J. Appl. Environ. Biol. Sci., 6(4S)218-226, 2016

ISSN: 2090-4274
Journal of Applied Environmental and Biological Sciences
www.textroad.com

Petal Anatomy: Can it be a Taxonomic Tool?

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Received: January7, 2016
Accepted: March 2, 2016

ABSTRACT

Epidermal anatomy of petals of 14 species belonging to family Rosaceae has been studied by using light microscope. Anatomical characters observed in petals and their reliability as a taxonomic marker has been discussed. The petal epidermal cells exhibit variations in shape and size. Such as *Prunus persica* was found to Isodiametric, *Pyrus pyrifolia* epidermal cells were Rectangular and *Rosa macrophylla* had rounded shaped epidermal cells. Diversified types of trichomes were observed among these species. Both glandular (*Rosmarinus officinalis*) and non-glandular (*Potentilla gelida*) types were reported. The presence and distribution of silica bodies and oil bodies also investigated. Shape and size of silica bodies varied from species to species. Moreover oil bodies were present throughout in all studied species, although these oil bodies showed remarkable variations in shape and size which suggest their importance as taxonomic tool. In this project taxonomic significance of petal anatomical structures in identification and elucidation of species affinity and relationship has been discussed.

KEYWORDS- Epidermal anatomy; Stomatal distribution; trichomes; silica bodies

I. INTRODUCTION

Rosaceae is cosmopolitan to sub-cosmopolitan family, but it is diversified particularly in the Northern hemisphere (Judd *et al*., 1999). It is moderately large one consisting of 125 genera and 3500 plus plant species (Landrein *et al.,* 2009). In Pakistan it is represented by 27 genera and about 160 species which possessed great significance as economic and medicinal perspectives. In traditional medicine, rose petals are used as a remedy for diarrhea, coughs and colds, sores, nervous tension, lethargy and painful joints. Rose seeds are effective as diuretic and to cure various urinary tract disorders (Faghire *et al*., 2012**).** Moreover in industries essential oils and vitamins containing species has been greatly used (Lu *et al*., 2003).

Taxonomically Rosaceae belongs to the Rosid Clade, Fabids Subclade, and Order Rosales. The Rosales contain 9 families and about 6300 species Rosaceae has been classified into four sub families Spiraeoideae (spirea subfamily), Rosoideae (rose subfamily), Amygdaloideae (peach subfamily), Maloideae (apple subfamily), (Vafadar *et al*, 2008).

Several international researchers had emphasized on petal anatomical feature in plant systematics such as Naz *et al*., 2009; Hameed *et al*., 2010; Riaz *et al*., 2010; Potter *et al* (2007). Gillani (2002) and they **s**uggested petal anatomical structure as an important taxonomic tool for delimitation of taxa. Significance of petal anatomy in systematics can also be realized by work of Akcin (2009) who investigated about the petal epidermis and petal anatomy of 11 taxa of *Onosma* L. by using the light and scanning electron microscopy and he concluded that micromorphological features on dorsal surfaces of petals could be useful for species-level diagnosis. In 2010 Tahir and Rajput studied petal anatomy of 8 species of *Sibbaldia* and showed the taxonomic significance of petal structure in the identification and elucidation of species affinity and relationship.

Above discussion signifies the importance of petal anatomy in plant systematics**.** Petal anatomy is a great field of interest in foreign countries, and they have done lot of work on this but unfortunately in Pakistan so far yet no attempt is made to use petal anatomical data for taxonomic purposes. So this project is lead with a purpose to study the petal anatomy of different species belonging to family Rosaceae and use this data for solving taxonomic problems. This present research was lead with the aims.

1) To provide a systematic approach for the easy identification of the species on the basis of the anatomical characters of petals.
2) To show the significance of petal anatomy for identification at species level.

II. MATERIALS AND METHODS

For anatomical studies fresh and herbarium dried specimen were used. Fresh specimens of Family Rosaceae were collected from different localities of Lahore (Garden of Lahore College for Women University, Bagh-e-Jinnah, Lawrence Garden, Jallo park).While dried preserved specimen were managed from Herbarium of Quaid-e-Azam University, Islamabad. For anatomical studies fresh petals were fixed in Lactic acid ammonia solution for 1-2 days. Lactic acid helped to remove the bright color of petals and softened the tissues of petals due to which it's peeling off was become easy. Abaxial and adaxial petal surfaces were prepared by placing petal on a tile and then it was flooded with 70% cold lactic acid. By the help of a sharp scalpel blade the epidermis was cut across the petal. Peeled epidermis was mounted on clean glass slide with a drop of 70%

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lactic acid. Microphotographs were taken by using CCD digital camera these micrographs were useful for identification and differentiation of different anatomical parameters on basis of microscopic feature.

