

Leaf anatomy and palynological differences among selected cultivars of *Vitis vinifera* and *Parthenocissus quinquefolia* (Vitaceae)

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ABSTRACT

Anatomical evaluation on eight cultivars (cv.) of *Vitis vinifera* (Kshms, Trespi, Taefi, Pasirani, Baesola, Deselanz and Sadani) and *Parthenocissus quinquefolia* (virginia creeper). Was achieved the results revealed leaf anatomy and palynological differences among the cultivars and between *Vitis vinifera* and *Parthenocissus quinquefolia*. Druses and raphid crystals were present in both. Starch grains were present in the petiole of (Taefi, Pasirani and Sadani). The shape of petiole, midrib and margin were anatomically different as well as the presence of trichome in Pasirani and absent in other sample. The structure of the leaf epidermis was investigated using light microscope (LM) and scanning electron microscope (SEM) which revealed the polygonal shape of the leaf epidermal cells and the anticlinal walls which were straight and straight to wavy from the surface view. The structure of the stomata was present only in abaxial surface and anomocytic type. LM and SEM were used to investigate the pollen grains tricolpate prolate-spheroidal, spheroidal and spheroidal but with an absence of acolpus. Exine ornamentation is reticulate to micro-reticulate.

Key words: *Vitis vinifera*, *Parthenocissus quinquefolia*, anatomical evolution, *Parthenocissus*.

1. INTRODUCTION

Vitaceae, the grape family contains around 700 species, assigned to 13-15 genera and are mostly lianas distributed in tropical and temperate areas throughout the world (Najmaddin, 2013; Lombardi, 2007). Vitaceae leaves are simple, lobed or unlobed or compound; 1-3 pinnately compound and alternate (Chen and Wen, 2007; Najmaddin, 2011c). Leaf opposed tendrils with unarmed stems and the inflorescence is panicles and corymbs (Najmaddin, 2011a, 2011c).

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Vitaceae contains *Vitis vinifera* which is commercially important species for the production of wine grape. However, little is known about the rest of the family especially from a botanical perspective, although the genera distinguish each species largely on the basis of their floral characters (Gerrath et al. 2004; Najmaddin, 2011b). The stomata apertures in the epidermis, bounded by two guard cells, their main function is to allow gases as carbone dioxide, water vapours oxygen to move rapidly into and out of the leaf (Tay and Furukawa 2008; Najmaddin, 2011 b, 2011c). *Parthenocissus quinquefolia*, which has the common name Virginia creeper, is the most attractive deciduous vines and fast growing, climbing by tendrils and adhesive disks, the leaves are compound containing five leaf lets (Gilman, 1999).

2. MATERIALS AND METHODS

Plant materials used in this research included *Vitis vinifera* cultivars (Kshms, Trespi, Taefi, Pasirani, Baesola, Deselanz and Sadani) and *Parthenocissus quinquefolia* (virginia creeper). The samples were collected from the Agriculture Research Center and plantation in Erbil-Iraq. The specimen was fixed in the fixative Formalin, acetic acid and alcohol. The petioles, midribs, lamina and margins were sectioned transversely (T.S) on a sliding microtome, stained in Safranin and Fast green dehydrated in series of alcohol, mounted on slides using Canada balsam (Ruzin, 1999). Epidermal peels were prepared by mechanical scraping, stained, dehydrated and mounted on the same way. For pollen morphological studies, the pollens gathered from open flowers or mature flower buds were prepared for light microscope (LM) by methods described by Erdtman (1952) and were washed three times with phosphate buffer solution (PBS), the samples later were dehydrated using series of acetone concentration percentages ranging from 50, 70, 80, 85, 90, 95% and three times in 100% acetone for 30min each, dried at critical point, coated with gold in a sputter coater and were observed under a Philips XL30 Scanning Electron Microscope (SEM), at 10 to 20 KV according to magnification requirements.

3. RESULTS

Anatomical description

T.S petioles

The vascular tissue system is closed and a fibrous layer occurs next to the phloem tissue which is present in both genera. Secretory cells are present in the cortex, accessory vascular bundles are present in *Vitis vinifera* but absent in *Parthenocissus*. Collenchyma cells present in the cortex beneath the epidermis. Druses are present in the cortex and pith, while raphides are present only in pith. Starch grains are present in the pith in *Vitis vinifera* cv. Taefi (Figure 1 and 2).

