Pak. J. Bot., 46(5): 1653-1661, 2014.

SYSTEMATIC SIGNIFICANCE OF ANATOMICAL CHARACTERIZATION IN SOME EUPHORBIACEOUS SPECIES

NADIA BATOOL ZAHRA[1], MUSHTAQ AHMAD[*], ZABTA KHAN SHINWARI[1], MUHAMMAD ZAFAR AND SHAZIA SULTANA

Department of Plant Sciences, Quaid-i-Azam University Islamabad Pakistan
[1]Department of Biotechnology, Quaid-i-Azam University Islamabad Pakistan
[*]Corresponding author e-mail: mushtaqflora@hotmail.com

Abstract

The study was aimed to explore the systematic potential of anatomical characters for identification and delimitation among *Euphorbia* species. Eight species of leafy spurges of genus *Euphorbia* L. (Euphorbiaceae) were evaluated for variations in micro morphological characters of foliar epidermal anatomy. While anatomical observations are of importance in the assessments and appraisals, use of these characters as an effective tool in interpreting phyletic evaluations and systematic delineations has its limitations too. The epidermal cell wall in majority of species was wavy to undulate on both adaxial and abaxial surfaces. The observations made in this study indicate that there is not a single type of stomata which appears as characteristic of the genus *Euphorbia*. Also their distribution whether epistomatic or hypostomatic is not a genus-characteristic. The trichomes found were simple, unicellular or multicellular, uniseriate. Present investigation revealed the utility of both qualitative and quantitative characters in systematic studies; also the potential influence in the delimitation of species cannot be ignored. Our results show that the micro-morphology of anatomical characters play an important role in definition of taxa at species and sectional levels.

Key words: *Euphorbia*, Anatomical characters, Epidermal cell wall, Stomata, Trichomes.

Introduction

The family Euphorbiaceae also known as spurge family is one of the most diversified families of flowering plants which is considered as sixth largest. There are about 300 genera and over 8000 species constituting the spurge family (Radcliffe-Smith 1986; Webster 1987). It is largely diversified family, made up of all kind of plants including large woody trees, climbing lianas and simple weeds which have a prostrate habit. The distribution of its members is all around the globe constituting old world and new world species, several of which are yet to be identified. This occurrence of the family is primarily in the tropics, with the wide distribution of the species in the Indo-Malayan region and the second most populated region is tropical America. The third most diverse region is tropical Africa, but contains less varied and less populated species as compared to the other two regions.

Euphorbia is included in the five most species rich genera of angiosperms (Frodin, 2004) having around 2000 species (Govaerts *et al.,* 2000) occurring in all temperate and tropical regions, occupying a wide range of habitats and exhibiting great diversity in growth forms. They are annual weeds to trees having a unique flower structure. However, it also has many species in non-tropical regions such as southern USA, South Africa, the Mediterranean Basin and the Middle East making it cosmopolitan. The genus *Euphorbia* is characterised by the presence of milky latex, being more or less toxic (Singia & Pathak, 1990). This toxic latex is made up of diterpene esters and may cause inflammation and a blistering rash if it comes in contact with the skin. The significant percentage of euphorbias is succulent. The astonishing diversity found within this genus ranges from spurges which are low-growing garden weeds to giant succulents which are cactus-like. South African euphorbias have evolved succulent, spine-covered stems that greatly resemble North American cacti, a biological phenomenon known as "convergent evolution". Until you do not examine the blossom of showy garden euphorbia called poinsettia (*Euphorbia pulcherrima*) carefully, it is hard to believe that all these diverse forms belong to the same genus. The poinsettia bears showy, red, modified leaves which are not petals actually. These modified leaves are not even part of the true flowers. They surround clusters of small, greenish, cup-shaped structures called cyathia. Each cyathium is actually a flower cluster or inflorescence containing unisexual, apetalous male and female flowers. The occurrence of this genus is majorly reported from tropical and subtropical regions of Africa and America (Radcliffe-Smith, 1986) but also in temperate zones worldwide. Succulent species are primarily originated from Africa, the America and Madagascar.

Euphorbia species have found wide application as medicinal plants including the use for the treatment of skin diseases, gonorrhoea, migraine, cough, dysentery, intestinal parasites and wart cures (Kinghom and Evans, 1975; Singia & Pathak, 1990; Usher, 1974). It is economically an important family and various applications were published (Khalil *et al*., 2014). In addition several macrocyclic diterpenoids with antibacterial, anticancer, Prostaglandin E2 inhibitory, anti multidrug resistant, prolyl endopeptidase inhibitory, antifeedant, anti Human Immunodeficiency Virus and analgesic activities have been recently reported from a number of *Euphorbia* species. Members of *Euphorbia* are rich in phenolics (Ahmad *et al.,* 2002a), aromatic esters (Ahmad *et al.,* 2002b), steroids (Jassbi *et al.,* 2004), diterpenoids (Ahmad *et al.*, 2002a; Vasas *et al.*, 2004, Pervaiz *et al.,* 2004, Corea *et al.*, 2005, Ahmad *et al.,* 2006), tetracyclic triterpenoids (Jassbi *et al.,* 2004), pentacyclic triterpenoids (Ahmad *et al.,* 2002b), essential oils (Feizbakhsh *et al.,* 2004) and several bioactive constituents (Ravikanth *et al.,* 2002; Hohmann *et al.,* 2003).

