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Macro- and micro-morphological characteristics of *Plantago* seeds and its implication for species identification

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ABSTRACT

Seed morphology of five species of *Plantago* (*Plantago psyllium, Plantago vate, Plantago lanceolata, Plantago major,* and *Plantago arenaria*) was examined under magnifying glass, compound microscope and scanning electron microscope. Macro- and micro-morphological characters, including seed shape, color, size, shape of cavity, surface ornamentation, and epidermal cell shape are presented. Marked difference among the species noted with respect of considered traits and a key for the identification of the investigated taxa based on seed characters is provided.

KEY WORDS: *Plantago,* Seed coat, scanning electron microscope

INTRODUCTION

The seed coat (testa) derived from the outer and inner integuments of the ovule, composed of a palisade layer, layers of crushed parenchyma cells, and a single layer of aleurone cells, is a multifunctional organ that plays vital roles in embryo nutrition during seed development, and in protection against various biotic and abiotic stresses afterward (Mohamed-Yasseen *et al.*, 1994; Weber *et al.*, 1996). Based on morphological and anatomical studies, (Sulaiman, 1995; Beeckman *et al.*, 2000) seed coat morphology is known to be an excellent character for taxonomic and evolutionary studies (Vaughan and Whitehouse, 1971; Algan and Büyükkartal, 2000; Zou *et al.*, 2001). At present, seed coat patterns have been used for various purposes: to solve classification problems, to establish evolutionary relationships, to elucidate the adaptive significance of the seed coat, and to serve as genetic markers for the identification of genotypes in segregating hybrid progenies (Lersten, 1979; Gopinathan and Babu, 1985; Rejdali, 1990). Thus, understanding its structure and development has been an important goal for workers and its worth increased for persons who deal with taxonomy and phylogeny of species.

Plantago, a monogeneric to family *Plantaginaceae* consist of 200 species worldwide (Rahn, 1996; Haddadian *et al.*, 2014). Seeds of these species are used for treatment of chronic constipation, dysentery, abdominal pain, piles, and rheumatism. Now, its potential to lowering cholesterol level also has also been documented (Husain *et al.*, 2008). Because of its immense importance in remedial field, increased market demand, use of allied species as adulterant, there is need for identifying characteristics of the seeds of *Plantago* species. Therefore, an effort has been made for similar objective.

MATERIALS AND METHODS

Seeds of five species of *Plantago*, namely, *Plantago psyllium* L., *Plantago ovata* Forssk., *Plantago lanceolata* L., *Plantago major* L. and *Plantago arenaria* Waldst. & Kit, were procured from various resources of India and their seed coat morphology was worked out in terms of shape, size, surface appearance, and pattern of cavity, etc., using magnifying glass, compound microscope and scanning electron microscope (SEM). For SEM sample preparation, first healthy dried seeds of the mentioned species were selected and placed on double-sided adhesive tape, followed by thin layer coating of gold using ion sputtering. For each

sample, photographs of micro-sculptures were taken using QUANTO 200, FEI 500 SEM. The anterior and posterior surface was examined at various magnifications, namely, ×40, ×60, ×150, and ×2500. The terminology of seed coat microcharacters in our present study was adopted from the descriptions used by Stearn (1966) and Barthlott (1981). For the study of similarity between species/ linkage between species, phenotypic data of species were entered into binary matrix as discrete variable (1) for the presence of phenotypic character and (0) for the absence of phenotypic character and this matrix were subjected to further analysis. Scores of individual phenotypic character were used to create data matrix. A dendrogram constructed based on Nei and Li's coefficient with unweighted pair group method and arithmetic average analysis (UPGMA) using Fig Tree Version 1.3.1 software.

RESULTS

In our experiment, variation in seed coat color was recorded. *P. ovata* which is more economically important among the considered species shared a unique seed coat color and also being the largest in terms of seed dimensions (2.89 ± 0.24 × 1.29 ± 0.03 mm). In all the species, the shape of cavity present on concave surface was variable.

