Journal of Medicinal Plants Research Vol. 5(13), pp. 2857-2862, 4 July, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals

Full Length Research Paper

Taxonomic potential of foliar epidermal anatomy among the wild culinary vegetables of Pakistan

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Accepted 8 March, 2011

The present study is focused on the taxonomic screening of ten wild culinary vegetables that is *Amaranthus viridus* L*., Bauhenia verigata* L*., Chenopodium album* L*., Malva parviflora* L*., Medicago falcate* L*., Medicago polymorpha* L*., Melilotus indicus* L*., Portulaca oleracea* L*., Portulaca quardifida* L and *Solanum nigrum* L. The objective of the present study is to use the foliar epidermal features for taxonomic identification of these culinary vegetables. The study based on light microscopic (LM) characterization of foliar epidermis. Shape of epidermal cells, presence and type of stomata and trichomes and stomatal index plays a key role in the identification of these species. Although detailed characterization of these species at molecular and genetic level is still needed.

Key words: Ethnobotany, foliar epidermis, culinary vegetables.

INTRODUCTION

The organic world is sustained by plants through the fundamental process of photosynthesis. The way in which plants influence life on earth is remarkable. Plants have provided all the basic needs of man ever since his birth and evolution. Plants are the source of food, medicine, fuel, fiber etc. As civilizations developed people identified and used many other plants that yielded spices, oil and selected forage and fodder grasses for the animals that they domesticated (Buksh et al., 2007). Traditional (wild culinary) vegetables grow wild and are readily available in the field as they do not require any formal cultivation. They represent inexpensive but high quality nutrition sources for the poor segment of the population and account for 10% of the world's higher plants (Chweya and Eyzaguirre, 1999). According to modern nutritional studies the consumption of leafy vegetables brings numerous health benefits, and their everyday consumption in diet is highly recommended (Block, 1991). Leafy vegetables are a source of vitamin C, folic acid, antioxidants, carotenoids and many other valuable chemicals (Hasler, 2002; Ogle et al., 2001; Pieroni et al., 2002; Tapsell et al., 2006). Wild leafy vegetables may be a particularly rich source of these compounds and are more likely to be free of agricultural pollutants. Edible weeds, however, are scarcely used in many countries and weed gathering is more of a weekend hobby than a regular source of food supply (Diaz-Betancourt et al., 1999). This is in spite of the fact that these vegetables grow spontaneously and in abundance around the rural homesteads.

The consumption of vegetables has however been linked to reduction in the incidence of oxidative-stress related diseases due to beneficial health functionality of phenolic antioxidants present in them (Halliwell, 1995; Squadriato and Peyor, 1998; Yildrim et al., 2001; Gulcin et al., 2002). The foliar epidermis is one of the fundamental taxonomic characters from biosystematics point of view and taxonomic studies of a number of plant families are made on the basis of leaf epidermis anatomy. Leaf epidermal features like shape of epidermal cells, stomata and trichomes are useful anatomical tools. Length and width of epidermal cells is a useful aid in distinguishing verities (Kadiri, 2005, 2006). The epidermis possess a number of important diagnostic character that

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offer valuable clues for identification, like size, shape and orientation of stomata, guard cells and subsidiary cells, structural peculiarities of epidermal cell walls, distinctive or specialized form of trichomes (Dickison, 2000). Edeoga (1991), Edeoga and Osawe (1996), and Mbagwu and Edeoga (2006) constantly reaffirmed the point that epidermal and cuticular traits of plants could serve as vital tools exploitable in the systematics of the present day angiosperms. Also, different shapes of epidermal cells, type and arrangement of stomata, size and shape of trichomes and number of vascular bundles are all vital in systematic botany (Nwachukwu and Mbagwu, 2006).

The present study is focused on the foliar epidermal characteristics of ten wild culinary vegetables by using light microscopy (LM). However, the core objectives of the present study were to: (a) identify and compare the variation of different stomatal types in different species of wild culinary vegetables, (b) variation of qualitative and quantitative characters of epidermal cells, trichomes and stomatal complex, and (c) to study the stomatal index of these species. All these characters might be useful for classification of their taxonomic hierarchy.

