

Full Length Research Paper

Comparative study on the anatomy and palynology of the three variety of *Vitis vinifera* variety (family Vitaceae)

Chnar Najmaddin*, Khatijah Hussin and Haja Maideen

School of Environmental and Resource Sciences, Faculty of Science and Technology University Kebangsaan, Malaysia.

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Morphological and anatomical evaluation on three varieties of *Vitis vinifera* (Trerash, Rash meri and Baidhaw) commonly known as grape vine was reported. The samples were prepared in a mixture containing 95% of alcohol and glacial acetic acid. The result revealed differences among the varieties ranging from morphological to aesthetic features of the leaf. Although, starch grains, druses and raphid crystals were present in all the varieties, the shape of the stem, petiole, midrib, lamina and margin were anatomically different as well as the presence of trichomes among the varieties. The microstructure of the leaf epidermis was investigated using light microscope (LM) and scanning electron microscope (SEM) which confirmed the polygonal shape of the leaf epidermal cells and the anticlinal walls which were straight and arched from the surface view. The structure of the stomata apparatus was present only in the abaxial epidermis and anomocyt type. SEM was used to investigate the pollen grains tricolporate and sub prolate often known as prolate-spheroidal. The flower was bisexual having size ranging between 4 to 5 mm.

Key words: *Vitis vinifera*, morphological and anatomical evaluation, light microscope, SEM.

INTRODUCTION

Vitaceae family comprise woody climbers, vines, trees, shrubs and succulent's trees (Timmons et al., 2007; Chen and Wen, 2007) which are important food sources. Commercially, Vitaceae family are valuable raw materials for the production of wine, medicine and perfumery (Gashkova, 2009). The grape family (Vitaceae) is a relatively small family and comprises about 14 genera and 700 species (Gerrath et al., 2004). Notably, the species of Vitaceae are characterized as woody, vines with leaf opposed tendrils which also includes shrubs and succulents (Timmons et al., 2007), inflorescence a cyme, corymb or panicle, usually with leaf opposed (Townsend and Guest, 1980; Gashkova, 2009; Timmons et al., 2007). 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 distinguishes each species largely on the basis of their floral characters, nature of their flowers (flower are generally small), inconspicuous, and ephemeral which makes it difficult family to study Vitaceae family (Gerrath et al., 2004). The stomata apertures in the epidermis are bounded by two guard cells which primarily allow the rapid movement of carbon dioxide, water vapours and oxygen in and out of the leaf (Perveen et al., 2007; Tay and Furukawa, 2008). Among the common name of grape vine are INAB (Arabic), TIRI (Kurdish) and AUZUM (Turkish) (Townsend and Guest, 1980). However, this study focuses on morphology and anatomical evaluation of three varieties of Vitaceae namely *V. vinifera* var. Trerash, *V. vinifera* var. Rash meri and *V. vinifera* var. Baidhaw respectively.

MATERIAL AND METHODS

Plant materials used in this study comprises *V. vinifera* var. Trerash, *V. vinifera* var. Rash meri and *V. vinifera* var. Baidhaw. The species were collected from the Agriculture Research Center in Erbil- Iraq. The specimen was fixed in a mixture of 95% alcohol and glacial acetic acid. The middle part of petioles, midrib, lamina and

*Corresponding author. E-mail: chnar_najm@yahoo.com.

Abbreviations: LM, Light microscope; SEM, scanning electron microscope; PBS, phosphate buffer solution; LM, light microscope.

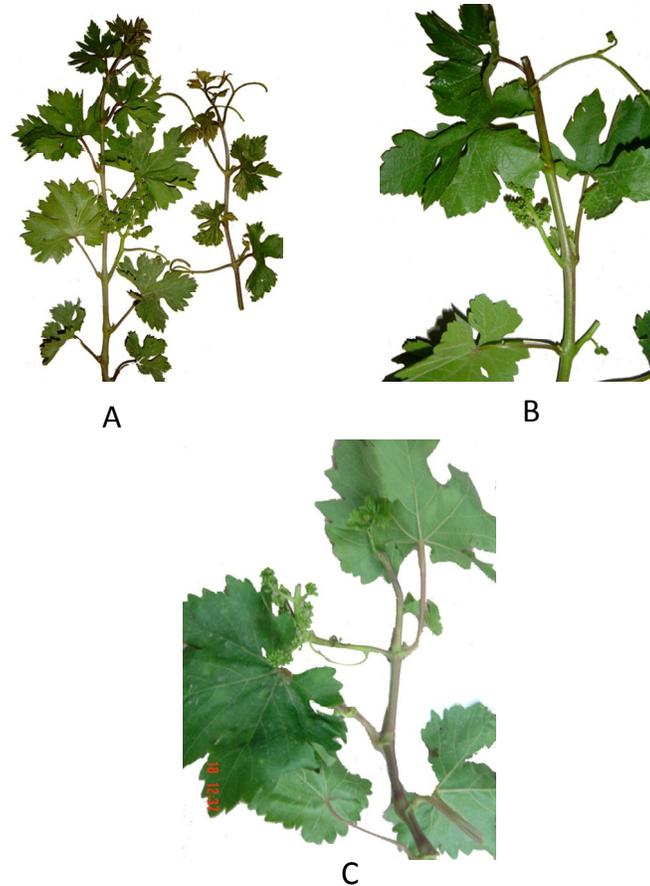


Figure 1. *V. vinifera* morphology, A-Trerash, B-Rash meri, C-Baidhawi.