It has been long time plant systematists deduced relationships among plant groups based upon a wide variety of biological characters. These characters include morphology at macro- morphology and micro-morphology, plant secondary metabolites, isozymes, and other characters. However, in some cases these characters are inconsistent and subjected to parallelism (Topik, 2005). Systematics plays a central role in the field of biology through providing the means for characterizing the organisms and the recognition in order to understand the biodiversity. Systematics is fundamentally aimed at discovering, describing and naming all the tips of the branches of the tree of life (Gravendel, 2000) as well as documenting the changes on the branches occurred during evolution and transform these into a predictive classification system that reflect evolution (Anon., 2000). Most important task in systematic is therefore the reconstruction of the historical relationships of groups of biological organisms. A correctly inferred phylogeny may provide a solid basis of the knowledge of relationships, and is a prerequisite for comparative investigations within such the fields as ecology and biogeography.

Remarkable diversification of morphological characters of the Euphorbiaceous species has been caused due to their occurrence in various topography, geography, climate, temperature and ecological interactions etc. Owing to this remarkable diversification and possible parallelism of vegetative and reproductive features, systematic and phylogenetic relationships among *Euphorbia* species are ill defined and they therefore await exploration. The present research work has been confined to genus *Euphorbia*, as it is cosmopolitan and has diverse economic and medicinal importance. *Euphorbia hirta* L., *Euphorbia pulcherrima* Willd. ex KL., *Euphorbia royleana* Boiss., *Euphorbia granulata* Forssk., *Euphorbia splendens* Bojer., *Euphorbia prostrata* Ait., *Euphorbia hamiltonii* Willd. (Syn. *E. heterophylla*) and *Euphorbia peplus* L. (Syn. *Euphorbia pilosa* L.) are undertaken as species of interest for systematic characterization through leaf epidermal anatomical characters. This approach may lead to phylogenetic characterization in future by using anatomical evidences at global perspective. In this study some new anatomical features have been reported first time with systematics significance used for taxonomic classification of Euphorbiaceous species. The future focus should be on molecular level as demonstrated for Liliaceae, Fabaceae and Solanaceae etc. (Shinwari, 1995; Shinwari *et al*., 2014 and Jamil *et al*., 2014).

Materials and Methods

The anatomical analysis was based on the study of fresh collection as well as herbarium specimens (ISL) of Quaid-i-Azam University, Islamabad (Table 1).

Table 1. Species and samples studied with current geographical distribution.

Taxa	Samples (Fresh & Herbarium) with voucher specimen No.	Distribution
E. hirta	N-ISl-140	A pantropical weed, near sea-level to 4500'/1370 m
E. pulcherrima	N-ISl-143	Central America from Mexico to Costa Rica. Now widely cultivated in the tropics and subtropics
E. royleana	N-ISl-144	North India (Himalayan foothills). 1500'/450 m - 6000'/1830 m.
E. granulata	N-ISl-147	From the Canaries and North Africa to Tropical Africa and eastwards to Central Asia and Northern India, near sea-level to 5000'/1525 m
E. splendens	N-ISl-149	Madagascar. Widely cultivated especially in the Old World Tropics
E. prostrata	N-ISl-150	Native to tropical and subtropical America introduced into many parts of the Old World. 1700'/518 m - 5900'/1800 m
E. hamiltonii	N-ISl-151	Throughout Tropical America from S. USA southwards; widely introduced into the Old World Tropics. 1800'/550 m -5000'/1525 m
E. peplus	N-ISl-152	Macaronesia, Europe, North Africa and South Western & Central Asia (extending Eastern to Northern Iran & Turkmenia). Introduced in South, South Eastern & East Asia, Australia and North & Central America, near sea-level to 8500'/2600 m

Foliar epidermal anatomy: For investigating foliar epidermal anatomy fresh leaves were taken from the available species in the field while few samples were taken from already collected species in dried form. Dried leaves were boiled for few minutes to soften the leaf. It allowed the leaves to unfold and became ready for epidermal scrapping. Fresh leaves were used directly for anatomical studies. The modified method of Clark (1960) was adopted for preparing specimens. The fresh or dried leaves were put inside a test tube containing 88% lactic acid placed in boiling water bath (Model, Memert D-91126- FRG, Germany) at 100°C for about 30 minutes. Lactic acid softens the tissue of leaf which resulted in easier peeling off. Three slides were prepared randomly from abaxial and adaxial surface of each species. For preparing the abaxial surface, the leaf was placed keeping its adaxial surface upward and then it was flooded with 88 % cold lactic acid. The adaxial epidermis was cut across the leaf by use of a sharp scalpel blade and scrapped away together with the mesophyll cells until only the abaxial epidermis of the leaf remained on the tile. The epidermis was placed on the glass slide and mounted in clean 88% lactic acid. For preparation of adaxial epidermis same procedure was followed. Microphotographs were taken by using CCD digital camera (Model: DK 5000) fitted on Leica Light Microscope (Model: DM 1000). Further study

of epidermal cells, stomata and trichomes was carried out by examining these micrographs. The length and width of the epidermal cells, stomata, guard cells and trichomes were recorded with the help of an ocular micrometer under 10X, 20X, 40X magnifications by using the above mentioned prepared slides from the abaxial and adaxial portions of the leaf epidermis.

Results and Discussion

Foliar epidermal anatomy: In many Euphorbiaceous species, the shape of epidermal cells was varying from undulating, irregular to variously shape. Most of the species had anomocytic and diacytic stomata, except in *E. hirta,* where stomata were anisocytic. While in *E. pulcherrima* they were totally absent (Figs. 9B & 10B). In *E. hamiltonii* and *E. peplus* stomata were not found on abaxial surface (Figs. 10G & 10H). Rest of the stomata possessing species had them on both surfaces. Trichomes were altogether absent in *E. royleana, E. granulata, E. splendens* and *E. peplus*. Glandular trichomes were seen in *E. hirta* only, *E. pulcherrima, E. prostrata* and *E. hamiltonii* had non-glandular trichomes (Table 2).