MATERIALS AND METHODS

Fresh samples of about 3 to 5 specimen of each plant were collected from different areas of Pakistan. Voucher specimens were deposited in Herbarium of Pakistan, Quaid-i-Azam University Islamabad. For the study of leaf epidermal features modified methodology of Ahmad et al. (2010) was followed. Pieces of fresh leaves were infused in 30% nitric acid and boiled with 2.0 g of potassium chloride in a test tube for 2 to 3 min. These leaf pieces were then washed down with distilled water twice. Epidermis was then peeled off and kept in 60% potassium hydroxide solution for 1 to 2 h for decolourization. Finally, thin sections of epidermis were suspended in lactic acid on glass slide for LM study. All the chemicals used are of Merck (Germany). Microphotographs were taken by using CCD digital camera (Model: DK 5000) fitted on Leica light microscope (Model: DM 1000). These micrographs were useful for the identification and differentiation among different plant species.

Stomatal index (SI)

The stomatal index (SI) was calculated using the formula described by Salisbury (1972) that is:

$$SI = \frac{S}{S + E} \times 100$$

Where S donates the number of stomata per unit area and E the number of epidermal cells in the same unit area.

RESULTS AND DISCUSSION

The data for the qualitative and quantitative micro morphological features of foliar epidermis of ten species of wild culinary vegetables is presented in Tables 1 and 2. Leaf epidermal tissue characteristics have an important role in taxonomy and determination of number of plant genera and species. Anatomical studies have been used successfully to clarify taxonomic status and help in the identification of different species (Scatena et al., 2005; Uphof, 1962).

Epidermal cells

Epidermal cells in all the examined species varied in their shapes from undulating-irregular to variously shape. The shape of epidermal cells on both surfaces that is, abaxial and adaxial surfaces show slight difference in their shape. Epidermal cells are undulating to irregular shaped in species like *Amaranthus viridus* (Figures 1A and B), *Chenopodium album* (Figures 1E and F), *Malva parviflora* (Figures 1 G and H), *Medicago falcata* (Figures 1L and M), *Medicago polymorpha* (Figures 1: I and J), *Portulaca oleracea* (Figure 1 Q and R), *Portulaca quardifida* (Figures 1S and T) and *Solanum nigrum* (Figures 1O and P) while in *Bauhenia verigata* (Figure 1C and D) and *Melilotus indicus* (Figures 1K and L) the epidermal cells are variously shaped. Ahmad et al. (2010) reported the presence of highly undulated epidermal cells in *M. indicus* and polygonal walls in *C. album*. This difference in results might be the consequence of edaphic and environmental factors. Stace (1965) noted that undulation of cell wall is a mesomorphic character and that environmental conditions such as humidity play a significant role in determining the pattern of anticlinal cell walls. Straight or curved walls were identified as characteristics of species growing in drier conditions while undulate walls were found mostly in species growing in areas of high humidity.

The quantitative analysis of all the selected species of wild culinary vegetables varies greatly as shown in Table 2. Apart from *M. indicus* and *C. album* all the examined species of wild culinary vegetables are reported the first time as far as we know no literature is available on the foliar epidermal features of *A. viridus, M. parviflora, M. falcata, M. polymorpha, P. oleraceae, P. quardifida and S. nigrum*.

Stomata

Five different types of stomata were observed in all the examined species of wild culinary vegetables. These types comprises of Anomocytic, Amphianisocytic, Staurocytic, Paracytic and Anisocytic. In all these species stomata are present on both surfaces but these are more abundant on abaxial surface as compared to adaxial surface. *M. polymorpha* posses staurocytic to Anomocytic type of stomata (Figure 1J), Staurocytic stomata in *A. viridus* and *C. album* (Figures 1B and F), Anomocytic stomata in *B. verigata, M. falcata* and *S. nigrum* (Figures

Table 1. Qualitative features of foliar epidermal cells characteristics of wild culinary vegetables.