margins were sectioned transversely on a sliding microtome stained in Safranin and Alcian blue dehydrated in a series of alcohol mounted on slides using Euparal (Johansen, 1940). Epidermal peels were prepared by mechanical scraping stained, dehydrated and mounted on the same way. Micrograph images were captured using Leitz Diaplan microscope fitted with a video camera connected to a computer using analysis Document software. For pollen morphological studies, pollens are gathered from open flowers or mature flower buds and were washed three times with phosphate buffer solution (PBS). The samples were later dehydrated using series of alcohol concentration percentages ranging from 50, 70, 80, 85, 90, 95 and three changes of 100% alcohol. The samples were immersed three times in 100% acetone for 30 min each, dried at critical-point, coated with gold in a sputter coater and were observed under a Philips XL30 Scanning Electron Microscope, at 10 to 20 KV according to magnification requirements.

RESULTS

This study focuses on morphology and anatomical study of the family Vitaceae (*V. vinifera*). The findings reported in this study were based on morphological, anatomical and palynological characters on which the variations among the family were revealed. The anatomical analysis conducted in this study focuses on the stem,

petiole, midrib, margin and lamina in transverse sections (cross sections), type of stomata, flowers, and morphology of the pollen.

The following variations were observed

Morphological description

Vitis: climbing tree, woody stems, presence of tendrils, leaf alternate, simple or lobed varies with respect to the species that were studied (Figure 1).

Anatomical description

T.S. Stem: Species stem shape was observed among the varieties however; the stem outline of each variety is smooth. In cortex layer, collenchymas tissue is present with closed vascular bundle surrounded by a fiber layer and the secretory cells are present (Figure 2).

T.S. petiole: Shapes of petioles differ according to the varieties. The trichome is present or absent; collenchyma is present in cortex, vascular bundle is closed and

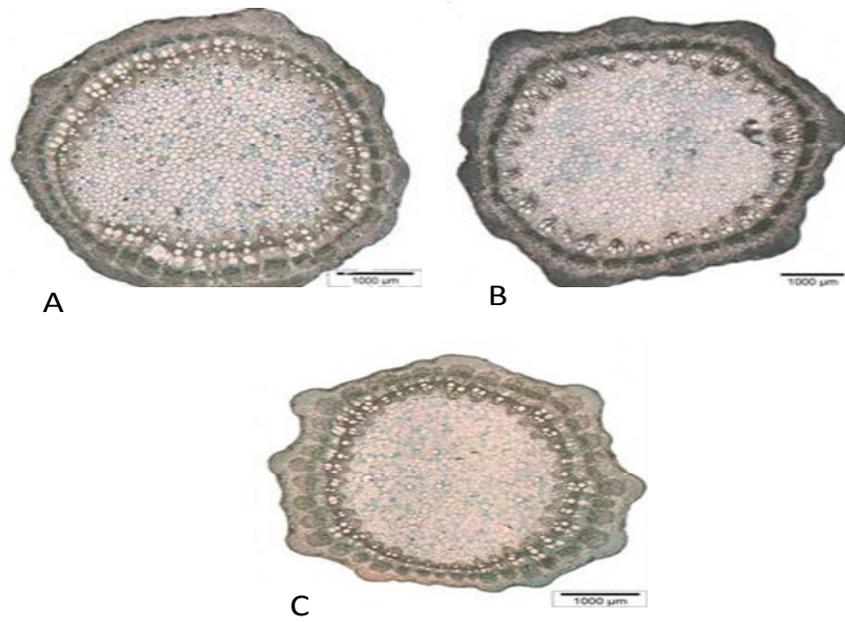


Figure 2. *V. vinifera* stems, A- Tre rash, B-Rash meri, C-Baidhawi.