The size of epidermal cell ranges from 20 µm (*E. hamiltonii*) to 76.5 µm (*E. royleana*) (Figs. 1 & 2). The length of stomata ranges from 09 µm (*E. hirta*) to 23.2 µm (*E. royleana* and *E. splendens*), while the width of stomata varies from 04 µm (*E. prostrata*) to 06 µm (*E. granulata*) (Figs. 3 & 4). The length of guard cells varies from 13.2 µm (*E. prostrata*) to 38.2 µm (*E. royleana*), while the width of guard cells varies from 7.5 µm (*E. prostrata*) to 30.7 µm in *E. royleana* (Figs. 5 & 6). The length of trichomes varies from 170.7 µm (*E. prostrata*) to 382.5 µm (*E. hamiltonii*) and its width ranges from 11.5 µm (*E. prostrata*) to 29 µm (*E. hamiltonii*) (Figs. 7 & 8) (Table 3).

Table 2. Distribution of Anatomical characters of Foliar Epidermis among the *Euphorbia* species studied.

Species	Epidermal cell adaxial/ abaxial	Type of stomata adaxial/abaxial	Trichomes adaxial/ abaxial
E. hirta	Irregular/ Irregular, variously shaped	Anisocytic/ Anisocytic	Bulbous base and glandular/ Bulbous base and glandular
E. pulcherrima	Undulating/Undulating	Absent/ Absent	Flat base and non-glandular/ Bulbous base and non-glandular
E. royleana	Variously shaped/ Variously shaped	Diacytic/ Diacytic	Absent/ Absent
E. granulata	Undulating/Rectangular, undulating, irregular	Anomocytic/ Anomocytic	Absent/ Absent
E. splendens	Undulating, irregular/ Undulating, irregular	Diacytic/ Diacytic	Absent/ Absent
E. prostrata	Variously/Undulating, variously, irregular	Anomocytic/ Anomocytic	Bulbous base and non-glandular/ Bulbous base and non-glandular
E. hamiltonii	Undulating, irregular/ Variously	Anomocytic/ Absent	Bulbous, flat base and non-glandular/ Absent
E. peplus	Undulating/ Variously	Diacytic/ Absent	Absent/ Absent

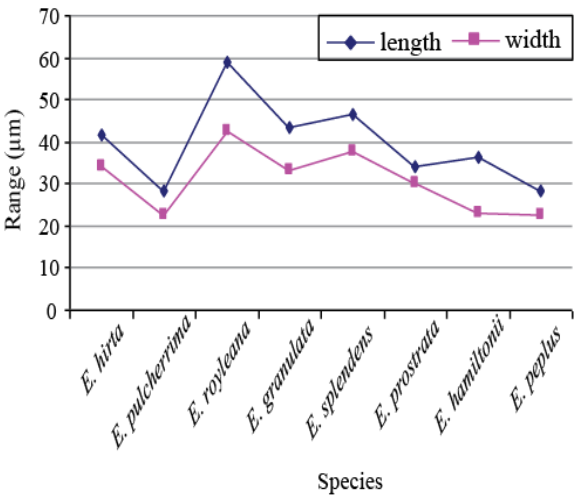

Fig. 1. Distribution of length and width of epidermal cells on adaxial side.

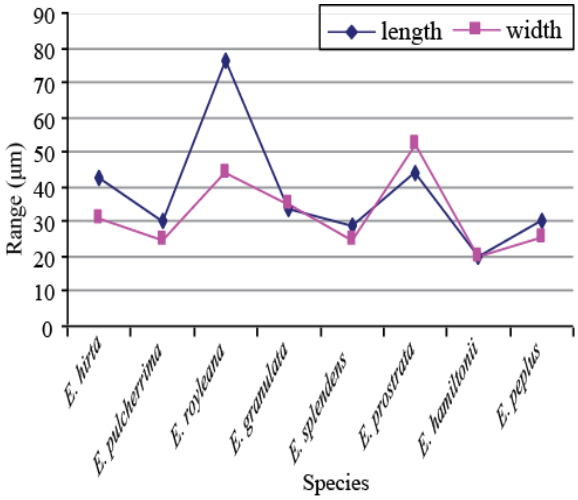

Fig. 2. Distribution of length and width of epidermal cells on abaxial side.

Table 3. Distribution of Foliar Epidermal Anatomical characteristics and measurement among the *Euphorbia* species studied.