T.S midrib

The midrib vascular tissue system is closed and a fibrous layer occurs next to the phloem tissue which is present in both genera. Secretory cells are present in the cortex. Collenchyma cells are present in the cortex beneath the epidermis. Trichomes are present in *Vitis vinifera* cv. Taefi which are simple multicellular non glandular. Druses are present in the cortex, near the vascular bundle and the pith. Raphides are present in the pith or in cortex (Figure 3 and 4).

T.S lamina

The palisade consists of two layers of cells; both raphides and druses are present while only raphides are present in the spongy mesophyll layer. Trichomes are present in *Vitis vnifera* cv. Taefi which are simple multicellular non glandular (Figure 5).

T.S margin

The shape of the margin is straight, pointing slightly downwards and rounded pointing downwards. Trichomes are absent (Figure 6).

Stomata

The adaxial anticlinal walls are straight slightly wavy, and abaxial anticlinal walls are straight to wavy; stomata are anomocytic. The cuticle is grooved in *Vitis vinifera* and scaly in *Parthenocissus quinquefolia* (Figure 7).

Pollen grains

Vitaceae pollen grains are tricolpate. The shape in equatorial view varies from prolate-spheroidal as in *Vitis vinifera* cv. Pasirani and *Vitis vinifera* cv. Deselanz, spheroidal as in *Vitis vinifera* cv. Trespi, *Vitis vinifera* cv. Taefi and *Vitis vinifera* cv. Sadani (Figure 8 A,B,G) and prolate as in *Parthenocissus quinquefolia* (Figure 8D,H). In the *Vitis vinifera* cv.

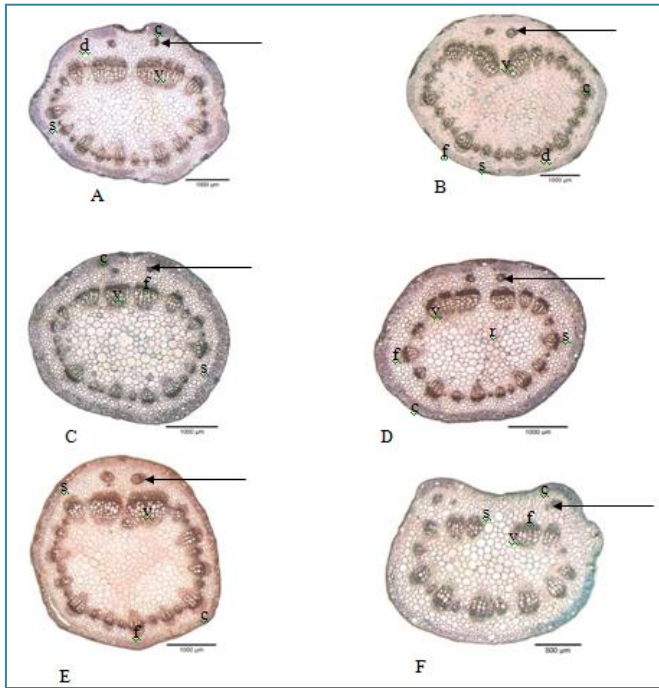


Figure 1

TS of petioles: A. *V. vinifera* cv. Kshmsb, B. *V. vinifera* cv. Trespi, C. *V. vinifera* cv. Taefi, D. *V. vinifera* cv. Pasirani, E. *V. vinifera* cv. Baesola, F. *V. vinifera* cv. Deselanz, s: secretory canal, d: druses, c: collenchyma cells, f: fibres, v: vascular bundles, accessory vascular bundles (black arrow).