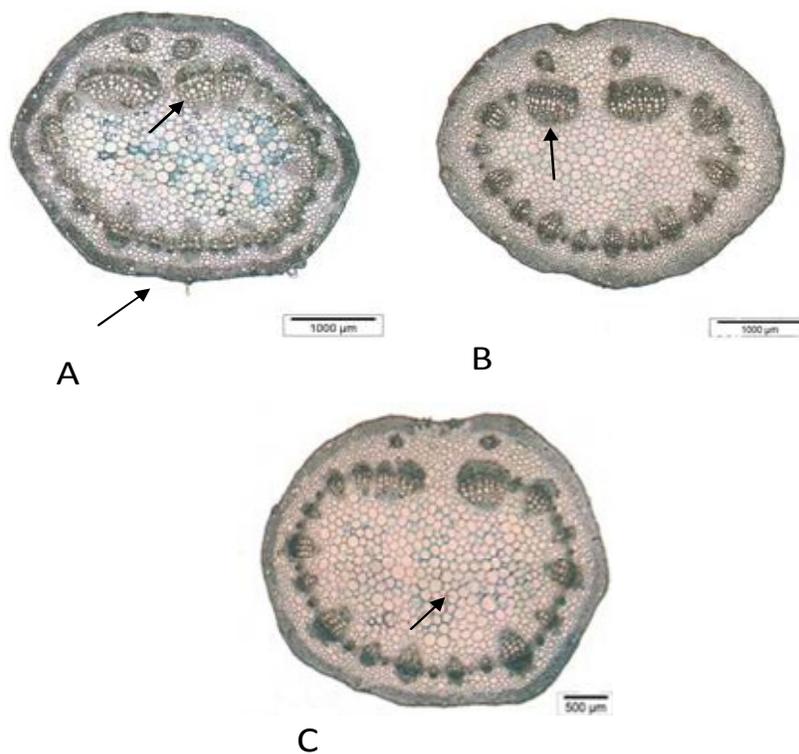


Figure 3. *V. vinifera* petioles, A-Tre rash petiole Trichomes (arrow). B-T. S. of Rash meri petiole showing large vascular bundle (arrow), C-Baidhawi petiole showing excesses vascular bundle (arrow).

surrounded by a fiber layer; two excesses vascular bundles are present in cortex and the large vascular

bundle exists underneath them and secretory cells are present (Figure 3).

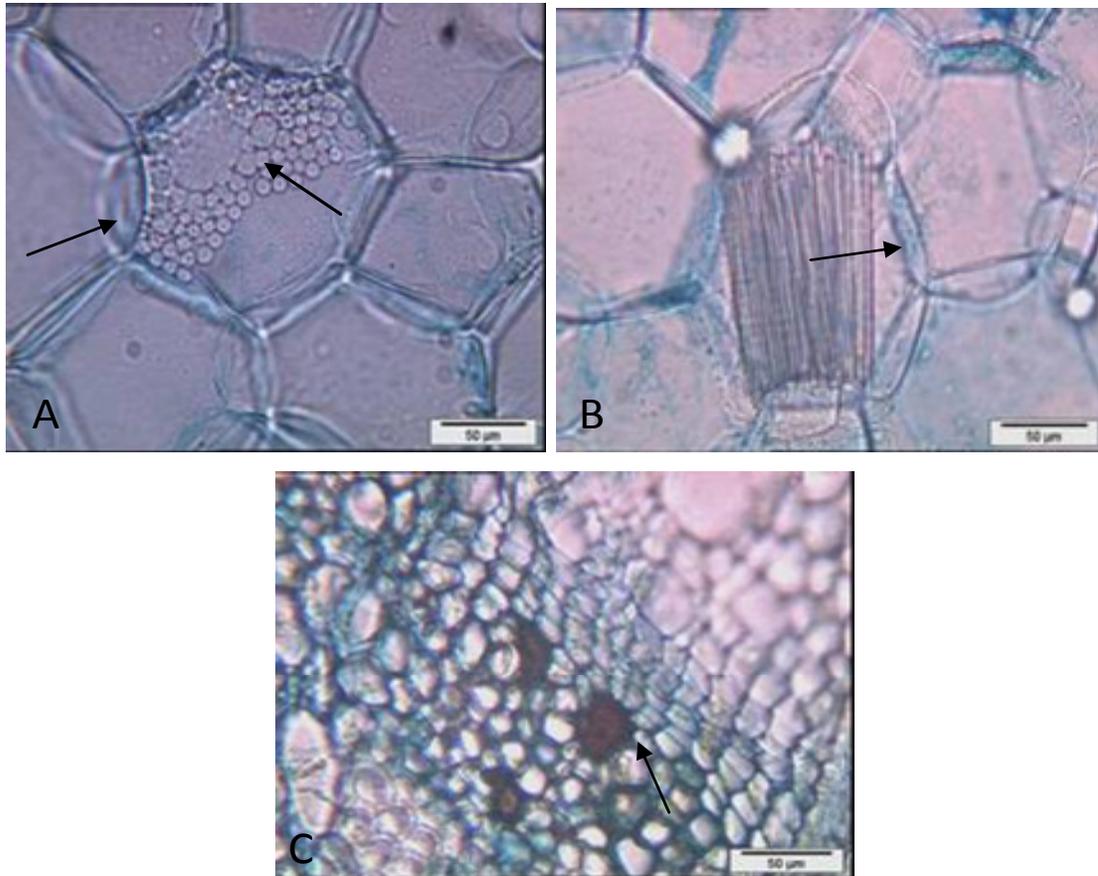


Figure 4. A-starch grains (arrow), B-raphid crystals (arrow), C-druses crystals (arrow).

