size of *Erythrina indica* Lamk. (38.49 µm) but larger than that of *Dalbergia sissoo* (24.3 µm) as reported by Tripathi and Mondal (2012). The guard cell size of *Prosopis cineraria, Alhagi maurorum* and *Crotalaria burhia* reported to be 27.39 ± 2.01, 15.16 ± 1.55 and 28.05 ± 6.93 µm in length, respectively on lower foliar epidermis (Bokhari and Dasti, 1991). *Bougainvillea glabra* is also reported to bear stomata of comparable size (32.5 µm) (Ahmad *et al.,* 2009). Stomata in legume herbs such as *Vicia faba, Melilotus indica, Lathyrus aphaca* are smaller (13 -14 µm) (Ahmad *et al.,* 2009). The stomata of tree legume species are generally larger than that of herbs (Tripathi and Mondal, 2012).

Table 6. Density of stomata and Capitate Glandular trichomes per mm^2 calculated on the basis of observation in 100 fields of vision on ventral surface of leaf of 20-day old seedling of *E. suberosa*.

Statistical Parameter of density	Density / mm^2	
	Stomata	Capitate Glandular trichome
Number of observations	100	100
Mean	110.28	6.1595
Standard error of Mean	2.065	0.64022
Median	108.119	9.8290
Standard Deviation	20.654	5.4443
CV (%)	18.73	90.01
Skewness	0.056	0.1765
SE of Skewness	0.478	0.277
Kurtosis	-0.6 54	-0.774
SE of Kurtosis	0.478	0.548
Minimum	68.80	0.0
Maximum	157.26	19.60
KS-d	0.0948 (NS)	2.881 ($p < 0.0001$)

Diversity of stomatal types even on the same surface (leaf) as noted in the present studies has also previously been reported (Shah and Gopal, 1969; Ahmed *et al.*, 2009). In spite of diversity, most frequent type of stomata can, however, be used as a taxonomic character. Epidermal (stomatal) studies may act as markers in taxonomic delimitation. Saheed and Illoh (2010) justified the separation of new genera *Senna* and *Chamaecrista* from their initial genus *Cassia* on the basis of epidermal surface ornamentation. Paliwal (1969) considered that stomatal studies may have little taxonomic value unless the development of different stomata types is studies. In Papilionaceae, Shah and Gopal (1969) reported that different types of stomata follow a similar pattern of development. The diversity of stomatal types, even on the same surface of an organ, indicates the weakness in using stomata as a taxonomic character (Pant and Kidwai, 1964). Shah and Gopal (1970), however, asserted that in spite of diversity, the most frequent type of stomata can be used as taxonomic character. Epidermal surface structure is reported to bear definite diagnostic features justifying the separation of the genera *Senna* and *Chamaecrista* from their initial genus *Cassia* (Saheed and Illoh, 2010).

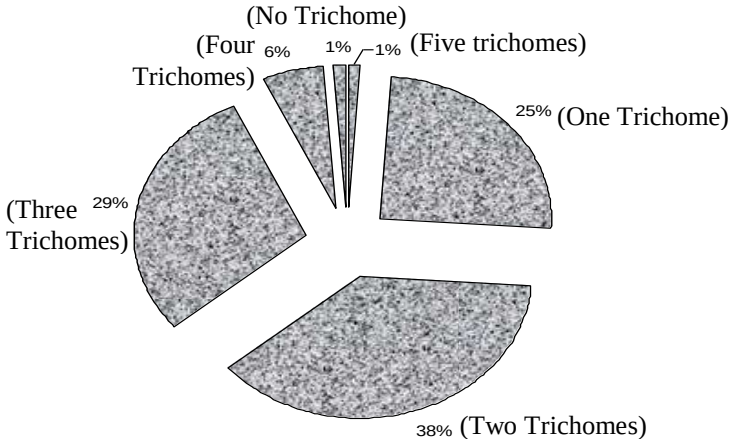

Fig. 12. Distribution of glandular trichome in 100 frames on ventral side of mature leaf (64.01 cm^2) of six-month old seedling. Each frame had a size of 0.10174 mm^2. The data distributed asymmetrically.

