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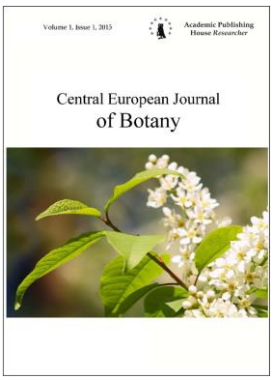

Taxonomic Study of Some Rare Species of Vitaceae from Pakistan by Foliar Micro-Morphological Approach

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Abstract

The present study is insight into foliar epidermal anatomy for characterizing species and their utility in the taxonomic separation of certain taxa of family of Vitaceae from Pakistan. The studied foliar micromorphology of 5 species; *Parthenocissus semicordata* (Wall.) Planch, *Parthenocissus tricuspidata* (Siebold & Zucc.) Planch, *Ampelopsis vitifolia* (Boiss.) Planch. subsp. *hazaraganjiensis* Nazim & Qaiser, *Cissus trifoliata* (L.) L., and *Cissus quadrangularis* L. was analysed and documented using Light microscopy (LM) for both qualitative and quantitative characteristics. Epidermal cells observed were either polygonal or irregular shaped, with straight or undulate anticlinal walls. Two types of stomata observed were paracytic and anomocytic with elliptical shaped guard cell all the studied species except adaxial surface of *Parthenocissus semicordata*, *Parthenocissus tricuspidata* and *Ampelopsis vitifolia* subsp. *hazaraganjiensis*. Trichomes were conical shaped, non-glandular either unicellular or multicellular. The quantitative parameters studied were size (length and width) of stomata, subsidiary cell, guard cells, stomatal pore, stomatal complex, trichomes. There was much variation in the size of all the parameters investigated. These anatomical features may help in the discrimination of studied taxa and are of much significance for the plant taxonomist in the correct identification of taxa.

Keywords: taxonomy, anatomical features, light microscopy, taxonomic implication.

1. Introduction

Vitaceae is an important family, well known among commercial fruits for the economic importance of the grapes (Karkamkar et al., 2010). The family is treated under order Rhamnale, divided into two major groups: 4-merous group and 5-merous group, comprised of 14 genera and 900 species distributed widely in tropical, subtropical regions and partially in temperate region. Mostly characterized by woody creepers or lianas, few are herbaceous or erect shrubs, rarely succulent trees categorized primarily as a source of food, wine, resins and also as ornamental (Manchester et al., 2013; Lu et al., 2013). A collection of 6 genera and 12 species are represented in the Flora of Pakistan, traced in wide range of habitat with dispersed location from coastal areas to higher altitude in north. The major genera of the family in Pakistan are *Vitis*, *Parthenocissus*, *Ampelocissus*, *Ampelopsis*, *Cissus* and *Leea*. Leaves are opposite, petiolate, stipulate or extipulate, pinnately compound, lobed or palmately compound, mostly unisexual apetalous flowers, few are bisexual, superior ovary, fruit is seeded or seedless berry (Perveen, Qaiser, 2008).

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Anatomical characteristics using Light microscopy (LM) are crucial to identify and resolve the problematic taxa at species level as well as generic level. Series of investigations are processed on genera of Vitaceae on the basis of morphological and anatomical features to confirm the placement of the family in plant phylogenetic tree (Gerrath et al., 2004). Present study is the first detailed investigation on foliar epidermal anatomy of Vitaceae from Pakistan based on light microscopy. The main objective of the study is to provide fine micro-morphological characteristics including both qualitative and quantitative features of family Vitaceae. This study may help in identifying problematic taxa to be placed in correct taxonomic rank.

2. Materials and methods

A total of 5 species belonging to 3 genera were collected from different regions of Pakistan, i-e Kaghan, Mansehra, Abbottabad, Ayubia and Islamabad during May 2017 to September 2017. Plants were photographed in field for identification purpose (Figure 1). Plants collected were identified with the help of Flora of Pakistan (http://www.efloras.org) and other available literature. Names of the plants were verified from Kew's Vascular Plants database-The Plant List (http://www.theplantlist.org/). Light microcopy was done by following the method adopted by Ullah et al. (2018) with little modification. Fully grown, undamaged leaves were selected from dried plant samples and dipped in lukewarm water for half an hour to avoid damage from drying. Then few leaves were boiled in 70 % lactic acid and 30 % nitric acid till the leaves become transparent. Excess of chemical was drained and then leaves were put in petri dish. Washed with water to remove debris separated from leaves. Both the abaxial and adaxial surfaces were taken carefully with the help of needle and placed on clean glass slide. Treated with lactic acid and cover slip was placed carefully to avoid air bubble. Cover slips were fixed at corners by using transparent nail polish. A total of 6-8 samples of both the surfaces of each plant species were prepared and studied using light microscope and photo-micrographs were also taken at different resolutions using Leica D-20. Epidermal cells shape and size (length and width), anticlinal walls, stomata type, size, stomatal complex, stomatal pore size, trichome type and size were measured (Tables 1, 2).