In pith (stems and petioles), some varieties contain starch grains which are absent in some; Druses crystal is present while some varieties in pith contain starch grains (Figure 4).

T.S. Midrib: The outline of the adaxial surface is slightly humped and the abaxial surface is arc shaped; trichome is present in some and are absent in other species; collenchymais is present in both epidermis layers; vascular bundle is closed and surrounded by fiber layer; secretory cells are present (Figure 5a), raphides, crystals, druses are present as well as starch grains (Figure 5b).

Margin: Margin is straight with slightly downwards as the tip is rounded or tapering. Trichomes are present or absent, druses and secretory cells are present in all the varieties (Figure 6).

T.S. Lamina: Palisade consists of two layers; the epidermis is uniseriate and their outlines are smooth or have trichome; raphide, druses crystals and secretory cells are present (Figure 7).

Stomata: The anticlinal walls are straight and are arched with stomatal anomocyt type (Figure 8).

Flower: Flowers are hermaphroditic, calyx-5, petal-5 (Figure 9).

Pollen grains: In *V. vinifera* var. Baidhawi, Pollen from male flower are prolate spheroidal and *V. vinifera* var. Tre rash, Rash meri, pollen from female flowers were spheroidal to oval and unaperturate with no colpi (figure 10).

DISCUSSION

The morphological and anatomical features of the family *V. vinifera* are reported. The family belongs to deciduous, woody climber (Townsend and Guest, 1980) or shrubs, climbing by the coiling of leaf opposed tendrils, leave simple, rounded, dentate which are usually palmate 3 to 5 lobed with rounded sinuses (Townsend and Guest, 1980; Jarad, 2007).

Findings from the present study revealed that different varieties of *V. vinifera* have different shape of stem within closed vascular bundle which is surrounded by a fiber layer. Among the evaluated family of *V. vinifera*, the presence of secretory cells, druses crystals, raphid crystals, starch grains in the pith or near vascular bundle and in cortex collenchyma tissue is present. Mechanical

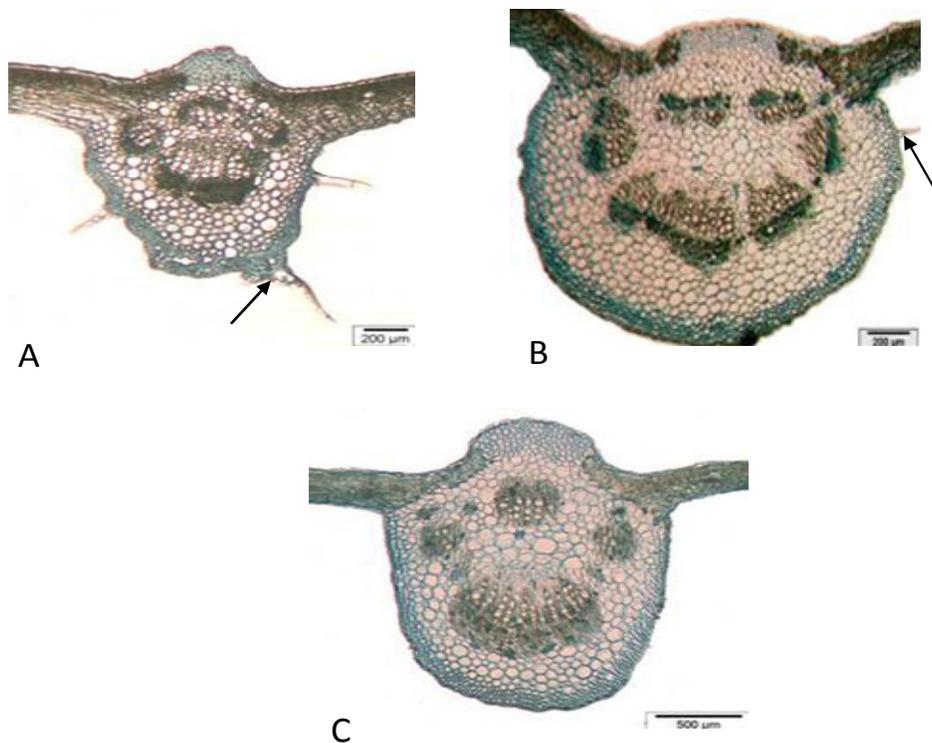


Figure 5a. *Vitis vinifera* midrib, A- Tre rash showing multicellular Trichomes (arrow), B- Rash midrib showing simple unicellular Trichome (arrow), C- Baidhawi.