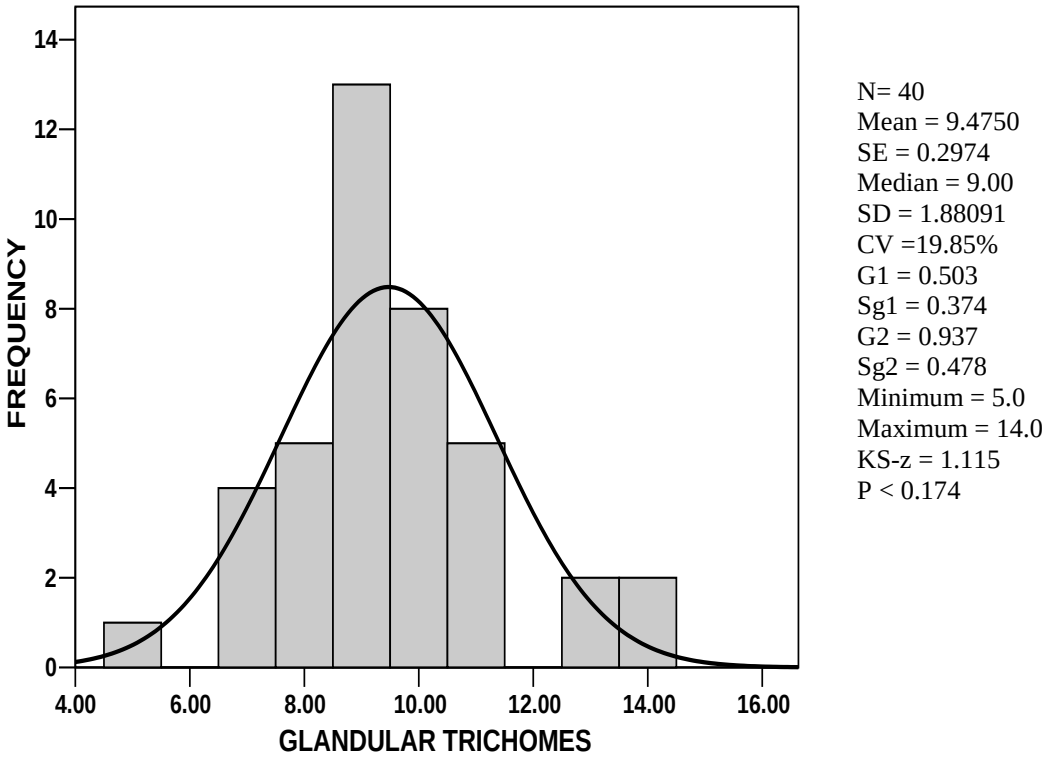

Fig. 13. Frequency distribution of number of glandular trichomes in 40 frames of vision on ventral surface of the young (4.2 cm^2) leaf of six-month old seedling of *E. suberosa*. Each frame was c 0.10174 mm^2 in size.

The stomatal abnormalities in *E. suberosa* included degenerate stomata, oblique stomata contiguously oriented stomata (abutting stomatal complexes) and stoma with single or no guard cells or incompletely developed guard cells (Fig.15).

The locality of collection of specimens is known to influence the epidermal structure and show wide variation in stomatal types e.g., specimens of *Heliotropium europium* collected from Quetta had anomocytic, anisocytic, brachyparacytic, staurocytic, cyclocytic and actinocytic stomata as common types while specimens collected from Pishin have no anisocytic but has an additional brachyparatetracytic stomata which didn't occur in the specimens of Quetta (Dasti *et al.*, 2003). Contiguous stomata were found *in Erythrina indica* but rarely. They were frequent in *L. sativus* (Shah and Gopal, 1969) and may be formed by budding. *Melilotus albus, Alysicarpus vaginallis, Aeschymonene indica, and Desmodium* spp. also reported to possess contiguous stomata (Kothari and Shah, 1975; Bora and Baruah, 1979). Aniesua and Silas (2012) have also reported un-open stomatal pores, two-stomata sharing one subsidiary cell, one guard cell, parallel contiguous and aborted guard cell in *Acalypha (Euphorbiaceae)*. Stomatal clustering on epidermis is reported in more than 60 species (Gan *et al.*, 2010). Drought and salinity increase the occurrence of contiguous stomata which indicates environmental- signaling-correlation with contiguous stomata (Gan *et al.*, 2010).