Species	Epidermal cells adaxial/ abaxial L×W(µm)	Stomata adaxial/ abaxial L×W(µm)	Guard cells adaxial/ abaxial L×W(µm)	Trichomes adaxial/ abaxial L×W(µm)
E. hirta	41.5(37.5-45) x 34.1(35-32.5)/ 42.5(32.5-55) x 30.7(27.5-35)	9(7.5-10) x 5(5-5)/ 9(5-12.5) x 7.5(7.5-7.5)	20(17.5-22.5) x 13.25(12.515)/ 19(17.5-22.5) x 12.5(10-15)	280(275-287.5) x 25(22.5-25)/ 163(150-185) x 15.7(12.5-20)
E. pulcherrima	28.2(25-32.5) x 22.5(25-37.5)/ 30(25-30) x 25(17.5-37.5)	Absent / Absent	Absent / Absent	250(237.5-275) x 28.2(25-30)/ 217.5(212.5-225) x 24(22.5-25)
E. royleana	59(52.5-62.5) x 42.5(35-50)/ 76.5(55-100) x 44(32.5-50)	22.5(20-25) x 8.2(7.510)/ 23.2(20-25) x 9(7.5-10)	38.2(35-42.5) x 28.2(25-30)/ 38.2(37.5-40) x 30.7(25-30)	Absent / Absent
E. granulata	43.2(37.5-50) x 33.2(30-40)/ 34(25-37.5) x 35(25-50)	15(12.5-17.5) x 9(7.5-10)/ 14(12.5-17.5) x 9(7.5-10)	16.5(12.5-17.5) x 10(12.5-20)/ 16.5(12.5-22.5) x 11.5(10-15)	Absent / Absent
E. splendens	46.5(37.5-57.5) x 37.5(25-50)/ 29(25-32.5) x 25(20-27.5)	23.2(20-25) x 5(1-5)/ 17.5(12.5-22.5) x 5.7(5-7.5)	32.5(27.5-37.5) x 25.7(20-32.5)/ 30.7(27.5-35) x 21.5(17.5-25)	Absent / Absent
E. prostrata	34(30-37.5) x 30(25-37.5)/ 44(37.5-50) x 52.5(45-62.5)	7.5(5-10) x 5.7(5-10)/ 6.5(5-7.5) x 4(2.5-5)	13.2(10-17.5) x 7.5(5-10)/ 18.2(15-22.5) x 10.7(7.5-15)	212.5(200-225) x 19(17.5-20)/ 170.7(150-187.5) x 11.5(10-12.5)
E. hamiltonii	36.5(27.5-45) x 23.2(17.5-30)/ 20(12.5-20) x 20(17.5-22.5)	17.5(15-20) x 8.2(7.5-10)/ Absent	27.5(25-30) x 21.5(20-22.5)/ Absent	382.5(325-425) x 29(25-35)/ Absent
E. peplus	28.2(25-32.5) x 22.5(20-25)/ 30(25-37.5) x 25.7(25-27.5)	23.2(20-25) x 9(7.5-10)/ Absent	Absent /Absent	Absent /Absent

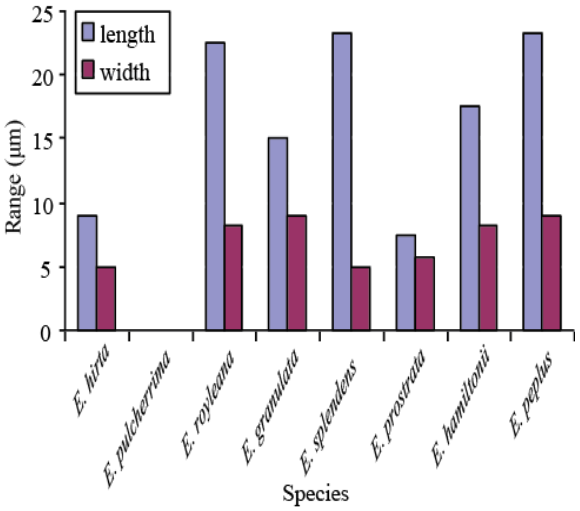

Fig. 3. Distribution of length and width of stomata on adaxial side.

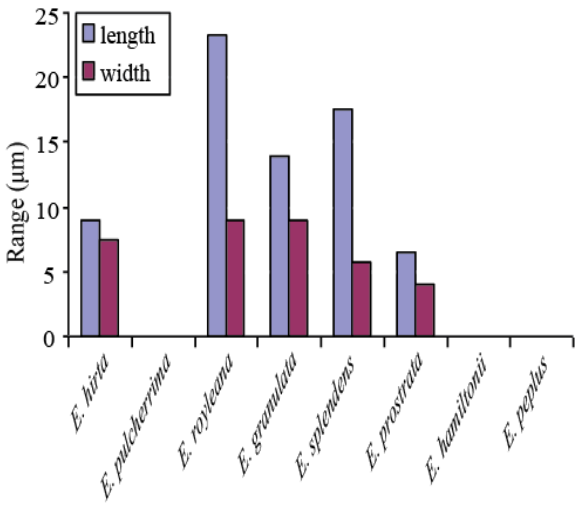

Fig. 4. Distribution of length and width of stomata on abaxial side.

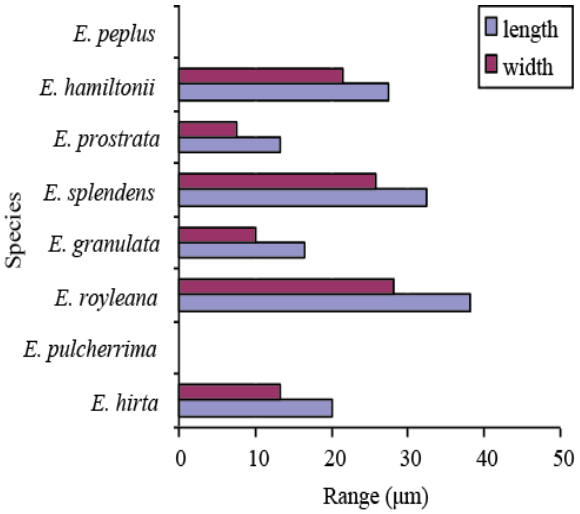

Fig. 5. Distribution of length and width of guard cells on adaxial side.