Fig. 1. Field photographs (A) *Parthenocissus semicordata* (B) *Parthenocissus tricuspidata* (C) *Ampelopsis vitifolia* subsp. *hazaraganjiensis* (D) *Cissus trifoliata* (E) *Cissus quadrangularis*

To find average, five consecutive values were noted. Mean value and standard for each feature was calculated by using statistical software IBM SPSS Statistics 20. Values are presented as

mean (minimum-maximum) ± Standard error in tabular form. Stomatal index was calculated using formula adopted by Shah et al. (2018) (Shah et al., 2018).

$$S.I = \frac{S}{S+E} \times 100$$

S.I = Stomatal index, S = number of stomata per unit area, E = number of epidermal cells per unit area.

3. Results and discussion

In the present study, 5 species belonging to 3 genera were investigated for the qualitative and quantitative micro-morphological features of leaf epidermis by using Light Microscopy (LM) (Tables 1, 2). Epidermal cells on both the adaxial and abaxial surfaces were noted in all the species. Significant difference in epidermal cell size was recorded on both the surfaces (Table 2). Epidermal cells of all the studied species appeared to be polygonal and irregular shaped (Table 1). On both the surfaces, the epidermal cells displayed similar dimensions all over the leaf, yet the adaxial epidermal cells were recorded comparatively larger than those of abaxial surface (Table 2). The maximum epidermal cell length was recorded for *Cissus trifoliata* on abaxial side (53.5 ± 2.8 µm) and on adaxial surface (60.5 ± 4.7 µm), and lowest for *Parthenocissus tricuspidata* adaxial surface (19.2 ± 0.4 µm). In this context, the largest sized epidermal cells were observed in *Cissus trifoliata*. Three types of anticlinal walls (Straight, slightly undulate, slightly undulate) were observed in the investigated plants. Majority of the species had epidermal cells with straight anticlinal walls, except *Cissus trifoliata* (undulate-both surfaces), and *Parthenocissus tricuspidata* (slightly undulate-adaxial). The highest number of epidermal cells counted per unit area were noted in the abaxial surface of *Parthenocissus semicordata* (435 cells/unit area). Among the studied anatomical characters, the most valuable one for distinguishing the species of different genera was the type and density of stomata. Stomata were observed to be arranged randomly throughout the epidermis on both the surfaces for *Cissus trifoliata* and *C. quadrangularis*, while in rest of the species stomata were either invisible or absent (Table 1 & Figure 1). The highest number of stomata were recorded for *Cissus trifoliata* (61), while lowest for the adaxial surface of *Cissus quadrangularis* (7). Stomatal index (SI) was found to be highest in *C. trifoliata* (42.9 %). Guard cells were elliptical shaped in all the species. According to the previous reports, stomatal parameters are influenced by the effect of ecological factors as light and temperature (Dickison, 2000).

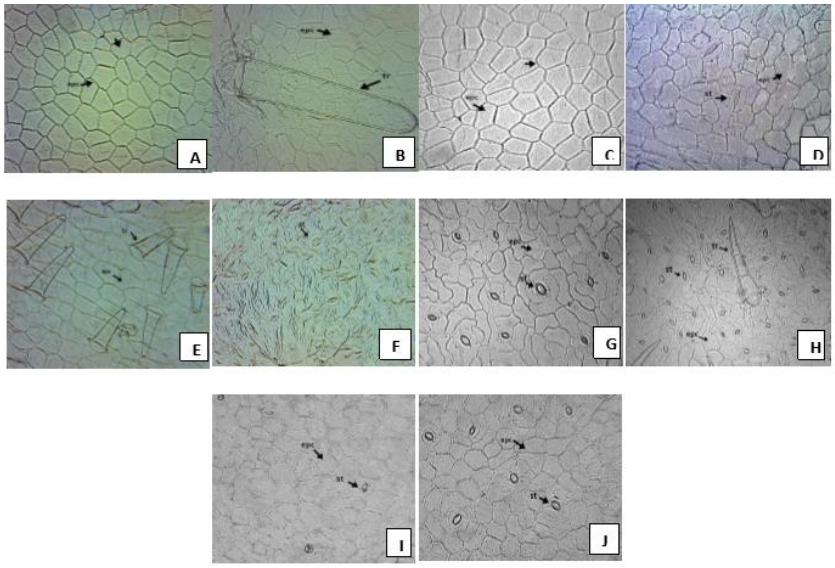

Fig. 2. Light Micrographs (LM) of Foliar epidermis, *Parthenocissus semicordata* (A) Adaxial (B) Abaxial; *Parthenocissus tricuspidata* (C) Adaxial (D) Abaxial; *Ampelopsis vitifolia* subsp. *hazaraganjiensis* (E) Adaxial (F) Abaxial; *Cissus trifoliata* (F) Adaxial (G) Abaxial; *Cissus quadrangularis* (H) Adaxial (I) Abaxial