Stomatal abnormalities are suggested to be the result of environmental perturbations as confirmed by Carr and Carr (1990a) and environmental stress like drought and salinity (Gan *et al.*, 2010). Carbon dioxide concentration and temperature may influence the stomatal density on the leaf (Beerling and Chaloner, 1993). Warming may significantly decrease the average nearest neighbour distance between stomata (Zheng *et* al. (2013). As structure, development and patterning of stomata on the leaf surface is the function of complex processes, they should be viewed from evolutionary, physiological, ecological and organ view-point (Croxdale, 2000). Great deal of research is needed with local flora from this view-point.

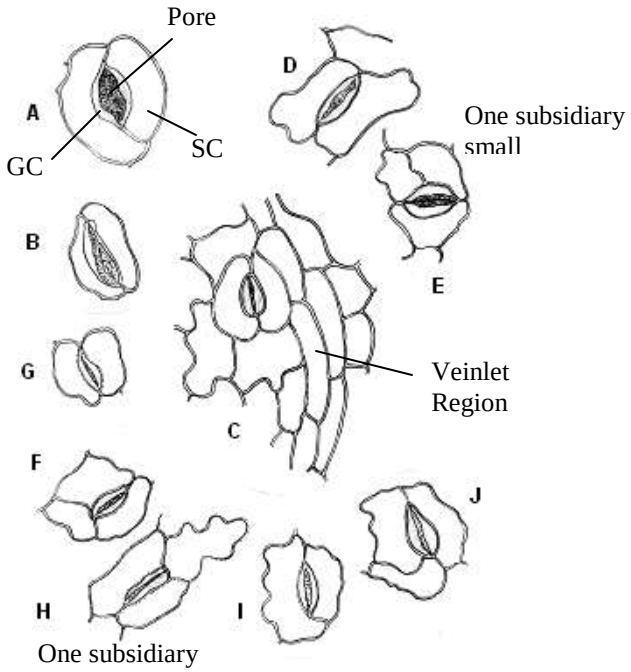

Fig.14. Dominant types of Cotyledonary and foliar stomata of *E. suberosa.* A and B, cotyledonary stomata; C, D and E, stomata from dorsal surface of leaf; F, G, H, I and J, stomata from ventral surface of leaf. A, B, C, D, G and I are Paracytic and E, and J are anisocytic type and F and H are Anisotricytic stomata (subsidiaries monocyclic. GC, Guard cells; SC, Subsidiary cells. Seen with 45x10 X magnification. Not drawn to scale.