III. RESULTS AND DISCUSSIONS

The present study was carried out to clear up the taxonomic position and delimitation of the taxa on the basis of petal anatomical features. Several specialists Agbagwa and Ndukwu (2012); Akcin (2009) Tahir and Rajput (2010) have stressed the taxonomic value of petal anatomical characters. Akcin (2009) had found that micromorphological features on dorsal surfaces of petals could be useful for species level diagnosis. The present study reported that petal anatomical features such as stomata, trichomes and other characters are useful anatomical tools and valuable in the identification of the plants. The petal anatomical features of the studied species were summarized in table 1&2.

TABLE 1 ABAXIAL ANATOMICAL VARIATIONS AMONG SELECTED PLANT SPECIES OF FAMILY ROSACEAE

Plant name	Petal epidermis cells	Stomata type & Guard cells	Subsidiary cells	Trichomes	Silica bodies	Oil bodies/ droplets
Geum montanum	Polygonal, smooth, thick walled, average length 190 (150-230) µm, average width 110 (80-140) µm.	Actinoctic Average length 48.1 (26.2-70) µm, average width 14.8(9.1-20.5) µm	Average length 120 (90-150)µm, average width 60 (40-90) µm.	Non glandular, enlongted, dichotomous, number of trichomes per unit area 4-8 average length 407.2 µm, average width 43.2 µm.	Narrow elliptate, 4-6 silica bodies per unit area, average length 40.4 µm, average width 38.9 µm.	Rounded, 55-60 oil droplets per unit area , average length 46.1 µm, average width 43.6 µm.
Potentilla gelida	Bilobed , smooth, thick walled, average length 219.4 (213.8-225) µm, average width 67.35 (61.1-73.6) µm.	Diacytic Average length 80.05(69.4-90.7) µm, average width 15.8(14.4-17.2) µm.	Average length112 (90.8-133.2) µm, average width 97.3 (80.6-114.0) µm.	Absent	Absent	Oval, 10-15 oildroplets per unit area , average length18.25 µm, average width 20 µm.
Prunus cerasus	Triangular, smooth, thick walled, average length 233.05 (173.5-292.6) µm, average width 206 (199.3-212.7) µm.	Paracytic Average length 62.85 (48.5-77.2)µm, average width 38.2(25.0-51.4) µm.	Average length 50.55 (32.6-68.5) µm, average width 37.2 (24.6-49.8) µm.	Glandular with spherical pluricellular or elipsoidal head, 4-6 trichomes per unit area, average length 452.4 µm, average width 92.4 µm.	Absent.	Rounded, 55-60 oildroplets per unit area, average length 29.9 µm, average width 22.9 µm.
Prunus persica	Isodiametric polygonal, smooth, thick walled, average length 102.7 (70.2-135.2) µm, average width 55.3 (44.3-62.3) µm.	Anisocytic Average length 57.9 (49.8-66.0) µm, average width 83.5(78.5-88.5) µm.	Average length 44.5 (30.8-58.2) µm, average width 30.1 (20.2-40.0) µm.	Non glandular, tubular, pointed end, 15-20 trichomes per unit area, average length 182.4 µm, average width 11.3 µm.	Bilobates, 6-8 Silica bodies per unit area , average length 36.5 µm, average width 34.2 µm.	Rod shaped, 4-6 oildroplets per unit area, average length 8.5 µm, average width 7.75 µm.
Pyrus pyrifolia	Rectangular, thick walled, average length 61.3 (36.0-86.6) µm, average width 47.5 (28.5-66.5) µm.	Anomocy-tic Average length 65.4(50.6-80.2)µm, average width 74.6(62.8-86.4) µm.	Average length 67.4 (56.6-78.2) µm,average width 56 (45.2-66.8) µm.	Absent	Absent	Triangular, number of oildroplets per unit area 10-12 , average length 23.8 µm, average width 18.1 µm.
Rosa indica	Rounded shaped , smooth, thick walled, average length 53.9 (37.3-70.5) µm, average width 45.5 (34.5-56.5) µm.	Tetracytic Average length 45.5(35.0-54.0) µm, average width 63.5(48.9-78.1) µm.	Average length 64.5 (46.8-82.2) µm, average width 76 (66.0-86.0) µm.	Glandular, capitate, mushroom like, 1-2 trichomes per unit area, average length 95.5 µm, average width 30.4 µm.	Saddle, 2-6 Silica bodies per unit area , average length 22.5 µm, average width 35.2 µm.	Rounded, smaller pores like, 40-45 oildroplets per unit area , average length 11.9 µm, average width 10.95 µm.
Rosa macrophylla	Rounded, smooth, thick walled, smaller in size, cells are compact like honey bees hive, average length 22 (18.0-26.0) µm, average width 18.5 (13.9-23.1) µm.	Anisocy- tic Average length 53(34-72) µm, average width 52.9(45.6-60.2) µm	Average length 51.8(42.4-61.2) µm, average width 69.5 (52.5-86.5) µm.	Non- glandular, tubular, blunt end, 2-4 trichomes per unit area, average length 573.3 µm, average width 29.3 µm.	Absent	Absent
Rosmarinus officinalis	Irregular undulating margins, smooth, thick walled, average length 173.8 (121-226.6) µm, average width 61.05 (41.1-81.0) µm.	Cyclocytic Average length 70.3(48.5-92.1) µm, average width 54(39.6-68.4.) µm.	Average length 50.8 (34.8-66.8) µm, average width 56.1 (42.5-69.7) µm.	Glandular, capitate, 20-25 trichomes per unit area average length 313.5µm, average width 54.65 µm.	Oval, 10-12 silica bodies per unit area, average length 105.5 µm, average width is 66.8 µm.	Oval , 8-10 oildroplets per unit area, average length 48.35 µm, average width 44.95 µm.