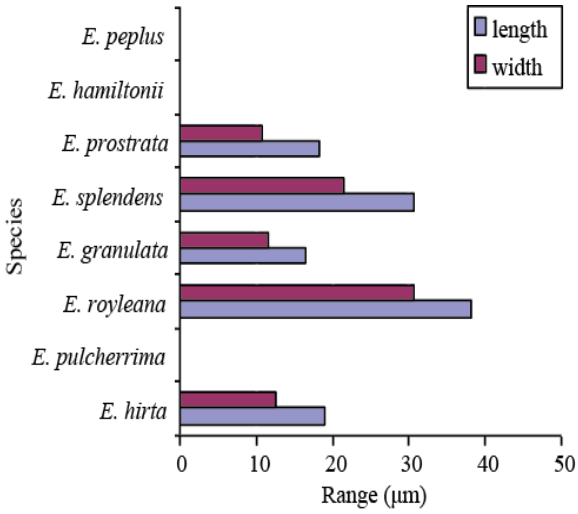

Fig. 6. Distribution of length and width of guard cells on abaxial side.

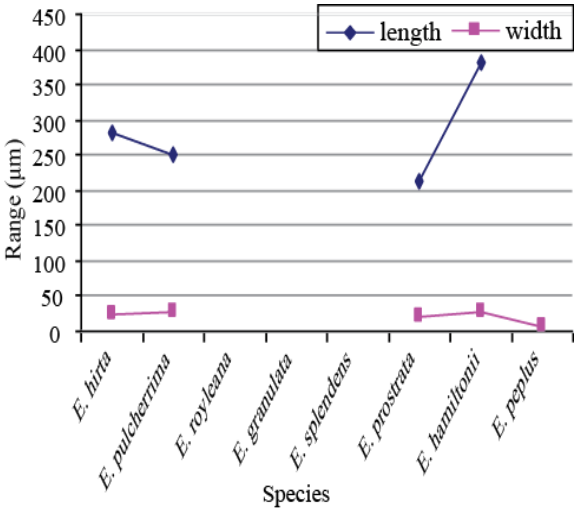

Fig. 7. Distribution of length and width of trichomes on adaxial side.

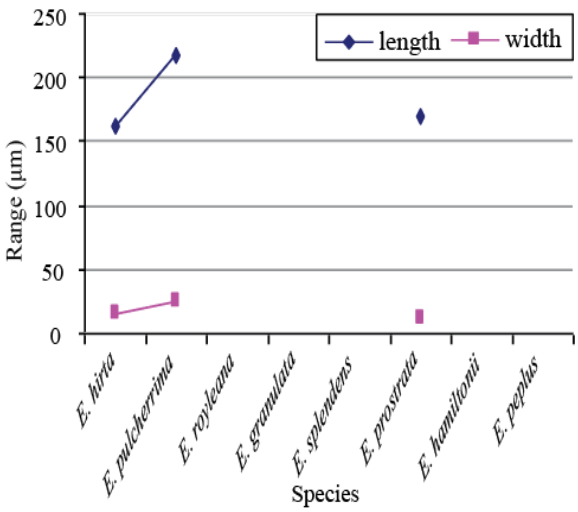

Fig. 8. Distribution of length and width of trichomes on abaxial side.