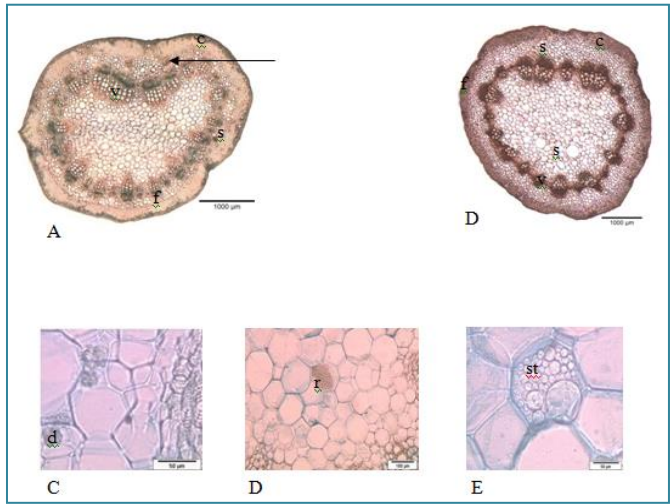


Figure 2

TS of petioles: A. *V. vinifera* cv. Sadani, B. *Parthenocissus quinquefolia*, C. druses, D. raphides, E. starch grains, s: secretory canal, d: druses, r: raphides, c: collenchyma cells, f: fibres, st: starge grains.

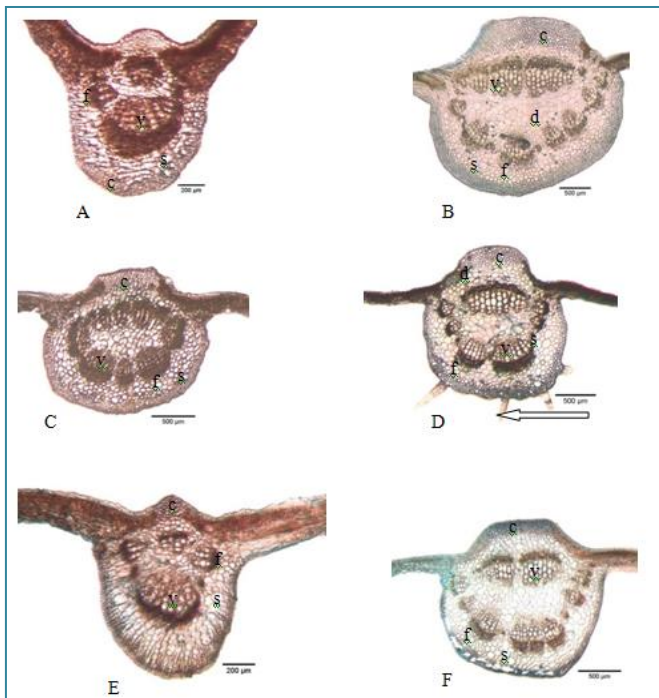


Figure 3

TS of midrib: A. *V. vinifera* cv. Kshmsb, B. *V. vinifera* cv. Trespi, C. *V. vinifera* cv. Taefi, D. *V. vinifera* cv. Pasirani, E. *V. vinifera* cv. Baesola, F. *V. vinifera* cv. Deselanz, s: secretory canal, d: druses, c: collenchyma cells, f: fibres, v: vascular bundles, trichomes (white arrow).