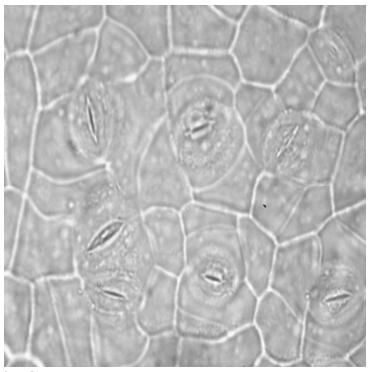

Fig.1 (a) *Geum montanum*

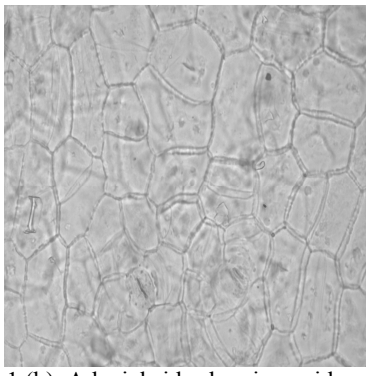

Fig.1 (b). Adaxial side showing epidermal cells

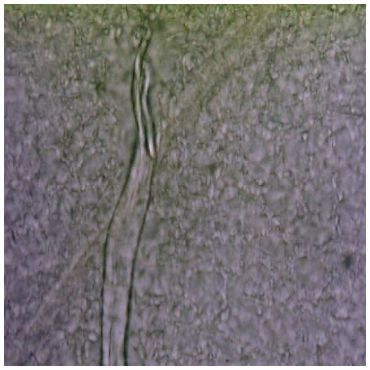

Fig.2 (a). *Potentilla gelida* (Trichome)