No.	Taxa	Leaf epidermis (Ad/Ab)		Stomata		Trichomes	
		shape	Margin	P/A (Ad/Ab)	Type	P/A	Type (glandular/non glandular)
1.	*Melilotus indicus*	Variously shaped	Straight	P on both	Anomocytic, amphianisocytic to staurocytic	Ab	Non-glandular and unicellular
2.	*Medicago polymorpha*	Undulating to irregular	Wavy	P on both	Staurocytic to anomocytic	Ab	Non-glandular and unicellular
3.	*Medicago flacta*	Undulating to irregular	Wavy	P on both	Anomocytic	Ab and Ad	Non-glandular and unicellular
4.	*Amaranthus viridus*	Undulating to irregular	Wavy	P on both	Staurocytic	Ab	Glandular and unicellular
5.	*Bauhenia veriagata*	Variously shaped	Straight	P on both	Anomocytic	Ab	Non-glandular and unicellular
6.	*Solanum nigrum*	Undulating to irregular	Wavy	P on both	Anomocytic	Absent	Absent
7.	*Chenopodium album*	Variously shaped	Straight	P on both	Staurocytic	Absent	Absent
8.	*Portulaca oleracea*	Undulating to irregular	Wavy	P on both	Paracytic	Absent	Absent
9.	*Portulaca quardifida*	Undulating to irregular	Wavy	P on both	Paracytic	Absent	Absent
10.	*Malva parviflora*	Undulating to irregular	Wavy	P on both	Amphianisocytic to anisocytic	Ab	Non-glandular and multicellular

Ab- Abaxial cells; Ad- Adaxial cell; P- Present; A- Absent.

Table 2. Quantitative data of foliar epidermal cells of wild culinary vegetables.

No.	Taxa	Surface	Leaf epidermis (µm)	Stomatal complex (µm)	Trichomes (µm)
		Ab/Ad	Length x width	Length x width	Length x width
1.	*Melilotus indicus* L.	Ab	36.66 (25 - 50) x 22.5 (20 - 25)	25.4 (22.5 -2 7.5) x 14.5 (12.5 - 17.5)	230 (200 - 375) x 11.66 (7.5 -15)
		Ad	73.33 (65 - 80) x 31.66 (25 - 40)	26.66 (25 -27.5) x 16.66 (12.5 - 20)	Absent
2.	*Medicago polymorpha* L.	Ab	37.5 (27.5 - 45) x 25.33 (23.75 - 27.25)	15 (12.5 -17.5) x 10.25 (7.75 - 12.5)	200 (150 - 210) x 12 (10 - 15.72)
		Ad	48.33 (37.5 - 62.5) x 28.33 (22.5 - 37.5)	13.16 (12.25 - 17.25) x 15.83 (13.75 - 17.5)	Absent
3.	*Medicago flacta* L.	Ab	90.83 (32.5 - 102.5) x 45.41 (35 - 55)	53.33(47.5 - 62.5) x 29.16 (23.75 - 36.25)	51.66 (27.5 - 87.5) x 23.66 (21.25 - 25)
		Ad	75 (72.5 - 77.5) x 28.33 (26.25 - 31.25)	19.5 (18.75 - 20) x 17.16 (16.25 - 18)	Absent
4.	*Amaranthus viridus* L.	Ab	52.58 (47.5 - 57.75) x 36.66 (27.5 - 45)	14.5 (9.6 - 15.7) x 6.5 (4.2 - 7.2)	89.91 (64.75 - 130) x 5.83 (4.75 - 7.5)
		Ad	65 (55 - 72.5) x 34.91 (30 - 37.5)	17.41 (12.25 - 22.5) x 12.41 (10 - 14.75)	Absent
5.	*Bauhenia verigata* L.	Ab	17.1 (12.5 - 22.5) x 10.95 (9.75 - 12.5)	16.25 (15 - 17.5) x 11.875 (10 - 12.5)	63.5 (37.5 - 87.5) x 10.45 (7.5 -12.5)
		Ad	31 (25 - 40) x 18.75 (13.75 - 22.5)	17 (12.5 - 20) x 10.95 (7.5 - 12.5)	Absent
6.	*Solanum nigrum* L.	Ab	64.16 (60 - 70) x 41.58 (37.25 - 50)	34.25 (25.25 - 45) x 20.75 (17.5 - 25)	Absent
		Ad	69.16 (62.5 - 75) x 34.16 (25 - 40)	40.83 (37.5 - 45) x 23.91 (24.5 - 24.75)	Absent
7.	*Chenopodium album* L.	Ab	70.83 (52.5 - 90) x 38.33 (35 - 40)	35.08 (34.75 - 35.5) x 24.08 (22.5 - 25)	Absent
		Ad	62.5 (50 - 72.5) x 52.91 (48.75 - 57.5)	32.5 (30 - 35) x 18.33 (15 - 20)	Absent
8.	*Portulaca oleraceae* L.	Ab	134.16 (125 - 150) x 64.16 (57.5 - 72.5)	40.83 (32.5 - 47.5) x 24.08 (20 - 27.5)	Absent
		Ad	128.33 (120 -140) x 66.58 (62.25 - 75)	44.16 (42.5 - 47.5) x 27.91 (26.25 - 27.5)	Absent