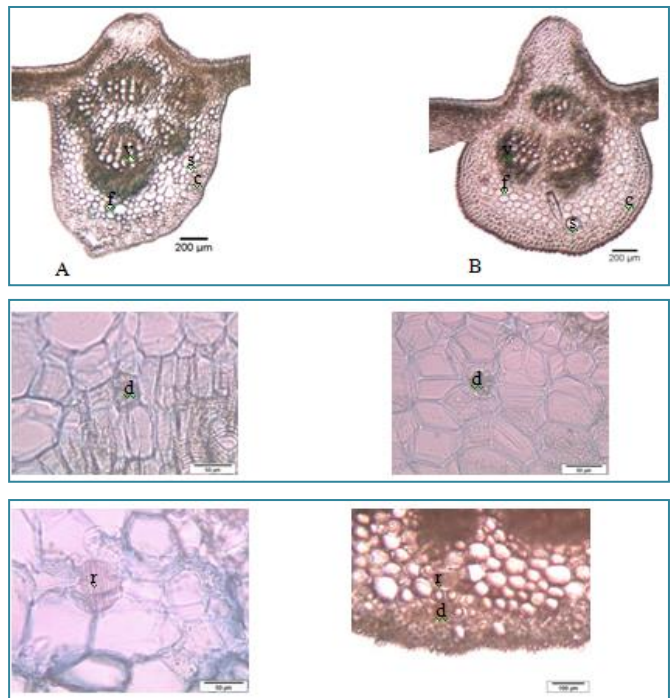


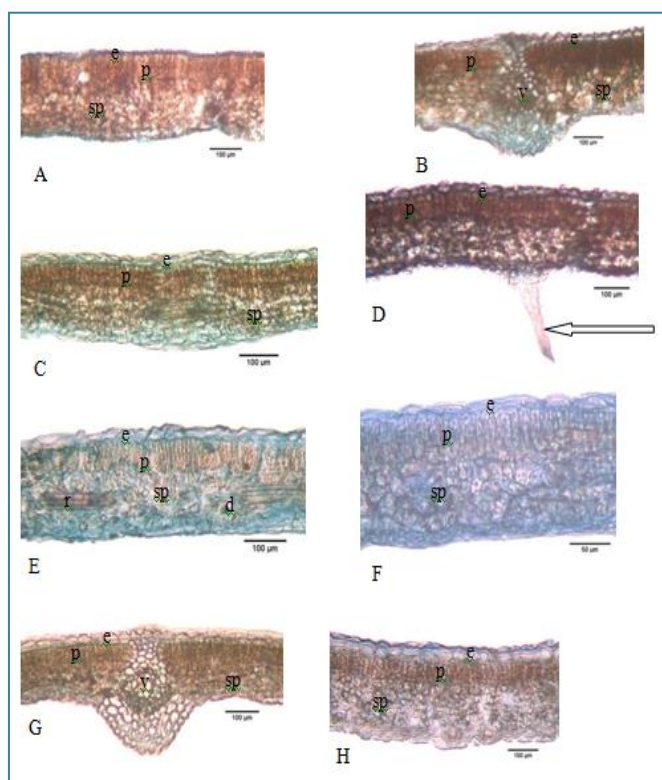
Figure 4

TS of midrib: A. *V. vinifera* cv. Sadani, B. *Parthenocissus quinquefolia*, C and D druses, E and F. raphides, s: secretory canal, d: druses, r: raphides, c: collenchyma cells, f: fibres, st: starge grains.