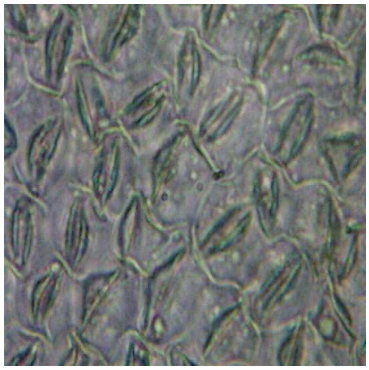

Fig.2 (b). Adaxial side showing stomata

Fig.3 (a). *Prunus cerasus*

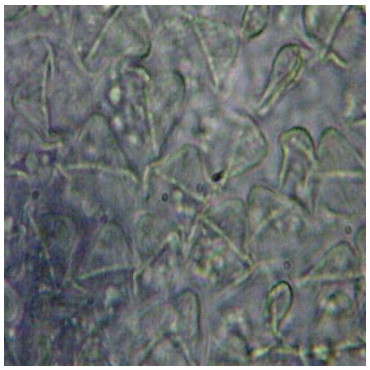

Fig.3 (b) epidermal cells

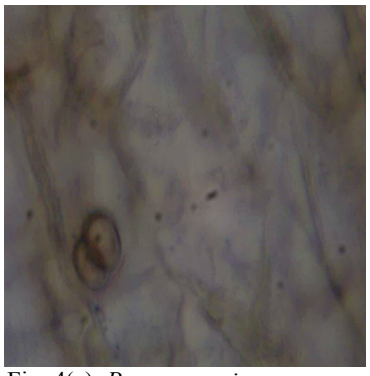

Fig. 4(a). *Prunus persica*

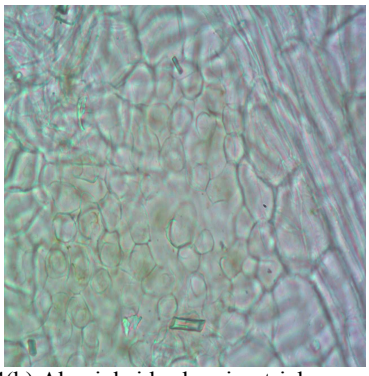

Fig. 4(b) Abaxial side showing trichomes

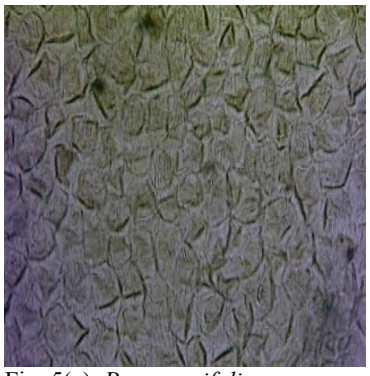

Fig. 5(a). *Pyrus pyrifolia*

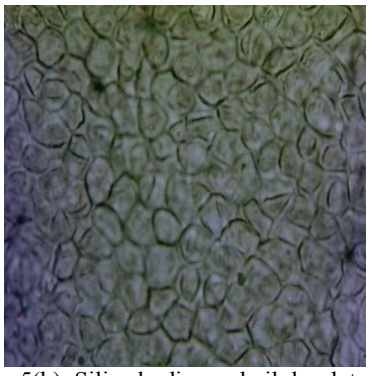

Fig. 5(b): Silica bodies and oil droplets

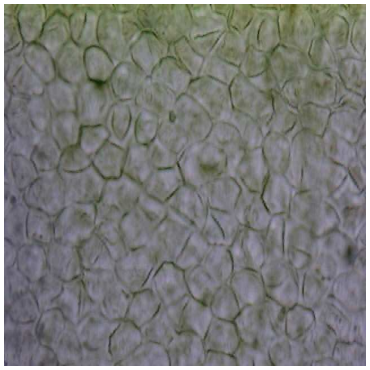

Fig. 6(a). Rosa alba

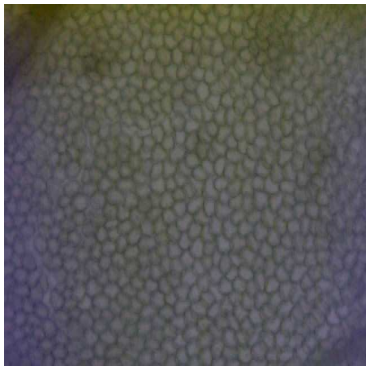

Fig. 6(b). Adaxial side showing epidermal cells

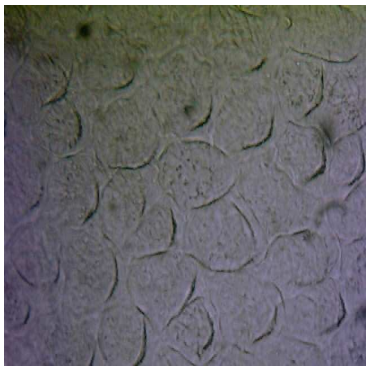

Fig. 7(a). R*osa banksiae*

Fig. 7(b).Abaxial side showing trichome

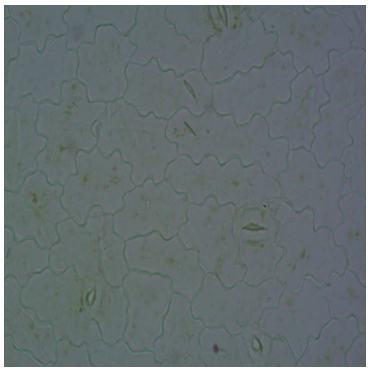

Fig. 8 (a). *Rosa benvenuto*

Fig. 8 (b). Adaxial side showing oil droplet

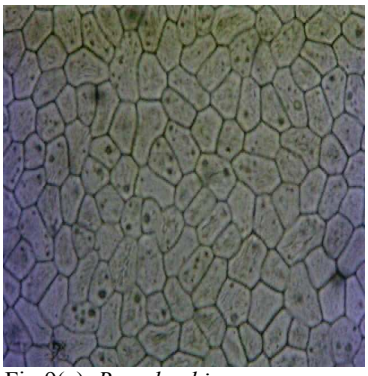

Fig.9(a). *Rosa bucbi*

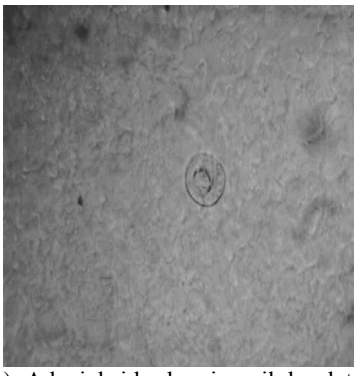

Fig.9(b): Adaxial side showing oil droplet

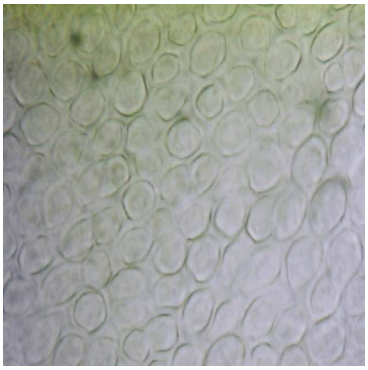

Fig.10(a). *Rosa indica*

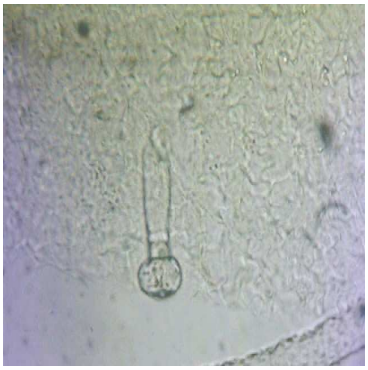

Fig.10(b). Adaxial side showing trichome

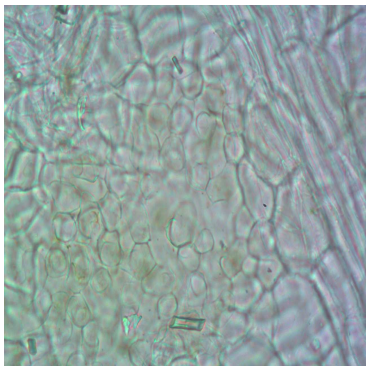

Fig. 11 (a). *Rosa macrophylla*

Fig. 11 (b): Trichome

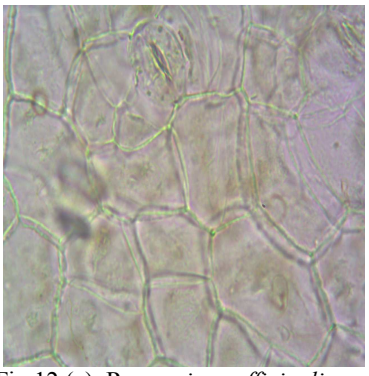

Fig.12 (a). R*osmarinus officinalis*

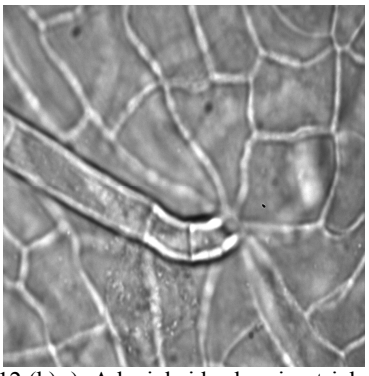

Fig.12 (b):): Adaxial side showing trichome

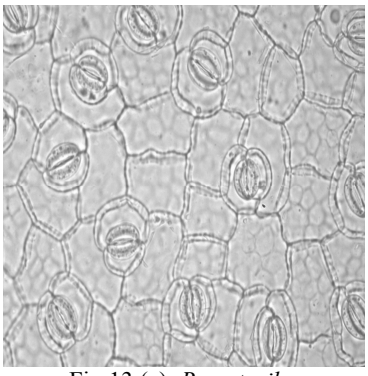

Fig.13 (a). *Rosa troika*

