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***Corresponding author:**

Madiha Zaynab;

Email:

madiha.zaynab14@gmail.com

Khalida Bahadar and Madiha Zaynab equally contribute this manuscript.

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The Anatomical Examination of Leaf Epidermis of Genus *Plantago* L. (Ispaghol) from Pakistan

Khalida Bahadar¹, Mahpara Fatima³, Ali Noman³, Safdar Abbas⁶, Ammar Raza⁷, Tahira Saleem², Rubab Sarfraz⁴, Kinza Khan⁶, Madiha Zaynab^{5*}

¹Department of Plant Sciences, Faculty of Biological Sciences Quaid-I-Azam University, Islamabad Pakistan.

²PARC Institutes of Advanced Studies in Agriculture, NARC, Islamabad, Pakistan.

³College of Crop Science, ⁴College of Resource and Environment, ⁵College of Life Science, Fujian Agriculture and Forestry University, Fuzhou 350002, PR. China.

⁶Department of Biochemistry, Quid-e-Azam University, Islamabad, Pakistan.

⁷Department of Horticulture Bahaudin Zakrya University, Multan, Pakistan.

Abstract

Anatomical observations of 17 different species of *Plantago* commonly known as Ispaghol (Family *Plantaginaceae*) obtained from Pakistan, were carried out with the help of a light microscope. The major objective of this study was to evidently describe the anatomical features of these species because there were a large number of overlapping characters between them. Amphistomatic type of leaf epidermis was found. Stomata were anomocytic or anisocytic. Both the upper and lower epidermis cells were double walled, irregular, elongated, oval and tetragonal to hexagonal in shape. Cell walls were found smooth or wavy anticlinal. Epidermal hairs were glandular, simple or multicellular. Stellate types of trichomes were also observed in one member of *Plantago* genus.

Keywords: *Plantago* species anatomy, light microscopy, stomata, traditional medicine.

INTRODUCTION

Plantaginaceae family contains 275 annual and perennial species all over the world. Most of the *Plantago* species have small elliptic leaves and spikes with tiny flowers. For long time it has been used in medicine because it contains diverse medicinal properties (Samuelsen, 2000). Some species are used to improve intestinal health (Lutterodt and Cheng, 2008). The *Plantago* species have been used in wound healing and in traditional medicine. *P. major* was used for wound healing in the first century A.D by Greek physicians (Roca-Garcia, 1972). It is still used in many countries for the skin infections treatment, prevention of cancer and enhances the circulation and reproduction (Samuelsen, 2000). *Plantago* species contains key metabolites known as Phenylethanoid glycosides which are derived from benzoic acid and have a phenylethylring linked to glycopyranose via an ester or glycosidic bond. Iridoid glycosides are useful chemotaxonomic markers also produced by *Plantago* species (Grubestic *et al.*, 2013). The large fleshy roots of this species are reputed to have great medicinal value (Michigan Flora Online, 2018). In Pakistan only, a single genus *Plantago* with 20 different species exhibits (Kazmi, 1974). The vegetative anatomy of the genus *Plantago* was unsatisfactory. There is need for new evidences from range of characters for revision of this genus. Therefore, the current study was aimed at examination of leaf epidermal anatomy of 17 species of the genus *Plantago*.

MATERIALS AND METHODS

Collection of Plant samples

Leaves samples were taken from specimens contained in different Herbaria i.e. Quaid-I-Azam University (ISL), Pakistan Museum of Natural History (PMNH), Pakistan Council of Scientific and Industrial Research (PCSIR) and some collections was made from the field. Voucher specimens were placed in the Herbarium of Quaid-I-Azam University Islamabad.

Study of leaf epidermis

The leaf epidermis was arranged for light microscopy (LM) by the standard Shultz's method of maceration with modified techniques described by Subrahmanyam (1996). For light microscopy, leaves were boiled in 100 ml distilled water amended with 4ml of concentrated nitric acid and 0.2g of potassium chloride until they regained their original shape. Once leaf became soft, it was fixed on slide, adaxial and abaxial leaf surfaces were obtained from the middle part of fully grown leaves, then the epidermis was peeled with the help of needle under microscope. The peeled epidermis was washed with water and placed on glass

slide, and then 1-2 drops of bleach was added for 30 Sec to remove the chloroplast. After bleaching, the specimens were washed again with water, and then amended with 1-2 drops of lactic acid. Mounted it in unstained glycerin jelly and covered with a cover slip. The permanent slides were photographed with a camera attached with Nikon Type-2 microscope under (E40) and oil immersion (E100). For the measurement of each specimen, 10 numbers of slides were used. Cell length and width, stomata length and width, guard cells length and cell wall thickness were measured by this method.