Trichome micromorphology have been reported to be valuable for comparative systematic studies at every level of taxonomic hierarchy, because of their various shapes, ease of observation and commonly occurrence in various plant families (Mannethody, Purayidathkandy, 2018). Trichomes were present on both the surfaces in *Parthenocissus semicordata* and *Vitis trifoliata* and adaxial surface of *Ampelopsis vitifolia* subsp. *hazaraganjiensis*. While trichomes were absent on both the surfaces of *P. tricuspidata*, and *C. quadrangularis*. Two types of conical shaped trichomes were recorded, (i) unicellular non-glandular, (ii) multicellular non-glandular. Anatomical features are usually as helpful as morphological features for plant diagnostics, and they often are useful in the separation of closely related taxa (Esfandani-Bozchaloyi, Zaman, 2018; Karamian et al., 2012).

Table 1. Foliar epidermal characters of family Vitaceae-Qualitative

Taxa	Ab×Ad	Epidermal cell shape	Anticlinal walls	Stomata P/A	Type of Stomata	Shape of Guard cell	Trichome P/A	Type of Trichome
Parthenocissus semicordata (Wall.) Planch.	Ab	Polygonal	Straight	P	Anomocytic	Elliptic	P	Conical, Unicellular, Non-glandular
	Ad	Polygonal	Straight	A	-	-	P	Conical, Unicellular, Non-glandular
Parthenocissus tricuspidata (Siebold & Zucc.) Planch.	Ab	Irregular	Slightly undulate	P	Anomocytic	Elliptic	A	-
	Ad	Polygonal	Straight	A	-	-	A	-
Ampelopsis vitifolia subsp. *hazaraganjiensis* Nazim & Qaiser	Ab	Polygonal	Straight	P	Paracytic	Elliptic	A	-
	Ad	Polygonal	Straight	A	-	-	P	Conical, Unicellular, Non-glandular
Cissus trifoliata (L.) L.	Ab	Irregular	Undulate	P	Anomocytic	Elliptic	P	Conical, Multicellular, Non-glandular
	Ad	Irregular	Undulate	P	Anomocytic	Elliptic	P	Conical, Multicellular, Non-glandular
Cissus quadrangularis L.	Ab	Polygonal	Straight	P	Paracytic	Elliptic	A	-
	Ad	Polygonal	Straight	P	Paracytic	Elliptic	A	-

Notes: Ab = abaxial; Ad = adaxial; P = present; A = absent

Table 2. Foliar epidermal characters of family Vitaceae-Quantitative

Botanical Name	Ab/ Ad	Epi cell		Stomata		Subsidiary cell		Guard cell		Stomatal pore		Stomatal complex		Trichome		Avg. No. of Epi cells	Avg.No. of stomata	Avg. No. of trichomes	SI
		L	W	L	W	L	W	L	W	L	W	L	W	L	W				
		Mean (min-max) ±SE (µm)																	(%)
Parthenocissus semicordata	Ab	34 (27.5-40) ± 2.3	25.5 (20-37.5) ±3.1	32.4 (29-39) ±1.9	31.2 (26-37) ± 1.9	22.6 (21-26) ± 1.1	13.8 (15-17) ± 1.3	35.2 (32-40) ± 1.3	10.6 (10-12) ± 0.4	26 (24-28) ± 0.7	9.6 (8-12) ± 0.7	45 (37-51) ± 2.8	32.5 (21.5-35.5) ±2.1	360 (312-400) ±17.4	71 (65-75) ±1.9	435	27	2	5.8
	Ad	53 (37.5-62.5) ± 4.2	38 (30-47.5) ± 3.5	-	-	-	-	-	-	-	-	-	-	288 (280-347) ±17.5	61.5 (47.5-75) ±5.4	223	-	1	-
Parthenocissus tricuspidata	Ab	38.5 (32.5-42.5) ±2.0	28 (25-30) ±0.9	24 (22.5-27.50) ±1	7.5 (5-10) ±0.8	33 (30-37.5) ±1.5	9 (7.5-10) ±0.6	22.5 (20-27.5) ±1.4	2.5 (2.5-2.5) ±0.0	16 (12.5-20) ±1.5	3 (2.5-5) ±0.5	31.5 (22.5-37.5) ±2.9	24.5 (20-27.5) ±1.5	-	-	424	27	-	6
	Ad	19.2 (18-20) ±0.4	13.2 (9-17) ± 1.4	-	-	-	-	-	-	-	-	-	-	-	-	97	-	-	-
Ampelopsis vitifolia subsp. *hazaraganjiensis*	Ab	35.83 (30-42.5) ±3.63	19.17(17.520) ±0.83	26.67 (25-27.5) ±0.83	1.67 (1-2.5) ± 0.42	30 (27.5-32.5) ±1.44	0.5 (0.5-0.5) ± 0	26.67(25-27.5) ±0.83	0.58 (0.5-1) ± 0.08	1.08 (1-1.5) ± 0.08	0.33 (0.2-0.5) ± 0.08	29.17(27.5-32.5) ±1.67	3.5 (2.5-5) ± 0.75	-	-	187	23	-	11
	Ad	36.7 (32.5-40) ± 2.2	20 (17.5-22.5) ±1.44	-	-	-	-	-	-	-	-	-	-	48.3 (37.5-62.5) ±7.41	28.33(25-30) ± 1.67	230	-	4	-