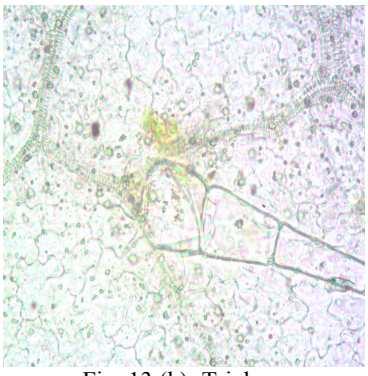

Fig. 13 (b): Trichome

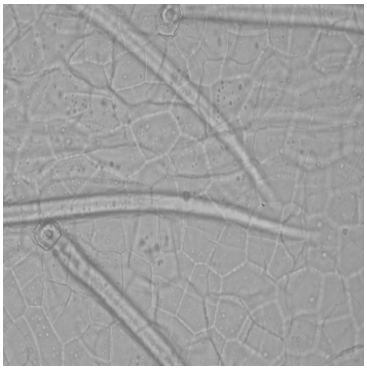

Fig.14 (a). *Rosa Tropicana*

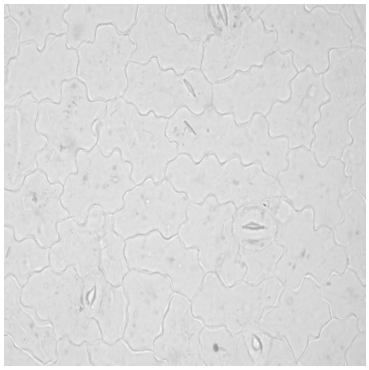

Fig.14 (b): Thick walled epidermal cells

A. Petal epidermis cells variations

At abaxial surface the shape of petal epidermal cells varied from isodiametric polygonal, bilobed, triangular, rectangular, rounded and irregular shaped. Wall patterns also showed diversity from smooth thick walled to undulate anticlinal walls. Length of epidermal cells varied from 22 (18.0-26.0) µm to 233.05 (173.5-292.6) µm and variations among width ranged from 18.5 (13.9-23.1) µm to 206 (199.3-212.7) µm (Table 1). The lower values belonged to species *Rosa macrophylla* while the *Prunus cerasus* had the highest value. While at the adaxial surface epidermal cells shapes had shown great variation, they could be isodiametric polygonal, triangular, irregular, polygonal rectangular, or even rounded shaped. Most of the epidermal cells were smooth and thick walled. The length of epidermal cells ranged from 23.55 (16.1-31.0) µm to 316.6 (246.6-386.6) µm. *Rosa macrophylla* represented the lower value whereas highest value belonged to *Prunus cerasus*. (Table 2).Variations in epidermal cells in relation to their shape and size can be regarded as useful taxonomic character. Campbell *et al*., 2007 had stressed upon the reliability of petal epidermal cells as taxonomic key. Similarly Akcin (2009) had also proved that petal epidermal cell variations can be used as an identification key.