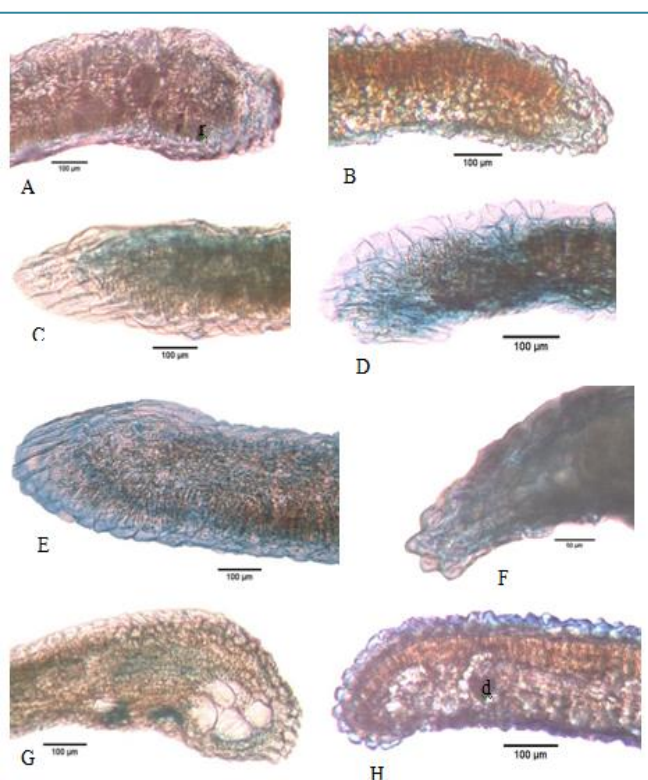
Table 1

Pollen grains feature

Species	Polar view	Equatorial view	Size P/E	Sculpture ornamentation	Colpus
<i>Vitis vinifera</i> cv. Kshmsb	circular	spheroidal	1.052	micro-reticulate	absent
<i>Vitis vinifera</i> cv. Trespi	circular	spheroidal	1.026	reticulate	present
<i>Vitis vinifera</i> cv. Taefi	trigonal	spheroidal	1.096	reticulate	present
<i>Vitis vinifera</i> cv. Pasirani	trigonal	prolate-spheroidal	0.889	reticulate	present
<i>Vitis vinifera</i> cv. Baesola	circular	spheroidal	0.987	micro-reticulate	absent
<i>Vitis vinifera</i> cv. Deselanz	trigonal	prolate-spheroidal	0.935	reticulate	present
<i>Vitis vinifera</i> cv. Sadani	trigonal	spheroidal	0.988	reticulate	present
<i>Parthenocissus quinquefolia</i>	circular	prolate	1.116	reticulate	present

**Figure 5**

TS of lamina: A. *V. vinifera* cv. Kshmsb, B. *V. vinifera* cv. Trespi, C. *V. vinifera* cv. Taefi, D. *V. vinifera* cv. Pasirani, E. *V. vinifera* cv. Baesola, F. *V. vinifera* cv. Deselanz, G. *V. vinifera* cv. Sadani, H. *Parthenocissus quinquefolia*, d: druses, r: raphides, c: collenchyma cells, v: vascular bundles, e: epidermis, p: palisade layer, sp: spongy layer, trichomes (white arrow).

**Figure 6**

TS of lamina: A. *V. vinifera* cv. Kshmsb, B. *V. vinifera* cv. Trespi, C. *V. vinifera* cv. Taefi, D. *V. vinifera* cv. Pasirani, E. *V. vinifera* cv. Baesola, F. *V. vinifera* cv. Deselanz, G. *V. vinifera* cv. Sadani, H. *Parthenocissus quinquefolia*, d: druses, r: raphides.

Kshmsb and *Vitis vinifera* cv. Baesola there are also spheroidal but with an absence of a colpus and pores (Figure 8C). Exine ornamentation is reticulate to micro-reticulate (Figure 8 E,F), (Table 1).

4. DISCUSSION

The anatomical features of the *V. vinifera* and *Parthenocissus quinquefolia* were reported. The family belongs to deciduous, woody climber (Townsend & Guest, 1980) or shrubs, climbing by the coiling of leaf opposed tendrils, leaf simple, rounded, dentate which are usually palmate 3 to 5 lobed with rounded sinuses (Townsend & Guest, 1980; Jarad, 2007). Mechanical collenchyma tissues in the petiole were observed. The present investigation revealed that the petioles of *V. vinifera* have different specific shapes and different with *Parthenocissus*. The vascular bundle in *V.*