Table 2. Contd.

9.	*Portulaca quardifida* L.	Ab	112.5 (100 - 125) x 61.66 (47.5 - 72.5)	35.5 (32.5 - 39) x 23 (22 - 24.75)	Absent
		Ad	126.66 (99.75 - 155.25) x 60.83 (32.5 - 77.5)	45.83 (40 - 50) x 30.66 (22.5 - 39.75)	Absent
10.	*Malva parviflora* L.	Ab	62.5 (50 - 75) x 40.83 (35 - 50)	28.33 (25 - 27.5) x 13.33 (10 - 17.5)	93.33 (150-300) x 14.33 (32.5-40)
		Ad	88.5 (50 - 105) x 42.5 (22.5 - 60)	29.16(25 - 32.5) x 25.83 (20 - 35)	Absent

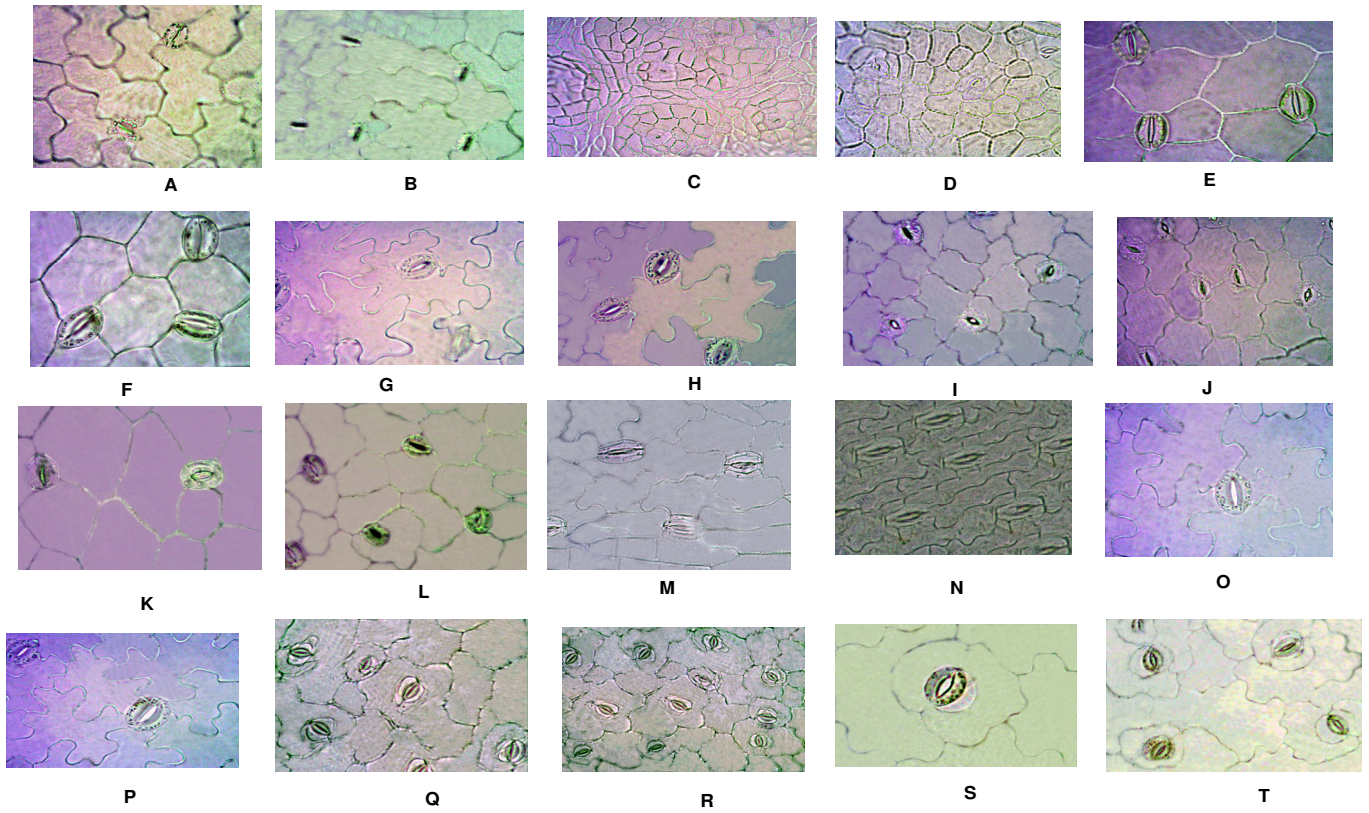

Figure 1. Epidermal cells and different types of stomata; A: *Amaranthus viridus* Adaxial surface (40×), B: *Amaranthus viridus* Abaxial surface (40×), C: *Bauhenia veriagata* Adaxial surface (10×), D: *Bauhenia veriagata* Abaxial surface (10×), E: *Chenopodium album* Adaxial surface (40×), F: *Chenopodium album* Abaxial surface (40×), G: *Malva parviflora* Adaxial surface (40×), H: *Malva parviflora* Abaxial surface (40×), I: *Medicago polymorpha* Adaxial surface (20×), J: *Medicago polymorpha* Abaxial surface (20×), K: *Melilotus indicus* Adaxial surface (20×), L: *Melilotus indicus* Abaxial surface (20×), M: *Medicago falcata* Adaxial surface (40×), N: *Medicago falcata* Abaxial surface (40×), O: *Solanum nigrum* Adaxial surface (40×), P: *Solanum nigrum* Abaxial surface (40×), Q: *Portulaca oleraceae* Adaxial surface (20×), R: *Portulaca oleraceae* Abaxial surface (10×), S: *Portulaca quardifida* Adaxial surface (40×), T: *Portulaca quardifida* Abaxial surface (20×).

Table 3. Stomatal index of wild culinary vegetables.

No.	Texa	Family	No. of epidermal cells		No. of stomata		Stomatal index	
			Ab	Ad	Ab	Ad	Ab	Ad
1.	*Amaranthus viridus*	Amaranthaceae	60	52	16	9	21.05	17.3
2.	*Bauhenia verigata*	Fabaceae	73	70	30	21	29.12	23.07
3.	*Chenopodium album*	Chenopodiaceae	70	65	25	18	26.31	21.68
4.	*Malva parviflora*	Malvaceae	25	22	19	17	43.18	43.59
5.	*Medicago flacta*	Papilionaceae	42	56	20	28	32.25	33.33
6.	*Medicago polymorpha*	Papilionaceae	48	57	15	13	23.81	18.57
7.	*Melilotus indicus*	Papilionaceae	36	55	21	38	36.84	40.86
8.	*Portulaca oleraceae*	Portulaceae	15	25	5	4	25	13.79
9.	*Portulaca oleraceae*	Portulaceae	16	14	5	2	23.8	12.5
10.	*Solanum nigrum*	Solanaceae	36	31	26	19	41.9	38