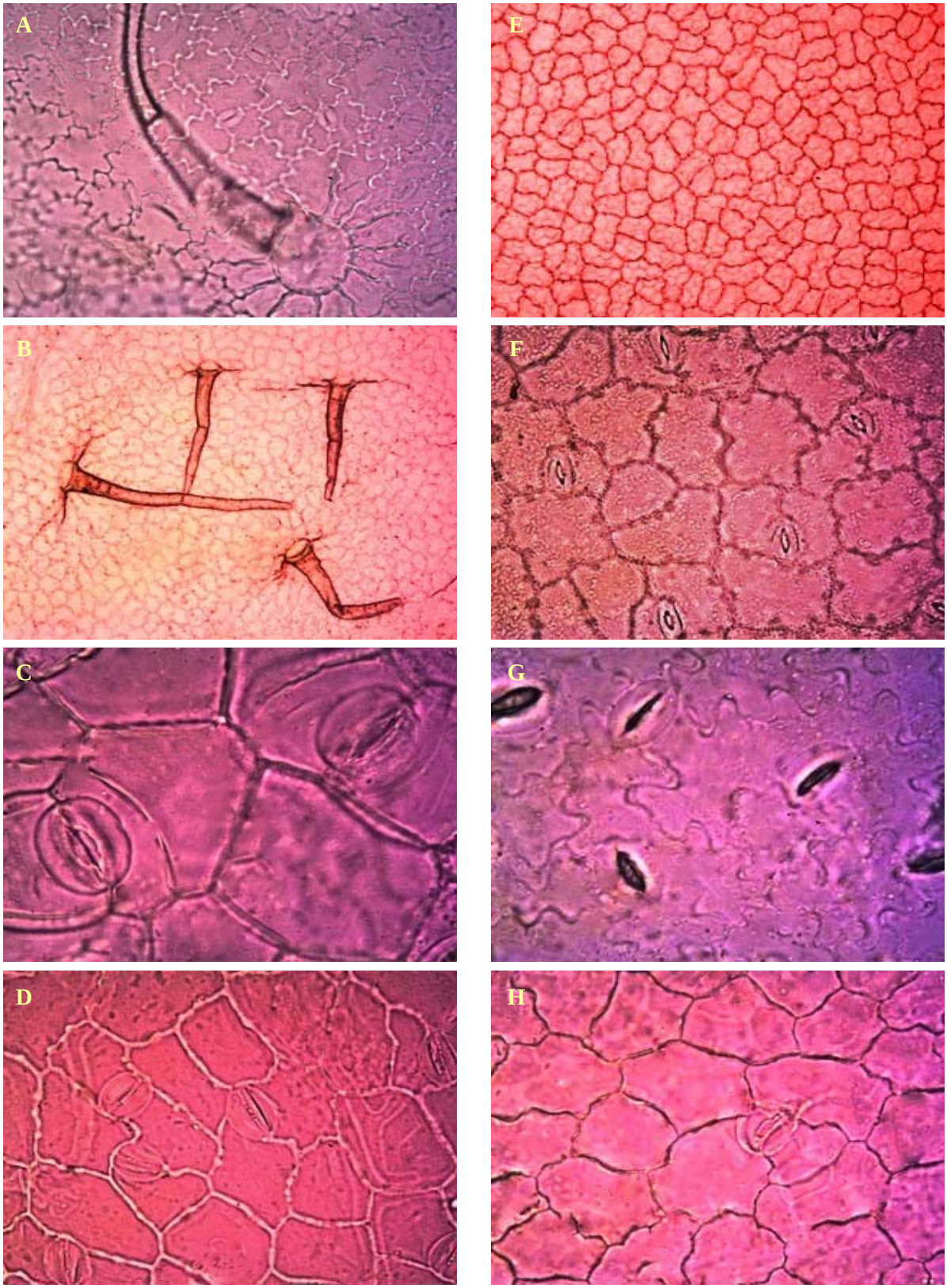

Fig. 9. Characteristics of adaxial foliar epidermal anatomy of *Euphorbia*: A- Glandular trichome of *E. hirta* (20x), B- Non- glandular trichomes of *E. pulcherrima* (10x), C- Stomata and epidermal cells of *E. royleana* (40x), D- Epidermal cell shape and stomata of *E. granulata* (40x), E- Epidermal cell shape of *E. splendens* (10x), F- Epidermal cell shape and stomata of *E. prostrata* (40x), G- Stomata of *E. hamiltonii* (40x), H- stomata and epidermal cells of *E. peplus* (40x).

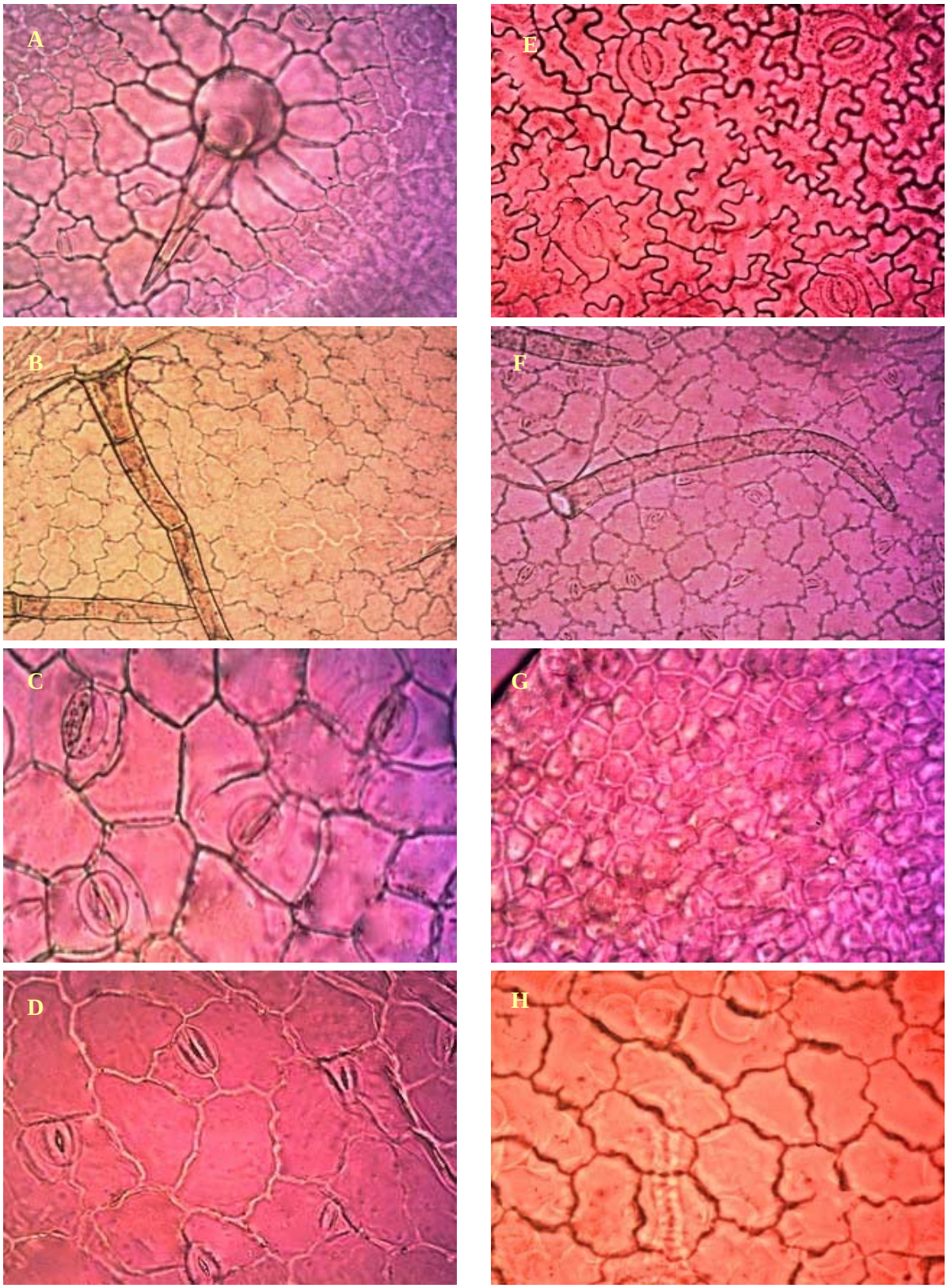

Fig. 10. Characteristics of abaxial foliar epidermal anatomy of *Euphorbia*: A- Glandular trichome of *E. hirta* (20x), B- Non- glandular trichomes of *E. pulcherrima* (20x), C- Stomata and epidermal cells of *E. royleana* (20x), D- Epidermal cell shape and stomata of *E. granulata* (40x), E- Epidermal cell shape and stomata of *E. splendens* (20x), F- Epidermal cell shape, stomata and non- glandular trichome of *E. prostrata* (20x), G- Epidermal cells of *E. hamiltonii* (20x), H- Epidermal cells of *E. peplus* (40x).