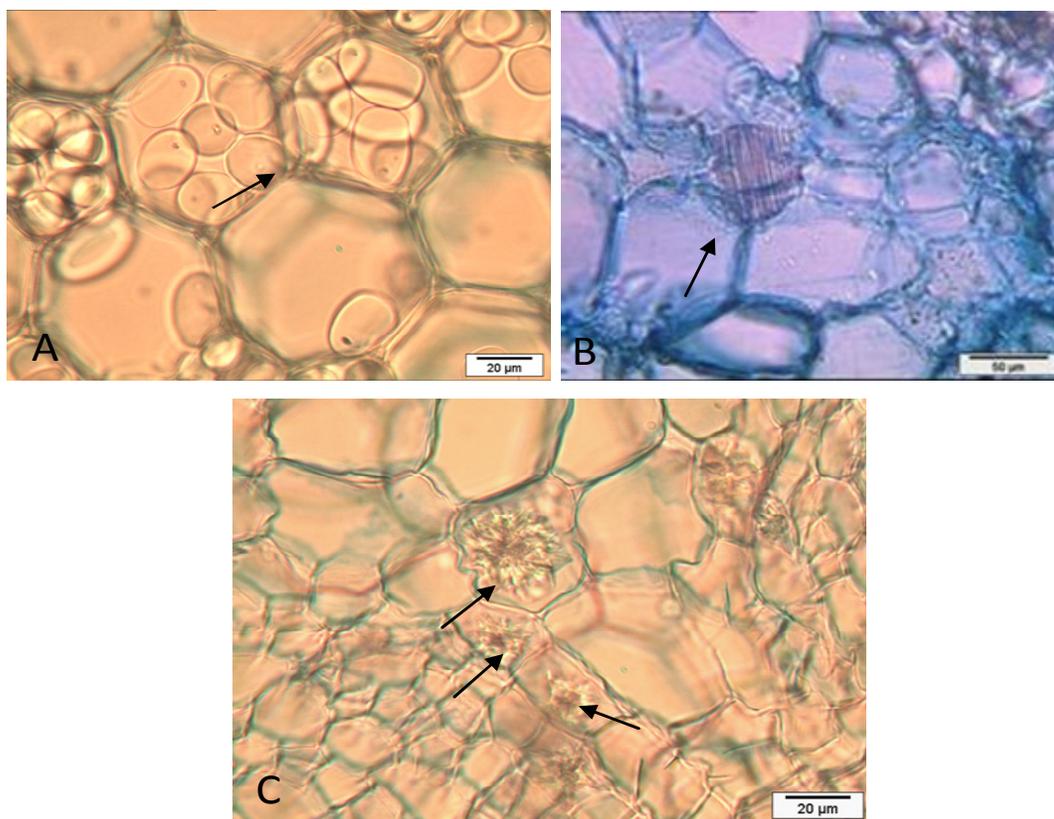


Figure 5b. A-Starch grains (arrow), B-Raphid crystals (arrow), C-Druses crystals (arrow).

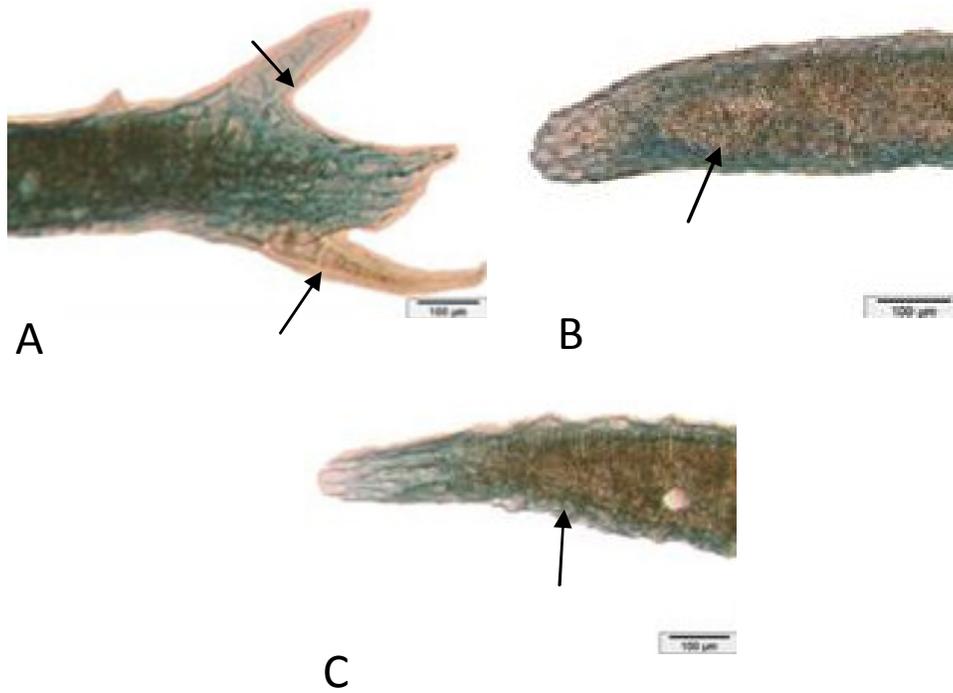


Figure 6. *Vitis vinifera* petioles, A-Tre rash midrib showing Trichomes (arrow), B-Rash meri, C-Baidhawi midrib showing secretory cell (arrow).