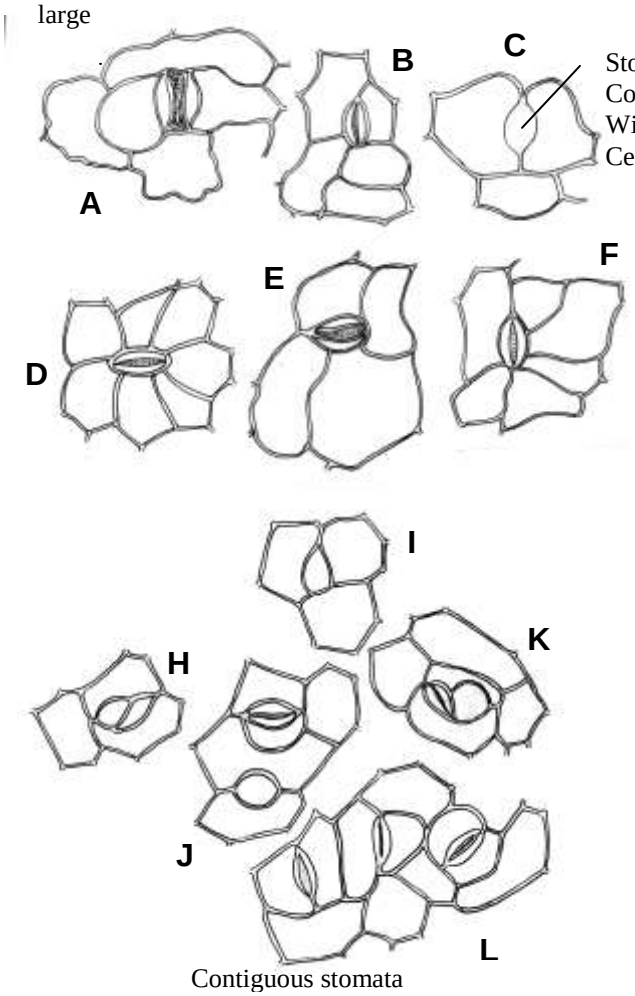

Fig.15. Some rare and unusual stomata of *E. suberosa* leaf. A, Disjoint guard cells on dorsal surface of leaf making the pore dumbbell shape in stead of spindle like; B and E are the staurocytic stomata on ventral surface of the leaf – these are rare in occurrence; C, The stoma with arrested cell on ventral side of leaf; D and F, anomocytic stomata G, paracytic stoma on the rib of leaf (ventral side). H, I, J and K are the ill-developed or abnormal stomata from ventral side of mature leaf of 6-month old sapling. L, the contiguous stomata. Magnification – 45 x 10X. Not drawn to scale.

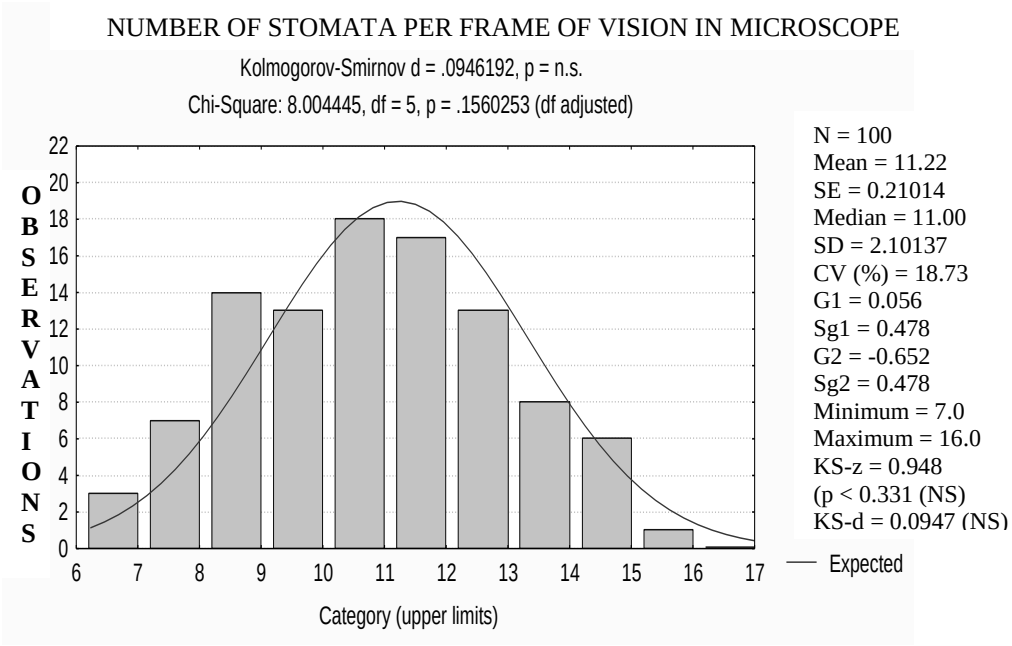

Fig.16. Frequency distribution of number of stomata per field of vision (N = 100) on ventral side of leaf of 20-day old seedling of *E. suberosa*. Each circular field of vision was of 0.10174 mm^2 in size. The distribution tended to be normal.