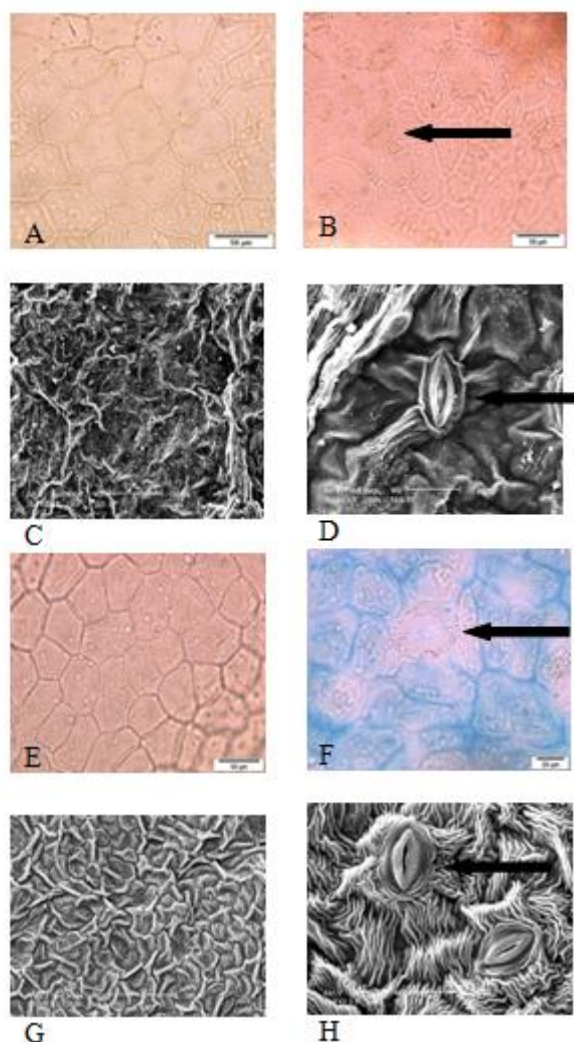


Figure 7

leaf abaxial and adaxial surface showing the stomata and the cuticle layer. A. *Vitis vinifera* LM of adaxial epidermis, B. *Vitis vinifera* LM of abaxial epidermis, C. *Vitis vinifera* SEM of adaxial epidermis, D. *Vitis vinifera* SEM of abaxial, E. *Parthenocissus quinquefolia* LM of adaxial epidermis, F. *Parthenocissus quinquefolia* LM of abaxial epidermis, G. *Parthenocissus quinquefolia* SEM of adaxial epidermis, H. *Parthenocissus quinquefolia* SCM of abaxial epidermis, stomata (large black arrow).

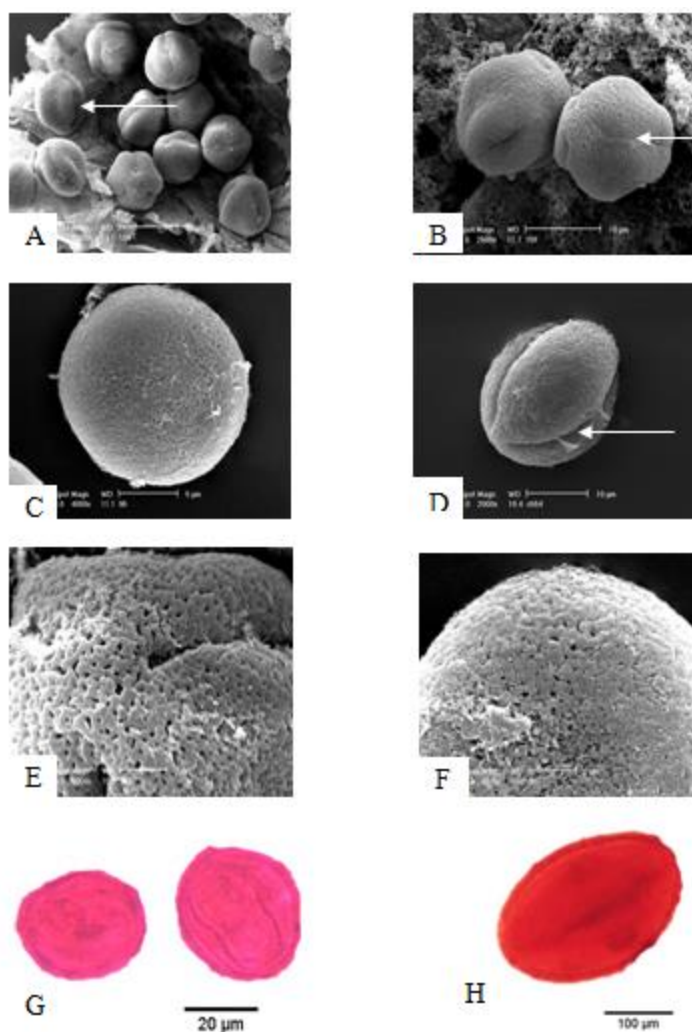


Figure 8

Pollen grains (SEM). A. polar and equatorial view of *Vitis vinifera*, B. polar and equatorial view of *Vitis vinifera*, C. equatorial view of *Vitis vinifera*, D. polar and equatorial view of *Parthenocissus quinquefolia*, E. pollen surface ornamentation of *Vitis vinifera* (SEM), F. pollen surface ornamentation of *Parthenocissus quinquefolia* (SEM), G. polar and equatorial view of *Vitis vinifera*, H. equatorial view of *Vitis vinifera*, colpus (small white arrow).