Plant morphology has a vital role and regarded as the first step to plant identification. While anatomical observations are of importance in the assessments and appraisals, use of these characters as an effective tool in interpreting phyletic evaluations and systematic delineations has its limitations too. The epidermal wall whether thick or thin is also helpful to some extent for identification. Ramassamy (1995) stated epidermal morphology and stomatal ontogeny are the characters which can distinguish between primitive and advance types and are helpful in morphological and ontogenetical classification. The epidermal cell wall in majority of species was wavy to undulate on both adaxial and abaxial surfaces. *E. royleana* was an exception where it was variously shaped on both sides (Figs. 9C &10C).

The presence of paracytic stomata in the tribe Euphorbieae were reported (Metcalfe & Chalk, 1950). Raju & Rao (1977) reported presence of paracytic stomata as most common. Same studies recorded anisocytic, anomocytic, and diacytic stomata in 50 species belonging to 17 different tribes of the Euphorbiaceae. In their opinions the paracytic type forms the basic stomatal type for the family Euphorbiaceae because of common occurrence in majority of tribes studied. Sehgal & Paliwal (1974) investigated 150 species of the genus *Euphorbia* and stated that most of the stomatal type recognized for dicotyledons met with in the genus *Euphorbia*. Anomocytic type being most preponderant. The authors in this study noted mostly anomocytic and diacytic type in *Euphorbia*, only *E. hirta* had anisocytic stomata (Figs. 9A & 10A) while absence of stomata was marked in *E. pulcherrima* (Figs. 9B & 10B). Kakkar & Paliwal (1974) observed anomo, aniso, para and cyclocytic type of stomata in various species of *Euphorbia*. The findings of the current study and the earlier reported work by different authors indicate that no stomata are characteristic of the genus *Euphorbia*. Also their distribution whether epistomatic or hypostomatic is not a genus-characteristic. However, it appears that anomocytic type is more commonly present in the *Euphorbia*. The other types such as paracytic, anisocytic and diacytic appear to have been derived from it in the euphorbiaceous alliance. Metcalfe and Chalk (1950) remarked that "the anatomical structure exhibit a wide range of variation in correlation with a diversity of habit and no important characters occurs throughout the numerous tribes into the families divided". The most comprehensive description of types of glandular and non-glandular hair and their distribution play an important role in correct identification of the specimen and its classification even to the specific level.

Metcalfe & Chalk (1950) documented basically three types of trichomes, viz., glandular, non-glandular and stinging types. A key was formulated based upon the presence or absence of trichomes in the two genera of family Euphorbiacaea (Baruah & Nath, 1997). Rao & Raju (1985) reported trichome types and their distribution in 250 species of the family Euphorbiacea. According to Gales & Toma (2006) trichomes found in *Euphorbia* were simple, unicellular or multicellular, uniseriate. Kakkar & Paliwal (1974) presented the same observations. Multicellular, uniseriate, glandular trichome was seen in *E. hirta* (Figs. 9A & 10A) whereas *E. pulcherrima* possessed multicellular, uniseriate, non-glandular trichome (Figs. 9B & 10B). In *E. prostrata* unicellular tapering end trichome was present (Fig. 10F) while rest of our species marked the absence of trichomes (Figs. 9C, 9D, 9E, 9G, 9H, 10C, 10D, 10E, 10G, 10H).

To summarize our efforts, it is so far indicated that leaf epidermal anatomy studies conducted in this project provide important data for identification as well as a contribution towards a better knowledge of the genus *Euphorbia*. In conclusion, it is obtained that leaf anatomy of *Euphorbia* species studied is of considerable value and prove the classification of this genus based on morphological results. Molecular systematics may resolve certain issues as reported for other angiospermic groups (Shinwari *et al*., 1994; Shinwari, 2002).

References

Ahmad, V.U., H. Hussain, J. Hussain, A.R. Jassbi, I.A. Bukhari, A. Yasin, M.I. Choudhary and A. Dar. 2002a. New bioactive diterpenoid from *Euphorbia decipiens*. *Z. Naturforsch*., 57: 1066-1071.

Ahmad, V.U., J. Hussain, H. Hussain, U. Farooq, F. Ullah, M.A. Lodhi and M.I. Choudhary. 2006. Two new diterpene polyesters from *Euphorbia decipiens*. *Natural Product Research*, 19: 267-274.

Ahmad, V.U., M. Zahid, T. Khan, M. Asim and A. Ahmad. 2002b. Chemical constituents of *Euphorbia heteradenia*. *Proceedings of Pakistan Academy of Sciences*, 39: 201-206.

Anonymous. 2000. Charting the biosphere, Technical Report. Systematics Agenda. New York.

Baruah, A. and S.C. Nath. 1997. Studies on the foliar epidermal characters of some members of the Euphorbiaceae. *Advances in Plant Sciences*, 10: 117-123.

Clark, J. 1960. Preparation of leaf epidermis for topographic study. *Stain Technology*, 33: 35-39.

Corea, G., C. Fattorusso, E. Fattorusso and V. Lanzotti. 2005. Amygdaloidins A-L, twelve new 13 α-OH jatrophane diterpenes from *Euphorbia amygdaloides* L. *Tetrahedron*, 61: 4485-4494.