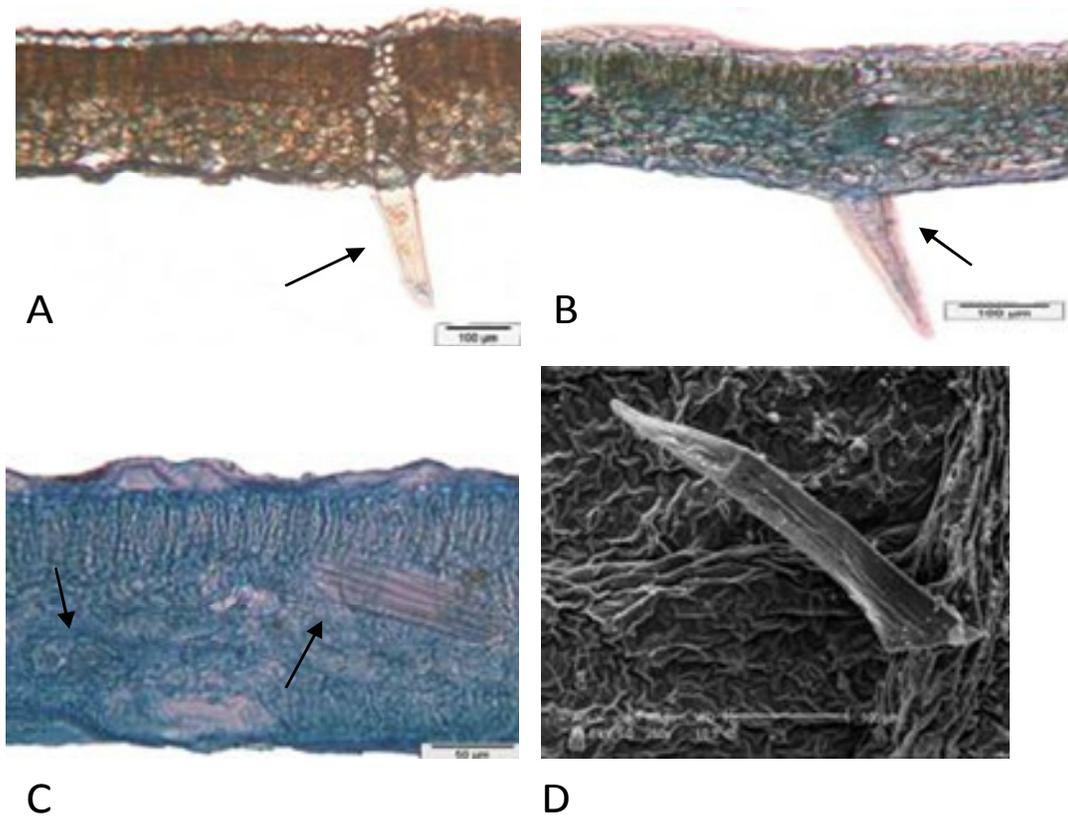


Figure 7. *V. vinifera* lamina, A-Tre rash lamina showing trichome (arrow), B-Rash meri lamina showing secretory cell (arrow), C-Baidhawi lamina showing druses and raphide crystals (arrow), D-Trichome (SEM).

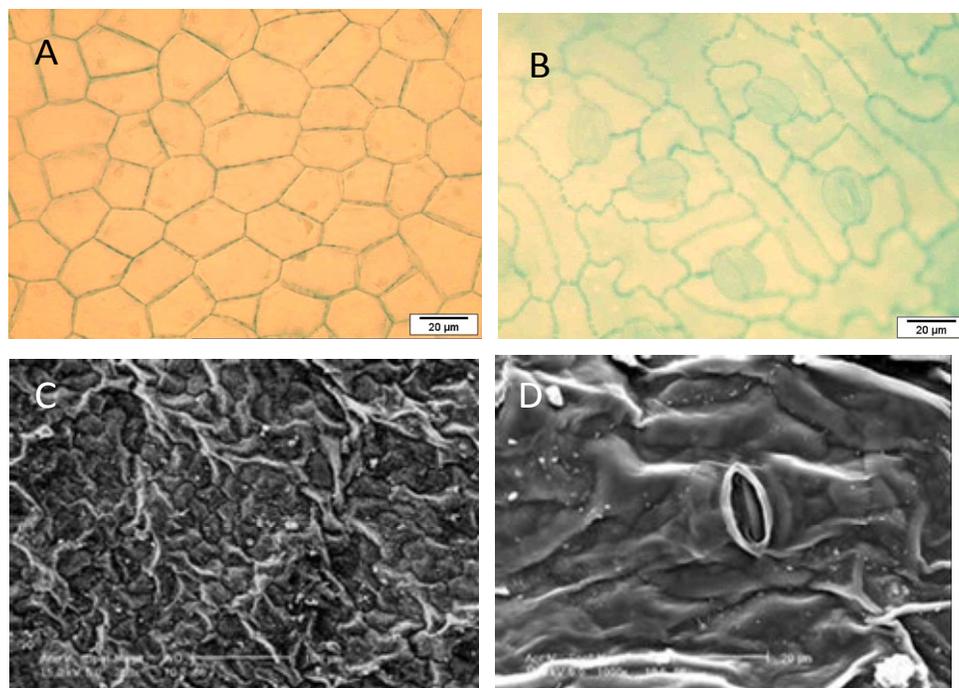


Figure 8. *V. vinifera* epidermis, A-Adaxial, B-Abaxial (Anomocyste), C-Adaxial SEM, D-Abaxial SEM.