B. Stomatal variations

At abaxial surface stomata were found in five species i.e. *Geum montanum*, *Potentilla gelida, Prunus cerasus, Prunus persica* and *Rosmarinus officinalis.* Among these *Potentilla gelida* showed highest number of stomata i.e., 35. actinocytic, diacytic paracytic, anisocytic and cyclocytic stomatal types were reported. While other three species had very less number of stomata. The guard cell length varied from 48.1 (26.2-70) µm to 80.05(69.4-90.7) µm and width ranged from 14.8(9.1-20.5) µm to 83.5(78.5-88.5) µm (Table 1). The lowest value of guard cells length and width was shown by *Geum montanum* while highest length was observed in *Potentilla gelida.* At adaxial surface stomata were observed only in species *Potentilla gelida* and *Prunus cerasus*. In both of these species the number of stomata ranged from 4-6. These stomata were anomocytic and diacytic type. The guard cells length ranged from 83 (60.4-105.6) µm to 183 (153.8-212.2) and their width varied from 14.3(13.6-15.0) µm. to 49.3 (38.8-59.8) µm (Table 2). The lower values belonged to *Potentilla gelida* and *Prunus cerasus* had the highest one. The discussed observations suggested that stomatal distribution is less common in petal epidermis. Chemli *et al* (2003) had investigated the petal epidermal structures of one common Rosaceae species *Sibbaladia* and they came up with conclusion that stomata were completely absent in petal epidermis.