Cissus trifoliata	Ab	53.5 (47.5-62.5) ±2.8	29.5 (25-37.5) ±2.4	19.5 (15-25) ±2	9 (7.5-12.5) ±1	31 (27.5-45) ±3.5	7.5 (7.5-7.5) ±0.0	19.5 (15-25) ± 2	2.5 (2.5-2.5) ± 0.0	13.5 (10-20) ± 2.2	6 (5-10) ± 1	31 (27.5-45) ± 3.5	22.5 (20-27.5) ±1.4	206.5 (137-292) ±32.1	42.5 (30-55) ± 4.9	245	61	11	15.3
	Ad	60.5 (50-75.5) ±4.7	45 (40-52.5) ±2.2	20 (15-25) ±1.8	8 (7.5-10) ± 0.5	32.5 (27.5-37.5) ±1.8	7 (5-7.5) ± 0.5	20 (15-25) ± 1.8	2.5 (2.5-2.5) ± 0.0	15.5 (12.5-20) ± 1.5	4.5 (2.5-7.5) ± 0.9	33.5 (30-37.5) ±1.3	22 (20-25) ± 0.9	148.5 (122-175) ±8.6	51.5 (47.5-55) ± 1.8	117	12	9	42.9
Cissus quadrangularis	Ab	45.83 (37.5-55) ± 5.07	28.33(22.5-37.5) ±4.64	2.08 (2-2.5) ±0.08	1.33 (1-1.5) ± 0.08	10.42(8.5-12.5) ±1.1	6.5 (6.5-6.5) ±0	2.25 (2-2.5) ± 0.14	0.58 (0.5-1) ± 0.08	2.08 (2-2.5) ± 0.08	0.67 (0.5-0.7) ±0.08	10.42(8.5-12.5) ±1.1	13.83(13.5-14) ± 0.08	-	-	314	20	-	6
	Ad	47.5 (37.5-55) ± 5.2	28.33(20-35) ± 4.41	2.08 (1.5-2.5) ± 0.17	1.17 (1-2) ±0.08	10.42(7.5-12.5) ±1.5	6.67 (6.5-7.5) ± 0.42	2.08 (1.5-2.5) ± 0.17	0.67 (0.5-1.2) ±0.08	0.85 (0.7-1.5) ±0.2	0.5 (0.5-0.5) ± 0	10.42(7.5-12.5) ±1.5	14.5 (13.5-16.5) ±0.88	-	-	230	7	-	3

Notes: Epi= epidermal cell, Ab= abaxial, Ad= adaxial, L= length, W= width, SI= stomatal index, Min= minimum, Max= maximum, SE= standard error, µm=micrometer

Anatomical studies have demonstrated that leaf epidermal characters are comparable and quite reliable in taxonomic studies (Yasmin et al., 2010). In the 20th century, various studies were conducted on the anatomy and morphology of individual species of Vitaceae, even fruit development (Hardie et al., 1996). The micro-morphological features of these species play significant role in identification of various anatomical features of leaf epidermis which may lead to the comparative study of the same species in other regions of the world and may provide aid to the unresolved phylogenetic status of Vitaceae. Taxonomic key was constructed on the basis of variation in microscopic characteristics observed under light microscope. Based on variations observed, taxonomical keys may be constructed so that one species can easily be identified and differentiated from the other.

4. Conclusion

Light microscopy of leaf and scanning electron microscopy of seeds of family Vitaceae has a distinguished role in identification at genus and species level and may provide evidence in the determination of taxonomic rank in the phylogenetic tree. Varied characters among species of seeds and diverse epidermal features such as shape and wall margins, stomata size and type, trichome size and type investigated in this study and their statistical analysis may possess great potential for plant taxonomist to further evaluate the chemical nature, vegetative growth and tissue development which is needed particularly for grape vine to increase the fruit.

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