RESULTS AND DISCUSSION

Leaf epidermis of genus *Plantago* L. (*Plantaginaceae*) was characterized by double walled, irregular, elongated, oval and tetragonal to hexagonal cells structures. Cell walls were smooth or wavy anticlinal. Epidermal hairs were mostly glandular, simple or multicellular. According to the present findings epidermis was amphistomatic; stomata were present on both adaxial and abaxial epidermis. Although *Plantago* is uniform in its epidermal characters for epidermis of all 17 species (Table 1). However, there were some differences in their stomata types. There were two distinct types of stomata recognized viz., anomocytic and anisocytic. Anomocytic stomata were surrounded by very limited number of cells which were readily distinguished by the absence of subsidiary cells in which five species viz., *P. amplexcaulis*, *P. depressa*, *P. stocksii*, *P. exigua*, and *P. ciliate* were included. Mostly anisocytic type of stomata were found in seven species i.e., *P. major*, *P. himalaica*, *P. lagopus*, *P. lenceolata*, *P. exgua*, *P. afra* and *P. indica*. These were dominant type and surrounded by subsidiary cells of unequal size. Cyclocytic stomata were found in 3 species i.e. *P. loeflingii*, *P. baltistanica* and *P. gentianoides*. Tetracytic stomata were recorded in 2 species i.e., *P. depressa* and *P. coronopus*, and amphianisocytic occurred in 2 species, *P. major* and *P. ovate*. The Paracytic stomata were found only in one specie *P. coronopus*. Stomata of two different structures were found in *Plantago lagopus* Linn. One type of stomata had cap like structure at their edges and the other type of stomata having bulb like swelling at their edges. Lower epidermal cells near the midrib were almost regularly elongated in shape. Upper epidermal cells were double walled and irregular in shape. Trichomes found in the specimens were mostly multicellular and glandular with narrow acute tip. However, in *Plantago lagopus* Linn, trichomes possessed an exceptionally elongated basal cell. But only a single species of this genus i.e *P. cordata* showed stellate trichomes. In the present study, stellate trichomes were reported for the first time in genus *Plantago* from Pakistan.

Table 1: Measurements of epidermis, stomata and guard cells of seventeen species of *Plantago* L. at 10X in micro meter under light microscope.