TABLE 2 ADAXIAL ANATOMICAL VARIATIONS AMONG SELECTED PLANT SPECIES OF FAMILY ROSACEAE

Characters	***Geum montanum***	***Potentilla gelida***	***Prunus cerasus***	***Prunus persica***	***Pyrus pyrifolia***	***Rosa indica***	***Rosa macrophylla***	***Rosmarinus officinalis***
Petal epidermis cells	Isodiametric polygonal, smooth, thick walled, average length 83.6 (66-101.2) µm, average width 33 (32.0-34.0) µm	Triangular, smooth, thick walled, average length 113.35 (98.2-128.5) µm, average width 63.35 (59.0-67.7) µm.	Irregular shaped, smooth, thick walled, average length 316.6 (246.6-386.6) µm, average width168.8 (127.3-210.3) µm.	Polygonal rectangular, smooth, thick walled, average length 102.9 (61.0-144.8) µm, average width 49.8 (43.0-56.6) µm.	Irregular, smooth, thick walled, average length 54.2 (38.1-70.3) µm, average width 45.6 (32.0-59.2) µm.	Polygonal rectangular, double layered, smooth, thick walled, average length 61.6 (37.1-86.1) µm, average width 50.8 (32.6-69.0) µm.	Rounded, smooth, thick walled, average length 23.55 (16.1-31.0) µm, average width 23.15 (14.3-32.0) µm.	Triangular, smooth, thick walled, average length 96.45 (80.6-112.3) µm, average width105.45 (89.8-121.1) µm.
Type of stomata	**Absent**	**Anomocytic**	**Diacytic or Anisoc-ytic.**	**Absent**	**Absent**	**Absent**	**Absent**	**Absent**
Guard cells	Absent	Average length 83 (60.4-105.6) µm, average width 14.3(13.6-15.0) µm.	Average length 183 (153.8-212.2) µm, average width 49.3 (38.8-59.8)µm.	Absent	Absent	Absent	Absent	Absent
Subsidiary cells	Absent	Average length 91.8 (78.0-105.6) µm, average width 60 (51.5-68.5) µm.	Average length 201.4 (178.2-224.6) µm, average width 164.2 (122.9-205.5) µm.	Absent	Absent	Absent	Glandular, mushroom like, 1-4 trichomes per unit area, average length trichome 387 µm, average width 21.1µm.	Absent
Trichomes	Absent	Non-glandular, tubular, pointed end, 2-4 trichomes per unit area, average length 347.8 µm, average width 29.6 µm.	Glandular with bulbose head or non glandular tubular with pointed ends, 10-15 trichomes per unit area, average length304.6 µm,average width 42.8 µm.		Absent	Non- glandular, enlongted, pointed, number of trichomes per unit area 2-4 , average length 281µm, average width 22 µm.	Absent	Non- glandular, enlongated, tubular, pointed, arrow like, number of trichomes per unit area 2-4, average length 312.3µm,average width 28.9 µm.
Silica bodies	Rounded, 4-6 silica bodies per unit area, average length 12.1 µm, average width 11.8 µm.	Absent	Absent	Conical, number of silica bodies per unit area 1-4,average length41.1 µm, average width 40.8 µm.	Rectangular, number of silica bodies per unit area 2-4, average length 122.6 µm, average width 120µm.	Oval, 10-15 silica bodies per unit area, average length155.5 µm, average width 134.4 µm.	Oval, 25-30 oildroplets per unit area, average length 14.85 µm, average width 12.55 µm.	Collapsed Saddle, 1-2 silica bodies per unit area, average length 46.1 µm, average width 38.3 µm.
Oil bodies/ droplets	Oval, 35-40 oil droplets per unit area, average length 15.9 µm, average width 13.9 µm.	Rounded, 10-15 oil droplets per unit area, average length 18 µm, average width 16.6 µm.	Rounded , 20-25 oildroplets per unit area, average length 20.3 µm, average width 18.6 µm.	Rounded, number of oildroplets per unit area 6-8, average length 13.5 µm, average width 13.45 µm.	Oval, number of oildroplets per unit area 4-6 , average length17.2 µm, average width 12.3 µm.	Rounded, 50-55 oildroplet per unit area, average length11.05 µm, average width 9.1 µm.		Rounded, 6-10 oildroplets per unit area, average length 36.5 µm, average width 34.45 µm.

C. Subsidiary cells variations

The specification in subsidiary cells also helps in identifying and classifying species. As it is already discussed that the occurrence of stomata in petal epidermis is rare so it is understood that presence of subsidiary cells will also be limited. On abaxial surface *Prunus cerasus* represented the lower values of subsidiary length and width i.e., 44.5 (30.8-58.2) µm and is 30.1 (20.2-40.0) µm. While highest length belonged to *Geum montanum* and highest width was the characteristic of *Potentilla gelida* (Table 1). On adaxial surface the length and width of subsidiary cells greatly varied. The lowest length and width was reported in *Potentilla gelida* i.e., 91.8 (78.0-105.6) µm and 60 (51.5-68.5) µm whereas the higher length and width were measured in *Prunus cerasus* i.e., 201.4 (178.2-224.6) µm and 164.2 (122.9-205.5) µm. (Table 1).