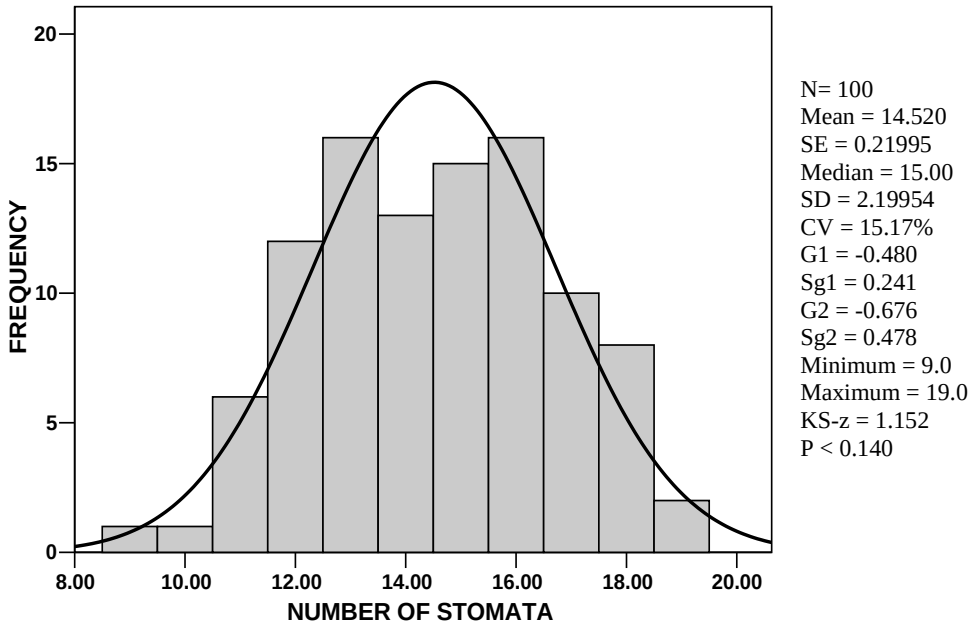

Fig. 17. Frequency distribution of number of stomata in 100 frames of 0.10174 mm^2 each) on ventral surface of mature leaf (64.01 cm^2) of six months old seedlings of *E. suberosa*.

REFERENCES

Abubakar, B.Y. and I.A. Yunusa (1998). Epidermal structure and stomatal ontogeny as an aid to the taxonomic identification of some species of Acacia (Leguminosae: Mimisoideae) from Nigeria. *Nigerian J. Bot.* 11: 117-123.

Ahmad, K., M.A. Khan, M. T. Ahmad, M. Zafar, M. Arshad, and F. Ahmad (2009). Taxonomic diversity of stomata in dicot flora of a district Tank (NWFP) in Pakistan. *African J. Biotech*. 8(6): 1052-1055.

Ali, S.I. (1977). Papilionaceae: *Flora of West Pakistan*. # 100. (Eds. E. Nasir and S.I. Ali)

Allen, N.A. (1981). The Leguminosae: A Source Book of Characteristics, Uses and Nodulation. University of Wisconsin Press, 812 pp.

Almeida, E.E. (2011). Caracterizaéão farmacotógica das folias de espécie *Erythrina speciosa* Andrews, *Bio Far* 5: 34-47.

Almeida, E.E. (2010). Caracteriza ção farmacognóstica da espécie *Erythrina falcata* Benth. Fabaceae. *Rev. Bras. Farmacogn* 20: 105.

Amritphale, Dilip and S.K. Sharma (2008). Seedlings of dicots: Form and Function. *Resonance J. Sci. Edu.* 13 (5): 468 -474.

Aniesua, E.U. and E.I. Silas (2012). Leaf epidermal studies of three species of *Acalypha* Linn. (Euphorbiaceae). *Adv. Appl. Sci. Res.* 3 (5): 3185-3199.

Bailey, I.W. and E.W. Sinnot (1916). The climatic distribution of certain types of angiosperm leaves. *Am. J. Bot.* 3: 24-39.

Beering, D.J. and W.G. Chaloner (1993). The impact of atomospheric carbon dioxide and temperature change on stomatal density: Observations from *Quercus rober* Lammas leaves. *Ann. Bot.* 71 (3):231-235.

Biradar, R.M., V.S. Gambhire, and A.S. Dhabe (2013). Pharmacognostic studies in *Erythrina suberosa* Roxb. and *E. variegata* L. *Bioinfolet* 10 (2b): 610-611.