Sr.	Botanical Name	Upper Epidermis Cell		Lower Epidermis Cell		Cell wall Thickness µm Mean±S.E	Stomata Cell		Guard cell	
		Length µm Mean±S.E	Width µm Mean±S.E	Length µm Mean±S.E	Width µm Mean±S.E		Length µm Mean±S.E	Width µm Mean±S.E	Length µm Mean±S.E	Width µm Mean±S.E
1	<i>Plantago major</i> Linn.	61.58±2.79 m	58.10±1.2 i	139.10±1.96 de	57.00±1.09 d	10.40±0.2 4 abc	30.0±0.85 kl	13.20±0.44 fg	41.60±0.87 i	5.20 ±0.34 g
2	<i>Plantago himalaica</i> pilger,	146.46±2.79 d	73.00±1.28 e	138.00±1.96 ef	68.20±1.09 c	9.30±0.24 cd	36.30±0.85 ghi	20.40±0.44 bcd	59.30±0.87 c	13.80±0.34 ab
3	<i>Plantago coronopus</i> Linn,	128.00±2.79 f	76.00±1.28 d	158.30±1.96 b	77.80±1.09 b	12.20±0.24 a	49.10±0.85 d	28.00±0.44 a	53.30±0.87 d	14.00±0.34 ab
4	<i>Plantago depressa</i> Willd,	100.07±2.79 k	53.00±1.28 j	118.00±1.96 i	50.40±1.09 e	10.00±0.24 abc	27.10±0.85 l	12.20±0.44 g	45.30±0.87 gh	11.50±0.34 bc
5	<i>P. gentianoides</i> Sibth. subsp. <i>griffithii</i> , Decne	107.00±2.79 j	57.40±1.28 i	106.30±1.96 k	77.00±1.09 b	8.80±0.24 cd	62.00±0.85 b	22.00±0.44 bc	75.00±0.87 b	14.00±0.34 ab
6	<i>P. amplexicaulis</i> Cav. subsp. <i>bauphula</i> Edgew.	140.30±2.79 e	85.40±1.28 b	135.40±1.96 fg	70.00±1.09 c	9.50±0.24 cd	40.70±0.85 e	15.00±0.44 fg	53.20±0.87 d	7.00±0.34 efg
7	<i>Plantago lanceolata</i> Linn.	169.00±2.79 b	88.20±1.28 a	197.00±1.96 a	87.10±1.09 a	10.00±0.24 abc	31.60±0.85 jk	15.00±0.44 fg	44.40±0.87 hi	6.10±0.34 efg
8	<i>Plantago lagopus</i> Linn.	145.00±2.79 d	68.00±1.28 f	144.00±1.96 c	68.00±1.09 c	12.10±0.24 ab	65.10±0.85 a	29.00±0.44 a	84.20±0.87 a	15.00±0.34 a
9	<i>Plantago ovata</i> Forssk	225.50±2.79 a	64.20±1.28 g	142.00±1.96 cd	69.10±1.09 c	8.20±0.24 cd	37.00±0.85 fgh	23.00±0.44 b	59.00±0.87 c	7.10 ±0.34 efg
10	<i>Plantago stocksii</i> Boiss. Ex Decne.	108.20±2.79 j	33.40±1.28 l	137.00±1.96 efg	33.40±1.09 g	8.40±0.24 cd	55.00±0.85 c	18.50±0.44 de	49.00±0.87 ef	8.30 ±0.34 de
11	<i>Plantago loeflingii</i> Linn.	82.00±2.79 l	40.60±1.28 k	110.00±1.96 j	46.00±1.09 f	9.00±0.24 cd	40.00±0.85 ef	5.10±0.44 fg	47.20±0.87 fgh	6.00±0.34 efg
12	<i>Plantago baltistanica</i> Hartmann.	159.20±2.79 c	74.00±1.28 de	142.00±1.96 cd	47.20±1.09 ef	12.00±0.24 ab	33.60±0.85 ij	19.00±0.44 cde	51.30±0.87 de	8.00±0.34 ef
13	<i>Plantago ciliata</i> Desf subsp. <i>lanata</i> Bioss.	112.40±2.79 i	62.10±1.28 gh	101.00±1.96 l	67.50±1.09 c	10.40±0.24 abc	33.60±0.85 ij	16.40±0.44 ef	48.00±0.87 fg	7.20 ±0.34 efg
14	<i>Plantago exigua</i> Murray.	122.00±2.79 g	29.10±1.28 m	130.00±1.96 h	69.00±1.09 c	9.70±0.24 bcd	37.30±0.8 fgh	20.00±0.44 bcd	45.60 ±0.87 gh	8.50 ±0.34 de
15	<i>Plantago afra</i> Linn.	158.00±2.79 c	61.00±1.28 h	134.30±1.96 g	76.10±1.09 b	10.50±0.24 abc	37.60±0.85 fg	19.00±0.44 cde	48.00 ±0.87 fg	5.30±0.34 fg
16	<i>Plantago indica</i> Linn.	127.00±2.79 f	80.00±1.28 c	107.20±1.9 jk	54.00±1.09 d	5.20±0.24 e	37.60±0.85 fg	19.00±0.44 cde	51.40 ±0.87 de	13.00±0.34 abc
17	<i>Plantago cordata</i> Lam.	118.60±2.79 h	75.20±1.28 de	81.20±1.96 m	50.00±1.09 e	7.40±0.24 de	34.50±0.85 hij	15.10±0.44 fg	47.40±0.87 fgh	11.00 ±0.34 cd

The anatomical features of leaf epidermis of genus *Plantago* were similar with peculiarities of the family *Plantaginaceae* studied by Metcalfe and Chalk (1950) and Filippa et al. (1999). The present findings correspond to the findings of Perveen and Qaiser (2004) and Bukhari (2009). A recent study revealed that *Plantago cordata* Lam. plant had irregular, mostly tetragonal, double walled upper and lower epidermal cells. Epidermis was amphistomatic while stomata were found as anisocytic (Paracytic). Trichomes were sessile or stalked stellate, three to five rayed and secondarily branched (Bahadar *et al.*, 2018).

CONCLUSION

From the present studies it is concluded that the leaf epidermis of seventeen species of *Plantago* (Ispaghul) are mostly amphistomatic. Both the upper and lower epidermis cells are double walled, irregular, elongated, oval and tetragonal to hexagonal in shape. Cell walls are smooth or wavy anticlinal. Stomata are of anomocytic or anisocytic type. Epidermal hairs are glandular, simple or multicellular. Stellate types of trichome are also recognized.

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CONFLICT OF INTEREST

Authors declare that there is no conflict of interest.

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