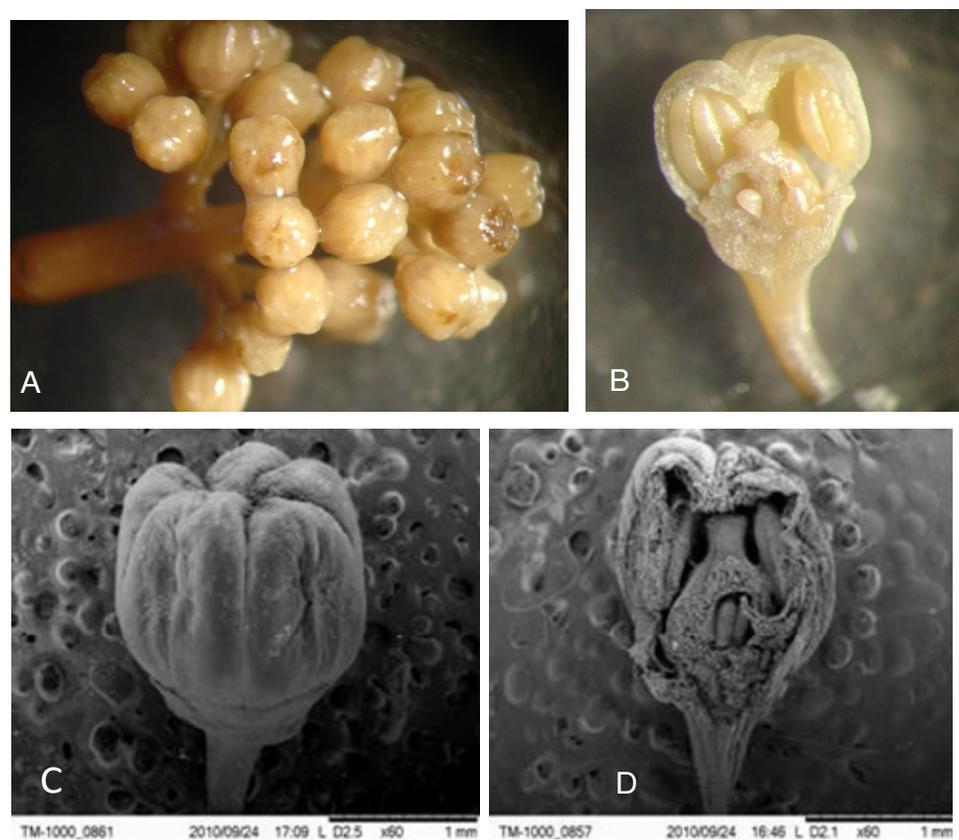


Figure 9. *V. vinifera* flower hermaphrodite, A-Cluster of flower, B-Cross section of flower C and D Flower (EM), 5-calyx (arrow), 5-corolla (arrow)