Bokhari, M.H. and A.A. Dasti (1991). *Ecological guidelines for exploitation of natural resources in Thal and Cholistan sand dunes*. Final Tech. Rep. (1990-91). Pak. Sci. Found. Res. Proj. PBZ-4/Bio-154. Inst. Pure & Appl. Biol. Bahauddin Zakariya Univ. Multan, Pakistan.

Bora, N. and P. Baruah (1979). Contiguous stomata in *Desmodium* Desv. (Papilionaceae). *Curr. Sci.* 487(1): 27-28.

Carr, D.J. and S.G.M. Carr (`1990b). Staurocytic stomatal complexes in species of *Monocalyptus* sensu Car and Car (Eucalyptus, Myrtaceae). *Aust. J. Bot.* 38 (1): 45-52.

Carr, S.G. and D.J. Carr (1990a). Cuticular features of the central Australian bloodwoods Eucalyptus section Corymbose (Myrtaceae). *Bot. J. Linnean Soc*. 102: 126-156.

Combalicer, M.S., D.K. Lee, S.Y. Woo, Y.K. Lee, and Y.H. Jang (2010). Early growth and physiological characteristics of planted seedlings in La Mesa Dam Watershed, Philippines. ISTF News, Maryland, USA (www.istf_bethesola.org). Originally published in the *Philippines Agricul. Scientist*. Vol 88 (3): 305-316. (2005).

Cratieri-Sosselsa, A.G. (2005). *Potentcialidade ornamemtal e paisagistica characterizaçâo morfo-anatömica e propagaçâo* Erythrina cristagalli *L. Rio grande do Sul*. 176 pp. Dissertaçâo de Mestradoem Ciẻncias Agronömicas Universidade de Passo Fundo. (seen in da Silva et al., 2013).

Croxdale, J.L. (2000). Stomatal patterning in angiosperms. *Am. J. Bot.* 87 (8):1069-1080.

Cutter, E.G. (1986). *Anatomia Vegetal: Celulas e tecidas*. Sâo Paulo. Roca. (Seen in da Silve *et al.*, 2013).

da Silva, M.M.B., Santana, A.S.C.O., Pimentel, R.M.M., Silva, F.C.L., Randan, K.P and Soares, L.A.L. (2013). Anatomy of leaf and stem of *Erythrina velutina.* Rev. Bras. Farmacognosia (*Braz. J. Pharmacognosy* = AOP 00713.

Das, D. Ch. and N.D. Paria, (1999). Seedling morphology in identification of some Indian species of *Bauhinia* L. (Caeselpiniaceae). *Fedes Repertorium* 110(5-6): 375-379.

Dasti, A.A., T.Z. Bokhari, S.A. Malik and R. Akhtar (2003). Epidermal morphology in some members of family Boraginaceae in Balochistan. *Asian J. Pl. Sci*. 2 (1): 42-47.

Deb, D.K. and N. Paria (1986). Seedling morphology of some economic species. *Ind. Agriculturist* 30(2): 133-142.

Dickson, W.C. (1975). Leaf Anatomy of Cunoniaceae. *Bot. J. Linn. Soc.*71: 275-294.

Ekenayake, I.J., Osini, D.V.S. and Porto, M.C.M. (1998). *Physiology of Cassava*. (http://www. Iita.org)

Elkhalifa, K.F and Aref, I.M.(2004). Morphological studies of fourteen Acacia species seedlings in Saudi Arabia. Res. Bullet. No. 122. Agr. Res. Center, King Saud University, pp. 5-11.

Esau, K. (1974). Anatomia des Plantas com Sementes. Sâo Paulo; Edgard Blücher.

Freire, S.E., A.M. Arambari, N.D. Bayon, G. Sancho, U Urtubey, C. Monti, M.C. Novoa and M.N. Colares (2005). Epidermal characteristics of toxic plants for cattle from Salado River Basin (Buenos Aires, Argentina). *Bol. Soc. Argent. Bot*. 40(3-4): 241-281.

Gan, Yi, L. Zhou, Zhong-Ji Shen, Yi-Qiong Zhang, Gen-Xian Wang (2010), Stomatal clustering, a new marker for environmental perception and adaptation in terrestrial plants. *Bot. Studies* 51: 325-336.

