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Morphological, Anatomical and Palynological Investigation of *Plantago cordata* Lam. (*Plantaginaceae*) from Pakistan

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Abstract

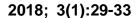
Plantago cordata Lam. (Plantaginaceae) collected from Hazara was investigated by its, morphology, leaf epidermis anatomy and pollen grain morphology. It is a threatened herb, having thickened elongated roots, heart shaped leaves with long petioles. Leaf epidermis anatomy and pollen grain morphology have been examined by the light microscope. Morphological and anatomical examination revealed that the test plant had double walled upper and lower epidermal cells which were irregular, mostly tetragonal. Epidermis was amphistomatic while stomata were found as anisocytic (Paracytic). Trichomes were sessile or stalked stellate, three to five rayed and secondarily branched. Pollen grains were free, medium-sized or small, radially symmetrical, tricolporate, apolar, spherical or prolate spheroidal in shape.

Keywords: Investigation, Epidermis, Stomata, Pollen grain, Shape.

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INTRODUCTION

Plantaginaceae is a cosmopolitan family of 3 related genera, and about 275 species are found in different habitats throughout the world (Cronquist, 1981; Heywood, 1993; Mabberley, 1997) or it is considered a monogeneric family with only Plantago as recognized by Rahn (1996). However, the relationships of the family, either with other families or between its genera, are still vague or need to be clarified (Heywood, 1993; Albach et al., 2004). In Pakistan it is represented by a single genus Plantago with 20 species (Kazmi, 1974). Genus Plantago L. was first introduced by Linnaeus in 1754. The genus Plantago is characterized by herbaceous habit; leaves in a basal rosette with prominent parallel veins; spicate or capitate inflorescences or wiry scapes; flowers 4-merous, polygamous, protogynous, bracteate, corolla membranous, stamens often exerted. Anther dorsifixed, Capsules membranous, circumscissile. Seeds of Plantago psyllium are used as a laxative. Seed production in natural populations of P. cordata was found to be the lowest in comparison to other species of Plantago (Primack 1979), and seedling mortality is reported as being high (NatureServe 2018). The large fleshy roots of this species are reputed to have great medicinal value (Michigan Flora Online, 2018). There are no reports on the leaf epidermal anatomy of genus Plantago found in Pakistan. Considerable work has been done on the pollen morphology of the family Plantaginaceae. The earliest report is that of Erdtman (1952). Kapp (1969), Serbanescu-Jitariu (1971), Solomon et al. (1973), Rao and Shukla (1975), Moore and Webb (1978), Kuprianova and Alysoshina (1978), Markgraf and Dantoni (1978) also studied the pollen of Plantaginaceae. Perveen and Qaiser (2004) studied pollen morphology of the 14 species of the genus Plantago by light and scanning electron microscope. Present study is based on Leaf epidermal anatomy and pollen morphology of Plantago cordata Lam. by light microscope for the first time from Pakistan. An attempt has been made to correlate the leaf epidermal anatomy and pollen characters with the taxonomy of the genus.

MATERIALS AND METHODS

Specimen Examined

Khyber Pakhtunkhwa, Hazara District: Bugnu Sharan, 6 - vii - 1979, Muhammad Zubair & Zahir Hussain, 115482/114 (ISL). Found primarily in central and northeastern U.S. and Canada, District of Colombia, Indiana, Virginia, New York and in Pakistan from Hazara District.

It is threatened perennial stem-less uncommon herb, having tuberous thick elongated king roots. Leaves are in basel rosettes, lamina heart shaped with scabrous petioles of 5-10.5 cm long, major veins appearing to diverge from the midvein above the base of the leaf. Inflorescence is

composed of compact dense cylindrical spike that is 3.5-5.5cm in length and scape 5-7cm in length. Flower is 4-merous, sessile, bracteates, bisexual, polygamous and protogynous. Bracts are 4.75mm long. Sepal's imbricate and 5mm long. Stamens are 4 epipetalous having dorsifixed anther. Fruit is a circumsessile capsule. Seeds are usually 4 in number, black in colour, trigonal in shape and 2.85mm long.

Methods

Plantago cordata Lam. specimen was obtained from Quaid-i-Azam University Herbarium, (ISL). Morphology was examined by naked eye and under Zeiss light microscope. For epidermal anatomical studies Shultz's method of maceration with modified technique was followed (Subrahmanyam, 1996). Leaves were boiled in 4ml of concentrated nitric acid to which 0.2g of potassium chloride and one ml of distilled water was added. The epidermis was peeled by hands under microscope. The permanent slides were photographed with Nikon Type-2 microscope at 40X. Pollen grains were dissected from herbarium specimens and placed on a clean microscope slides and added 2-3 drops of acetic acid to melt the resin and oil. The pollen grains were prepared for light (LM) microscopy by the standard methods described by Erdtman (1952). For light microscopy, the pollen grains were mounted with a drop of glycerin jelly stained with 1% safranine and observations were made with a Nikon Type-2 microscope, under (E40, 0.65) and oil immersion (E100, 1.25), using 10x eye piece. The measurements were based on 15-20 readings from each specimen.