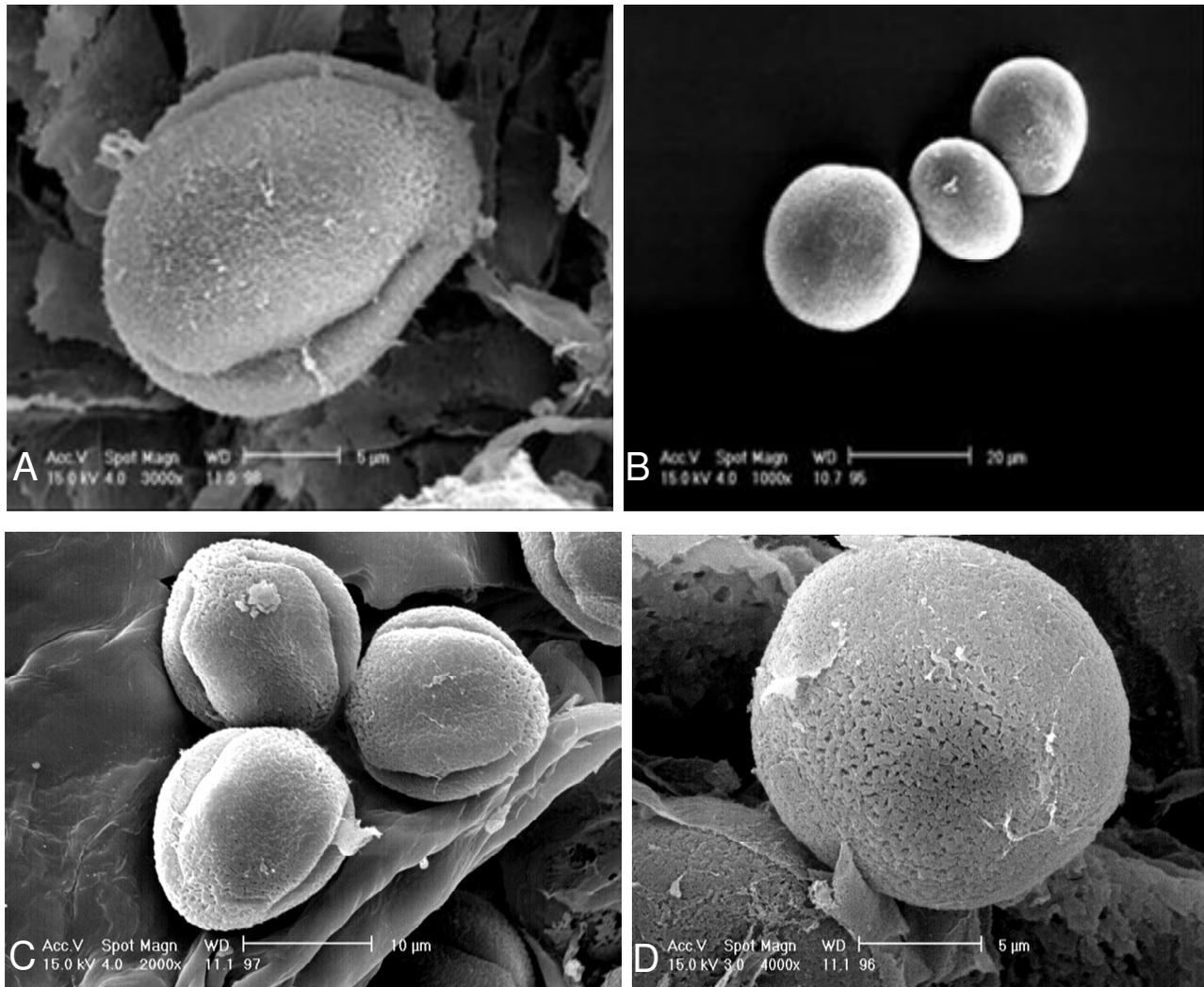


Figure 10. *V. vinifera* pollen grains, A-C pollen grain with colpi (Male), B-D pollen grain without colpi (Female).

collenchymal tissues in the petiole were observed.

The present investigation revealed that the petioles of *V. vinifera* have different specific shapes with respect to the species of the *V. vinifera* variety. The vascular bundle is closed and is surrounded by fiber layer, secretory cells are present. The present study further revealed that the midribs of *V. vinifera* are characteristics by the outline of the adaxial surface which is slightly humped by arc shape surface of the abaxial and the presence of collenchyma in both epidermis layers. The vascular bundle is closed and the druses, raphid crystals and starch grains are present in the pith.

However, the present study showed that the margin of *V. vinifera* was straight and slightly downwards with rounded or tapering tip. The trichomes are present or absent, druses and secretory cells are present. In addition, Vitaceae mesophyll layer contains calcium oxalate (druses) crystals and mucilage cells or secretory cells with raphides in bundle. Similarly, this has also be

reported in a study by Metcalfe and Chalk (1950), mucilaginous idioblasts in the mesophyll of leaf and cortex of the stem which was absent in the epidermis (Kannabiran and Pragasam, 1994).

The petiole, midrib, margin and lamina of Vitaceae features outline of these sections is smooth in some varieties while trichomes are present in some varieties and are glandular or non glandular which is an important feature in determining the varieties. The trichome complement of a particular organ can consist entirely of unbranched or branched hairs (Lombardi, 2007). For example, the present study showed that Baidhawi outline is smooth, but in Tre rash, trichomes are present, while in Rash mire the outline of the petiole and lamina trichomes are also present.

The present study revealed the presence of starch grains in pith and beside xylem tissue among Vitaceae family. 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 study reveals that Vitaceae leaf epidermis has different types of stomata which are anomocytic, hemicytic, cyclocytic and saturocytic and are in conformity with Hui et al. (2003) and Kannabiran and Pragasaam (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 colpi grains. However, sufficient variation is found between pollen shape class and exine pattern. Base on this features the family divided into three pollen types, *V. parvifolia* type, *V. jacquimontii* and *Ampelopsis vitifolia* subsp., *hazaraganjiensis* type (Perveen and 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 prolate-spheroidal (Perveen and 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).

Conclusion

The morphological and anatomical evaluation of the species of Vitaceae is reported. The result shows that starch grains, druses and raphid crystals were present among the varieties. Leaf epidermal cells in all the varieties were irregular or polygonal. The anticlinal walls were straight and arched. The stomatal apparatuses were present only on the abaxial epidermes and anomocytic type and trichomies were present in epidermes. The flower bisexual were 5-numerous and the size ranges between 4 to 5 mm. Pollen grains tricolporate, sub prolate or prolate-spheroidal present in the varieties were unaperturate with no colpi.

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