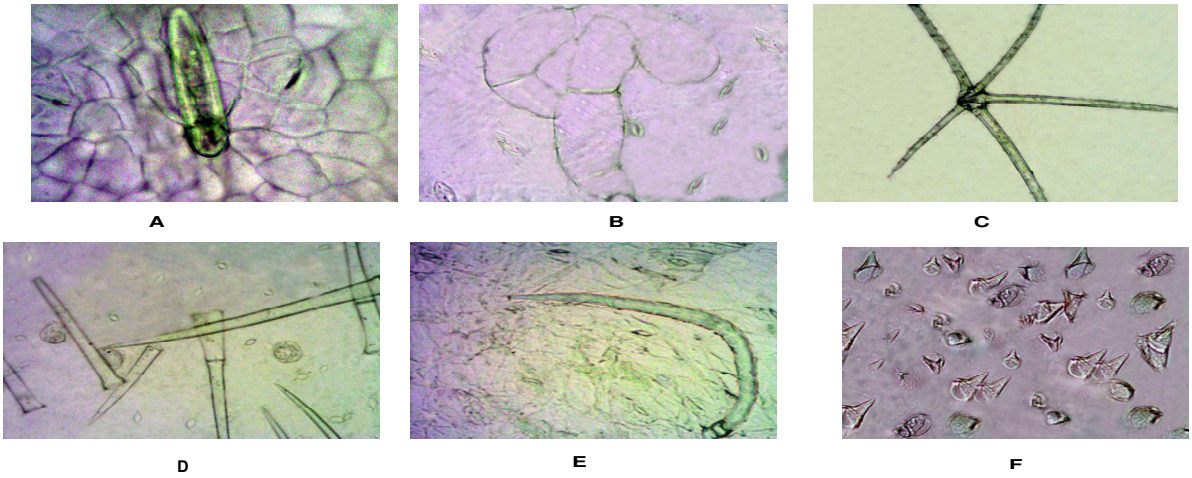

Figure 2. Different types of trichomes; A: *Bauhenia veriagata* Abaxial surface (40×), B: *Amaranthus viridus* Abaxial surface (40×), C: *Malva parviflora* Abaxial surface (10×), D: *Medicago polymorpha* Abaxial surface (20×), E: *Medicago falcata* Abaxial surface (10×), F: *Melilotus indicus* Abaxial surface (40×).

1D, M and P), Amphianisocytic to Anisocytic stomata in *M. parviflora* (Figure 1H), Paracytic stomata in *P. oleracea* and *P. quardifida* (Figure 1R and T) and Anomocytic, Amphianisocytic to Staurocytic type of stomata in *M. indicus* (Figure 1L).

All these species of wild culinary vegetables were reported for the first time in literature expect for *C. album* and *M. indicus* which according to Ahmad et al. (2009) possess anomocytic type of stomata in *M. indicus* and Staurocytic stomata in *C. album.*

Our results are exactly the same as reported in literature. Qualitative and quantative features of stomatal type, stomatal complex and stomatal apparture are presented in (Tables 1 and 2).

Stomatal index

The stomatal index of all the examined species of wild culinary vegetables show a wide rang of variation. This character is found to be very useful for differentiation of hybrid plants from parental type.

It is also useful to determine the level of atmospheric CO_2. Variation of stomatal index on adaxial and abaxial surfaces is presented in Table 3.

On adaxial surface stomatal index is found to be high in *M. parviflora* (43.5) and lowest in *P. quardifida* (12.5), while on abaxial surface it is high in *M. parviflora* (43.1) and lowest in *A. viridus* (21.05).

Trichomes

Two main type of trichomes were observed in all the selected species of wild culinary vegetables that is, glandular and non-glandular. Trichomes are present either on abaxial surface or adaxial surfaces, present on both surface and in some cases these are absent. In the present study trichomes are present on both surfaces in *M. falcata* and in *M. indicus, M. polymorpha, A. viridus, B. verigata,* and *M. parviflora* trichomes are only observed on abaxial surface (Figures 2A, B, C, D, E and F), while

Portulaca oleraceae, Portulaca quardifida, C. album and *S. nigrum* trichomes are absent. The quantitative data related to trichomes are also presented in Table 2.

Conclusion

All examined species of wild culinary vegetables show an immense variation in the epidermal cells characteristics at different level of taxonomy. Foliar epidermal anatomy with special reference to stomata type, types of trichomes and stomatal undex are very useful features form taxonomic point of view. Although there is a need to integrate and analyze this data at molecular level. This will provide a pathway for future workers to identify and recognize wild culinary vegetables in order to overcome food crises.

ACKNOWLEDGEMENT

We are very thankful to our Higher Education Commission (HEC) for financial support to complete this work.

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