Garwood, N.C. (1996). Functional morphology of tropical tree seedlings (pp. 59-129). In: *The Ecology of Tropical Forest Tree Seedlings* (Ed. M.D. Swaine), MAB Series, Vol.17, UNESCO, Paris.

Hickey, L.J. (1973). Classification of the architecture of dicotyledonous leaves. *Am. J. Bot.* 60(1): 17-33.

Hickey, L.J. (1979). A revised classification of the architecture of dicotyledonous leaves. Pp. 25-39. In (C.R. Metcalfe and L. Chalk, Eds.). *Anatomy of the Dicotyledons.* II Ed. Vol I. *Systematic Anatomy of the Leaf and Stem with Brief History of the Subject.* Oxford: Clarendon Press. 176 Pp.

Jain, D.K. (1978). *Studies in Bignoniaceae. III. Leaf architecture. J. Ind. Bot. Soc.* 57: 369-387.

Kass, D.L. (1998). *Erythrina* species-Pan tropical Multipurpose Tree Legume (Ed. R.C. Gutteridge and Shelton, H.M.). *Forage Tree Legumes in tropical Agriculture Publ. by Tropical Grasslands soc. Australia.*

Khan, F., Z. Yousuf, S. Rani, and Khan Farah (2011). Taxonomic treatment of medicinally important arboreal flora of tropical and subtropical region based on leaf epidermal anatomical markers. *J. Med. Pl. Res*. 5 (28): 6439-6454.

Kothari, M.J. and G.L. Shah (1975). Epidermal structure and ontogeny of stomata in the Papilionaceae (Tribe Hedysareae). *Bot. Gaz*. 136: 372-379.

Kozlowski, T.T. and S.G. Pallardy (1997). *Physiology of Woody Plants.* II Ed. San Diego, Acad. Press Inc., 411 pp.

Lu, H., W. Jiang, M. Ghiassi, S. Lee and M. Nitin (2012). Classification of *Camellia* (Theaceae) species using leaf architecture variations and patte4rn recognition technique. PLOS One 7(1): e29704. doi:10.1371/journalpone.0029704.

LWG (Leaf Working Group). (1999). *Manual of Leaf Architecture: Morphological description and Categorization of Dicotyledonous and Net-Veined Monocotyledonous Angiosperms.* Smithsonian Institution, USA. Pp. 65.

Matheus, M.T. and J.C. Lopes (2007). Fruit, seed and seedling morphology and seed germination in *Erythrina variegata* L. *Rev. Bras. Sementes* (online vol. 27(3): 8-12.

Metcalfe, C.R. and L. Chalk (1950). *Anatomy of the dicotyledons: Leaves, Stem and Wood in Relation to Taxonomy with Notes on Economic Uses.* Oxford, Clarendon Press.

Metcalfe, C.R. and L. Chalk (1979). *Anatomy of the Dicotyledons (Second Ed.). Vol. I. Systematics, Anatomy of Leaf and Stem with Brief history of the Subject*. Oxford, 176 pp.

Miller, J.T and C. Miller (2011). Acacia seedling morphology: phyllotaxy and its relationship for seed mass. Aust. J. Bot. 59 (2): 185-196.

Misra, M.K., D. Padamajyoti, N.S. Prakash, A.S. Rana C.S. Srinivasan and M.S. Sreenivassan (2010). Leaf architecture in Indian coffee (*Coffea arabica* L.) cultivars and their adaptive significance. *World J. Fungal and Pl. Biol.* 1(2): 37-41.

Nenggan, Ye (1983-84). Description of various seedlings of leguminous plants. *Phytologia* V.54 (3): 190-218.

Norfaizal, G.M., H. Khalijah and A.R. Muhammad Ruzi (2012). Leaf anatomical study of five *Macaranga* species (Euphorbiaceae). *J. Trop. Agric. & Food. Sci.* 40(2): 289-296.

Obiremi, E.O, and F.A. Oladale (2001). Water conserving stomatal systems in selected Citrus species. *South Afr. J. Bot*. 67: 258-260.