RESULTS

Morphological Characters

P. cordata Lam., Tabl. Encycl. Metho. Bot. 1: 338.1791; Deam., Fl. Indiana. 867-871. 1940; Gray, & Fernald, Man. Bot. Handb. Fl. Pl. & Ferns, C & NE, US & Adj. Can. 1632. 1987; (Figure 1 a-d).

Synonyms

P. canadensis Hort., P. kentukensis Michx.

Anatomical Characters

The upper and lower epidermis revealed the following elements (Table 1). Epidermis was amphistomatic, number of stomata were different and their size were recorded same for upper and lower surfaces, and stomata cells occured on the both surfaces at the same level with neighboring cells surrounding by subsidiary cells which were anisocytic type (Paracytic). Stomata length was found as 30.1 ± 0.82 to $34.50\pm0.85\mu m$. The cell walls of both epidermises were double walled and smooth, cell wall thickening was not uniform. Cells were irregular, mostly tetragonal. Upper epidermis cells were found more elongated than lower epidermis cells. Trichomes were

sessile-subsessile or stalked stellate with three to five rayed having secondary branching (Figure 2 a-d).

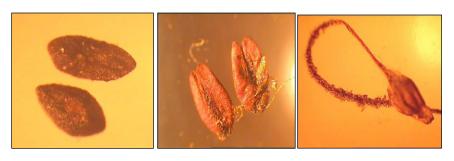
Palynological Characters

The pollens of *P. cordata* were free radially symmetric, 3- colporate, Polar axis (P) was between $93.0-122.0\mu m$, equatorial axis (E) $88.5-117.0\mu m$, and P/E ratio $1.1\mu m$

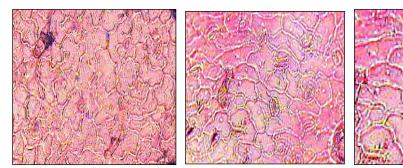
(Figure 3 a-b). Shape of pollen grain in equatorial view was spherical, spheroidal-subsphaeroidal, and in polar view was circular, semi-angular. The external wall thickness was observed between 3.1-3.5µm and exine surface was smooth. Internal wall thickness was found as 3.0-3.3µm. Colpi length was 15.0-39.4µm and width was 8.2-21.4µm.



a. General appearance of P. cordata Lam.



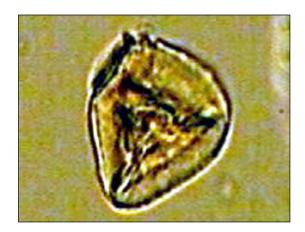
b. Seeds; c. Anthers; d. Carpel; Fig. 1 (a-d). General features of *P. cordata* Lam.



a. Upper epidermis;

Table 1. Epidermis	features upper and	d lower surface o)f <i>I</i>	P. cordata Lam.

Characters	Lower surface Mean±S.E	Upper surface Mean±S.E
Cell length (µm)	81.20 ± 1.96	118.60 ± 2.79
Cell width (µm)	50.00±1.09	75.20 ± 1.28
Cell wall thickness (µm)	7.40 ± 0.24	11.25 ± 0.27
Stomata length (µm)	30.1 ± 0.82	34.50±0.85
Stomata width (µm)	10.4 - 0.40	15.10 ± 0.44
Guard cell length (µm)	42.5 – 0.84	47.40 ± 0.87
Guard cell width (µm)	9.1 - 0.32	11.00 ± 0.34





a. Polar view, b. Equatorial view Fig. 3 (a-b). Pollen morphology of *P. cordata*

DISCUSSION

This is the first detailed morphological, anatomical and palynological report on P. cordata Lam. from Pakistan. Morphological characters such as characters of leaf and spikes, length of petiole and scape and pubescence are used to identify the species. P. cordata resembles to P. major but the thick horizontal rootstock and the early flowering period, distinguishes it sharply from the latter. Present results are generally in line with the description of Roland (1944) and Penskar (2010). The leaf epidermal anatomical features of this species are similar with pecularities of the family Plantaginaceae studied by Metcalfe and Chalk (1950). But the stellate trichomes are reported for the first time in genus Plantago. Stomata are of anisocytic type and the distribution of stomata on upper and lower epidermis is different. Pollens of P. cordata are free radially symmetric, 3- colporate; Shape of pollen grain in equatorial view is spherical, spheroidal and in polar view is circular, semiangular. The present results correspond to the findings of Perveen and Qaiser (2004) and Bukhari (2009). Pollen studies clearly indicate that the genus Plantago is a homogenous taxon in accordance with the morphology of the genus. Light microscopic observations

could not clearly indicate the exine sculpturing. It is recommended that scanning electron microscopy (SEM) of this species should be carried out for detailed study of external wall sculpturing.

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CONFLICT OF INTEREST

There is no conflict of interest.

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