Feizbakhsh, A., M. Bighdeli, M.S. Tehrani, A. Rustaiyan and S. Masoudi. 2004. Chemical constituents of essential oil of *Euphorbia teheranica* Boiss., a species endemic to Iran. *Journal of Essential Oil Research*, 16: 40-41.

Frodin, D.G. 2004. History and concepts of big plant genera. *Taxon*., 53 753-776.

Gale, R.C. and C. Toma. 2006. Comparative anatomy of the vegetative organs of some *Euphorbia* species (euphorbiaceae juss.) from the Romanian flora. *Romanian Journal of Biology-Plant Biology*, 51: 39-47.

Govaerts, R., D. Frodin and A. Radcliffe-Smith. 2000. World checklist and bibliography of Euphorbiacea (with Pandaceae). *Royal Botanic Gardens, Kew*, 2: 417-921.

Gravendeel, B. 2000. Reorganising the orchid genus *Coelogyne*: A phylogenetic classification based on morphology and molecules. National Herbarium Nederland.

Hohmann, J., D. Rédei, P. Forgo, J. Molnár, G. Dombi and T. Zorig. 2003. Jatrophane diterpenoids from *Euphorbia mangolica* as modulators of the multidrug resistance of L5128 mouse lymphoma cells. *Journal of Natural Products*, 66: 976-979.

Jamil, I., S. Qamarunnisa, A. Azhar, Z.K. Shinwari, S.I. Ali and M. Qaiser. 2014. Subfamilial relationships within solanaceae as inferred from *Atpβ-rbc*L intergenic spacer. *Pak. J. Bot*., 46(2): 585-590.

Jassbi, A.R., S. Zamanizadehnajari and S. Tahara. 2004. Chemical constituents of *Euphorbia marschalliana* Boiss. *Z. Naturforsch*., 59: 15-18.

Kakkar, L. and G.S. Paliwal. 1974. Studies on leaf anatomy of *Euphorbia* L. V. Epidermis. *Proceedings of Indian National Science Academy*, 40: 55-67.

Khalil, A.T., Z.K. Shinwari, M. Qaiser and K.B. Marwat. 2014. Phyto-therapeutic claims about euphorbeaceous plants belonging to Pakistan; an ethnomedicinal review. *Pak. J. Bot*., 46(3): 1137-1144.

Kinghorn, A.D. and F.J. Evans. 1975. Planta Medica. 28: 326-35.

Metcalfe, C. R. and Chalk, L. 1950. Anatomy of Dicotyledons Vol. II. Clarendon Press, Oxford, England.

Parvez, M., V.U. Ahmad, J. Hussain and U. Farooq. 2004. 3, 7, 17-Tri-*O*-acetyl-5-*O*-butanoyl-13, 15- dihydroxymyrisinol. *Acta Crystallographica*, 60: 148-150.

Radcliffe-Smith, A. 1986. Euphorbiaceae. In: *Flora of Pakistan*; (Eds.) E. Nasir & S.I. Ali. Shamim Printing Press, Karachi, Pakistan. 172: 1-170.

Raju, V.S. and P.N. Rao. 1977. Variation in the structure and development of foliar stomata in the Euphorbiaceae. *Botanical Journal of Linnean Society*, 75: 69-97.

Ramassamy, V. and B. Kannabiran. 1995. Stomata on earlier Origin; Morphology and Ontogeny. Beitraege Zur Biologie Der Pflanzen. 68: 1-9.

Rao, P.N. and V.S. Raju. 1985. Foliar trichomes in the family Euphorbiaceae. *Advances in Plant Sciences*, 128-136.

Ravikanth, V., V.L.N. Reddy, T.P. Rao, P.V. Diwan, S. Ramakrishna and Y. Venkateswarlu. 2002. Macrocyclic diterpenes from *Euphorbia nivulia. Phytochemistry*, 59: 331-335.

Sehegal, L.S. and G.S. Paliwal. 1974. Studies on the leaf anatomy of *Euphorbia* III the node. *Botanical Journal of Linnean Society*, 69: 37-43.

Shinwari, Z.K. 1995 Congruence between morphology and molecular phylogeneties in Prosartes (Liliaceae). *Pak. J. Bot*., 27(2): 361-369.

Shinwari, Z.K. 2002. Sequence divergence of *rbc*L gene and Phylogenetic relationships in Liliales. *Pak. J. Bot*., 34(2): 191-204.

Shinwari, Z.K., K. Jamil and N.B. Zahra. 2014. Molecular systematics of selected genera of family Fabaceae. *Pak. J. Bot*., 46(2): 591-598.

Shinwari, Z.K., R. Terauchi, F.H. Utech and S. Kawano. 1994. Recognition of the New World Disporum Section Prosartes as Prosartes (Liliaceae) based on the sequence data of the rbcL gene. *Taxon*, 43(3): 353-366.

Singia, A.K. and K. Pathak. 1990. Fitoterapia, LXI. 483-516.

Topik, H. 2005. Systematic study of subtribe Aeridinae (Orchidaceae). Ph.D. thesis, The University of Tokyo, Japan.

Usher, G. 1974. Dictionary of plants used by man. Tinling Limited, Prescot, Lanes, London.

Vasas, A., J. Hohmann, P. Forgo and P. Szabó. 2004. New tri- and tetracyclic diterpenes from *Euphorbia villosa. Tetrahedron*, 60: 5025-5030.

Webster, G.L. 1987. The saga of the spurges: a review of classification and relationships in the Euphorbiales *Botanical Journal of Linnean Society*, 94: 3-46.

(Received for publication 8 June 2013)