D. Variations in Trichomes

In the present work, the presence or absence of trichomes, as well as their types were seen useful in characterizing the species studied. Various researchers had proved the systematic value of trichomes as valuable anatomical markers. Cutler (2012) had revealed that floral trichome types can be used as key for the identification of plants. On abaxial surface trichomes were observed in six species i.e., *Geum montanum, Prunus cerasus*, *Prunus persica*, *Rosa indica*, *Rosa macrophylla* and *Rosmarinus officinalis.* Shapes of trichomes greatly varied, they observed to be non- glandular, tubular, blunt end, glandular with spherical pluricellular or elipsoidal head or glandular, capitate, mushroom like and even non glandular, tubular, pointed end. In *Rosmarinus officinalis* numerous trichomes were reported, almost 20-25 per unit area. While in other species it is normally ranged from 2-4 to 4-6. The smallest trichome was found in *Rosa indica* (95.5 µm) while the *Rosa macrophylla* possessed the largest one (573.3 µm) (Table 1). In relation to width trichomes showed variations from 92.4 µm to 11.3 µm. The lower limit was characterized by *Prunus persica* however upper limit was reported in *Prunus cerasus*. On adaxial surface trichomes were reported in five species i.e., *Potentilla gelida, Prunus cerasus*, *Rosa indica*, *Rosa macrophylla* and *Rosmarinus officinalis*. Among these species trichomes showed the great diversity which is significantly important in plant systematics. Some were glandular with bulbose head but some were non glandular and tubular with pointed ends. Normally 2-4 trichomes were present in per unit area however *Prunus cerasus* had shown the great number i.e., 10-15 per unit area. The lower length measured in *Rosa indica* i.e., 281µm while *Rosa macrophylla* came up with the highest length values i.e., 387 µm. The trichome width varied from 21.1µm (*Rosa macrophylla*) to 42.8 µm (*Prunus cerasus*) (Table 2). On the basis of above discussion it is clear that trichomes are undoubtedly a realiable taxonomic character and this is also supported by studies of Akcin (2009).

E. Variations Silica bodies presence

Silica bodies play an important role in the identification and classification of different taxa. On abaxial surface silica bodies were seen in *Geum montanum*, *Prunus persica*, *Rosa indica* and *Rosmarinus officinalis*. Shapes of silica bodies greatly varied, they observed to be Narrow elliptate, bilobates, saddle and oval .Their number varied from 2-4 but in *Rosmarinus officinalis* number is slightly higher i.e., 10-12. The average length ranged from 22.5 µm (*Rosa indica*) to 105.5 µm (*Rosmarinus officinalis*). Whereas variation in width ranged from 34.2 µm (*Prunus persica*) to 66.8 µm (*Rosmarinus officinalis*) (Table 1). On adaxial surface silica bodies were reported in *Geum montanum, Prunus persica*, *Pyrus pyrifolia*, *Rosa indica* and *Rosmarinus officinalis*. Shapes of silica bodies greatly varied, they observed to be rounded, conical, Oval, rectangular and Collapsed Saddle The average number of silica bodies ranged 2-4 but in *Rosa indica* number is slightly higher i.e., 10-15. The smallest silica bodies were observed in *Geum montanum* (12.1 µm) whereas largest was reported in *Rosa indica* (155.5µm). Width of silica bodies ranged from 11.8µm to 134.4 µm. *Geum montanum* possessed the lower value while higher value belongs to *Rosa indica* (Table 2). By observing these variations in regard to silica bodies we can conclude that presence of silica bodies emerged as a useful taxonomic feature to delimit different taxa in the present studies. Hummer (2007) stated that the silica bodies are of particular interest, as they possessed important morphological features, which have encouraged many investigators to identify plants.

F. *Variations Oil bodies/ droplets presence.*

On abaxial surface oil bodies were observed in all studied species except *Rosa macrophylla* (Table 1). Shapes of oil bodies greatly varied, they observed to be rounded, oval, rod shaped, triangular and their number showed variations in species. *Prunus persica* had the lowest number i.e., 4-6 while number of oil bodies was quite higher in *Geum montanum* and *Prunus cerasus i.e.,* 55-60. The length and width of oil bodies also remarkably varied from 8.5 µm to 48.35 µm and 7.75 µm to 44.95 µm respectively. The lower value belonged to *Prunus persica* whereas highest value was characteristic to *Rosmarinus officinalis*. (Table 1). On adaxial surface the oil bodies were reported in all studied species of Rosaceae. Shapes of oil bodies greatly varied, they observed to be rounded and oval and their number showed variations in species and number varied from species to species. Lowest number was reported in *Pyrus pyrifolia* i.e., 4-6 while 50-55 oil bodies were observed in *Rosa indica*. The length variations ranged from 11.05 µm (*Rosa indica*) to 36.5 µm (*Rosmarinus officinalis*) whereas width ranged from 9.1 µm (*Rosa indica*) to 34.45 µm (*Rosmarinus officinalis*) (Table 2). These variations give the confidence of utilizing oil bodies as a valuable taxonomic marker.

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