vinifera and *Parthenocissus quinquefolia* is closed, a fibrous layer occurs next to the phloem tissue and secretory cells there are present in both genera. The present study further revealed that the midribs of *V. vinifera* were characteristics by the outline of the adaxial surface which was slightly humped by arc to elongated shape surface of the abaxial and the presence of collenchyma in both epidermis layers, while in *Parthenocissus quinquefolia* the midrib was characteristic by the outline of the adaxial surface which was long humped by circular shape surface of the abaxial and the presence of collenchyma in both epidermis layers. The vascular bundle was closed and the druses, raphid crystals were present, starch grains were present in the pith *V. vinifera*, but absent in *Parthenocissus quinquefolia*. However, the present study showed that the margin of *V. vinifera* and *Parthenocissus quinquefolia* were straight and slightly downwards with rounded or tapering tip. The druses and secretory cells are present. In

addition, mesophyll layer contains calcium oxalate (druses) crystals and mucilage cells or secretory cells with raphides in bundle. Similarly, this has also been reported in a study by Metcalfe & Chalk (1950), mucilaginous idioblasts in the mesophyll of leaf and cortex of the stem which was absent in the epidermis (Kannabiran & Pragasam, 1994).

The petiole, midrib, margin and lamina outline of these sections was smooth, while in *V. vinifera* cv. Pasirani trichomes were present and non glandular which is an important feature in determining the cultivars. The trichome complement of a particular organ can consist entirely of unbranched or branched hairs (Lombardi 2007). For example, the present study showed that the outline of the petiole and lamina of *V. vinifera* cv. Kshms, Trespi, Taefi, Baesola, Deselanz and Sadani) and *Parthenocissus quinquefolia* trichomes were absent, but in *V. vinifera* cv. Pasirani, trichomes are present. The present study revealed the presence of starch grains in pith of petiole of cultivars. Starch grains were observed in xylem fibers and most ray parenchyma cells but not in axial parenchyma cells or ray parenchyma cells with direct lateral wall contact with vessels (Sun et al. 2008). Vitaceae leaf epidermal characters have been studied by Ren et al. (2003) using light and scan electron microscope. The shape of leaf epidermal cells was irregular or polygonal; the anticlinal walls are straight, arched or sinuate which are in conformity with the leaf epidermal cell of the present study. However, the present studies revealed that leaf epidermis has anomocytic and were in conformity with Hui et al. (2003) and Kannabiran & Pragasam (1994).

The flower of *Vitis* sp. has complex organ, arranged between 4 to 5 mm, bisexual, 5- numerous, pedicels mostly umbellate clustered (Townsend and Guest 1980; Al-Saady 1982). However, the symmetry of the flower is pentamerous while *Ampelopsis*, *Cayratia* and *Cissus* have tetramerous flowers; therefore, they may have closely related features (Patil, 1998). *Vitaceae stenopalynous* in nature and is characterized by three colporate grains. However, sufficient variation is found between pollen shape class and exine pattern. Based on these features the family is divided into three pollen types, *V. parvifolia* type, *V. jacquimontii* and *Ampelopsis vitifolia* subsp., *hazaraganjiensis* type (Perveen & Qaiser, 2008). The pollen grains of the species were studied using scan electron microscope which show Vitaceae pollen grains consist of isopolar and tricolporate, and mostly sub-prolate or prolate to prolatespheroidal (Perveen & Qaiser, 2008; Marasali et al. 2005; Inceoglu et al. 2000), exine foveolate-rugulate at the mesocolpia and distinctly reticulate towards the poles and at the poles (Inceoglu et al. 2000). In *V. vinifera*, two types of aperture observed were tricolporate pollen grains in male flower and unaperturate pollen grains in the female flowers (Gallardo et al. 2009).

5. CONCLUSIONS

The anatomical evaluation of the species of Vitaceae was reported. The result shows that starch grains, druses and raphid crystals were present among the cultivars. Leaf epidermal cells in all the cultivars and *Parthenocissus quinquefolia* were irregular or polygonal. The anticlinal walls were straight and straight to wavy. The stomatal apparatuses were present on the abaxial epidermis and anomocytic type and trichomes were present in epidermis in some cultivars. Pollen grains tricolporate prolate-spheroidal, spheroidal and spheroidal but with an absence of apolus. Exine ornamentation is reticulate to micro-reticulate.

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