Oyeleke, M.O., A.A. Abdul Rahman, and F.A. Oladele (2004). Stomatal anatomy and transpiration rate in some afforestation species. *Nigerian Soc. Exp. Biology Journal* 4 (2): 83-90.

Paliwal, G.S. (1966). Structure and ontogeny of stomata in some Acanthaceae. Phytomorphology 16: 527-532.

Pant, D.D. and P.K. Kidwai (1954). On the diversity in the development and organization of stomata in *Phyla nodiflora* Michx. *Curr. Sci*. 33: 653-654.

Patil, A.M. and D.A. Patil (2011). Investigations on foliar epidermal characteristics in some Acanthaceae. Curr. *Bot*. 2 (9):01-08.

Prabhakar, M. (2004). Structure, delimitation, nomenclature and classification of stomata. *Acta Botanica Sinica* 46 (2): 242-252.

Ravindran, P.N., K. Nirmal-Babu, and M. Shylaja (2003). *Cinnamon and Cassia: The genus Cinnamomum.* CRC Press, 384 Pp.

Reddy, P.K.R. and G.L. Shah (1979). Observations on the cotyledonary and hypocotyledonary stomata and trichomes in some Caesalpiniaceae with a note on their taxonomy. *Feddes Repertorium* 90: 239 -250.

Saheed, S.A and H.C. Illoh (2010). A taxonomic study of some species in Cassiinae (Leguminosae) using leaf epidermis characters. *Nortulae Bot. Hort. Agrobot*. Cluj. 38 (1): 21-27.

Shah, G.L. and B.V. Gopal (1969). Development of stomata in some Papilionaceae. Can. J. Bot. 47: 387-393.

Sinjushin, A.A. and J.A. Akopian (2011). On seedling structure in *Pisum* L., *Lathyrus* L. and *Vavilova* Fed. (Fabae: Fabaceae). *Wulfenia* 18: 81-93.

Soto-Hernández, R.M., Garcia-Mateos, R., Mignet-Chávez, R.S., Kite, G., Martinez-Vásquiz and A.C. Ramos-Valdiva, (2012). *Erythrina*, a potential source of chemicals from the Neotropics (Chap. # 9). In; Bioactive compounds in Phytomedicine (Ed. Iraj Rasooli). ISBN: 978-953-307-805-2 (http://www.intechopen.com/books/bioactive-compounds-in-phytomedicine/erythrina-a-poytential-source-of-chemicals-from-the n-neotropics). 218 pp.

Tripathi, S. and Mondal, A.K. (2012). Taxonomic diversity in epidermal cells (stomata) of some selected Anthophyta under the order Leguminales (Caeselpiniaceae, Mimosaceae and Fabaceae) based on numerical analysis: A systematic approach. *IJSN* 3(4): 788-798.

Vogel, E.F de (1980). Seedlings of dicotyledons: structure, development, types: Distribution of 150 woody Malesian taxa. Wageningen.

Wang, Xiu-Wei, Mao Zi-Jun, Choi, Kyung and Park, Kwang-Woo (2006). Significance of the leaf epidermis fingerprint for taxonomy of Genus Rhododendron. J. Forest. Res. 17(3): 171-176.

Wright, I.J., H.T. Clifford, R. Kidson, M.L. Reed, B.L. Rice and M. Westoby (2000). A survey of seed and seedling characters in 1744 Australian dicotyledons species: cross-species trait correlations and correlated trait-shifts within evolutionary lineages. *Biol. L. Linnean. Soc.* 69: 521-547.

Zamora-Carnelio, L.F., Ochoa-Gaona, G.V. Simon, J.C. Albores and B.H. Jong (2012). Seed germination and key to seedling identification for six native tree species of wetlands from Southeast Mexico. *Rev. Biol. Trop*. 58(2): 717 – 732.

Zar, J.H. (2010). *Biostatistical Analysis*. 5th Ed. Prentice-Hall, Englewood Cliffs. New Jersey, USA.

Zheng, Y., M. Xu, R. Hou, R. Shen, S. Qiu, and Z. Ouyang (2013). Effect of experimental warming on stomatal traits in leaves of maize (Zea mays L.). *Ecology and Evolution* 3(9): 3095-3111.

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