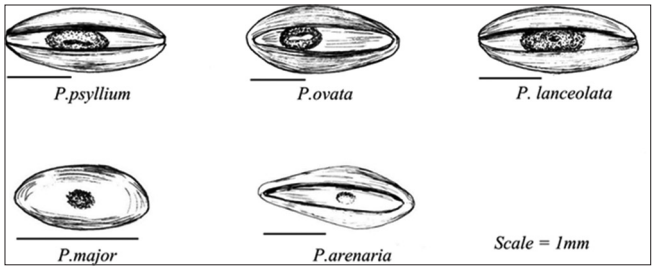

Figure 1: Line diagram of seeds of *Plantago species*

Bottle-shaped cavity with a flattened edge and naviculoid cavity with flattened edge was found in *P. arenaria* and *P. ovata,* respectively. Elliptic cavity with round edges was observed in *P. lanceolata* and *P. psyllium* while cavity in *P. major* was shallower and shared flattened edges. Regular scalariform to reticulate seed coat ornamentation with angular cells were found in all species except for *P. major,* that shared tuberculate type of ornamentation (Figure 1). The angular cell dimension was highest for *P. ovata* (62.67 ± 2.89 × 39.33 ± 0.88 μm) and lowest for *P. psyllium* (45.33 ± 1.52 × 27.67 ± 0.58 μm). Under high magnification (×2500) spindle shaped striations in *P. lanceolata* and rosette pattern of dark patches in seed coat *P. ovata* were observed and noted as unique feature (Table 1). UPGMA cluster analysis of all five species results a dendrogram that divided species into two main clusters: The first comprised *P. psyllum*, *P. lanceolata* and *P. major* and second comprised of *P. ovata* and *P. arenaria* (Table 2, Figures 2 and 3).

On the basis of observations for authentic identification of seeds of considered species a bracketed dichotomous key was constructed.

1a. Seed shape oval (2)
1b. Seed shape elliptical (3)

2a. Presence of rosette pattern of unique dark patches on seed coat the surface *P. ovata*
2b. Absence of unequal dark patches on seed coat *P. psyllium*

3a. Angular cell shape pattern forms the surface ornamentation (4)
3b. Non-angular cell shape pattern forms the surface ornamentation *P. major*

Table 1: Seed phenology of five *Plantago* species

Seed traits	Name of species				
	P. psyllium	*P. ovata*	*P. lanceolata*	*P. major*	*P. arenaria*
Seed color	Brown-to-dark brown	Whitish pinkish light brown	Copper brown	Black	Brownish black
Seed shape	Oval	Oval	Elliptical	Elliptical	Elliptical
Seed length (mm)	2.63±0.04	2.89±0.24	2.39±0.01	1.45±0.03	2.29±0.13
Seed breadth (mm)	1.24±0.03	1.29±0.03	1.06±0.11	0.74±0.04	0.97±0.08
Position of mucilage pore	Median	Sub-median	Median	Median	Sub-median
Mucilaginous behavior of seeds	Heavy	Heavy	Moderate	Mild	Mild
Cavity shape	Elliptic	Naviculoid	Elliptic	Elliptic	Bottle shaped
Nature of cavity	Broad	Narrow	Broad	Narrow	Narrow
Nature of cavity edges	Rounded	Flattened	Rounded	Flattened	Flattened
Overall seed coat pattern	Scalariform to reticulate	Reticulate to sclariform	Scalariform to reticulate	Tuberculate	Scalariform
Seed coat cell shape	Angular	Angular	Angular	Irregular	Rectangular
Length of seed coat cell (μm)	45.33±1.52	62.67±2.89	50.67±1.15	-	58.67±0.67
Breadth of seed coat cell (μm)	27.67±0.58	39.33±0.88	29.00±1.02	-	32.33±0.57
Striations/other unique ornamentations of seed coat	-	Black-colored resettle patches	Spindle shaped striations	-	-

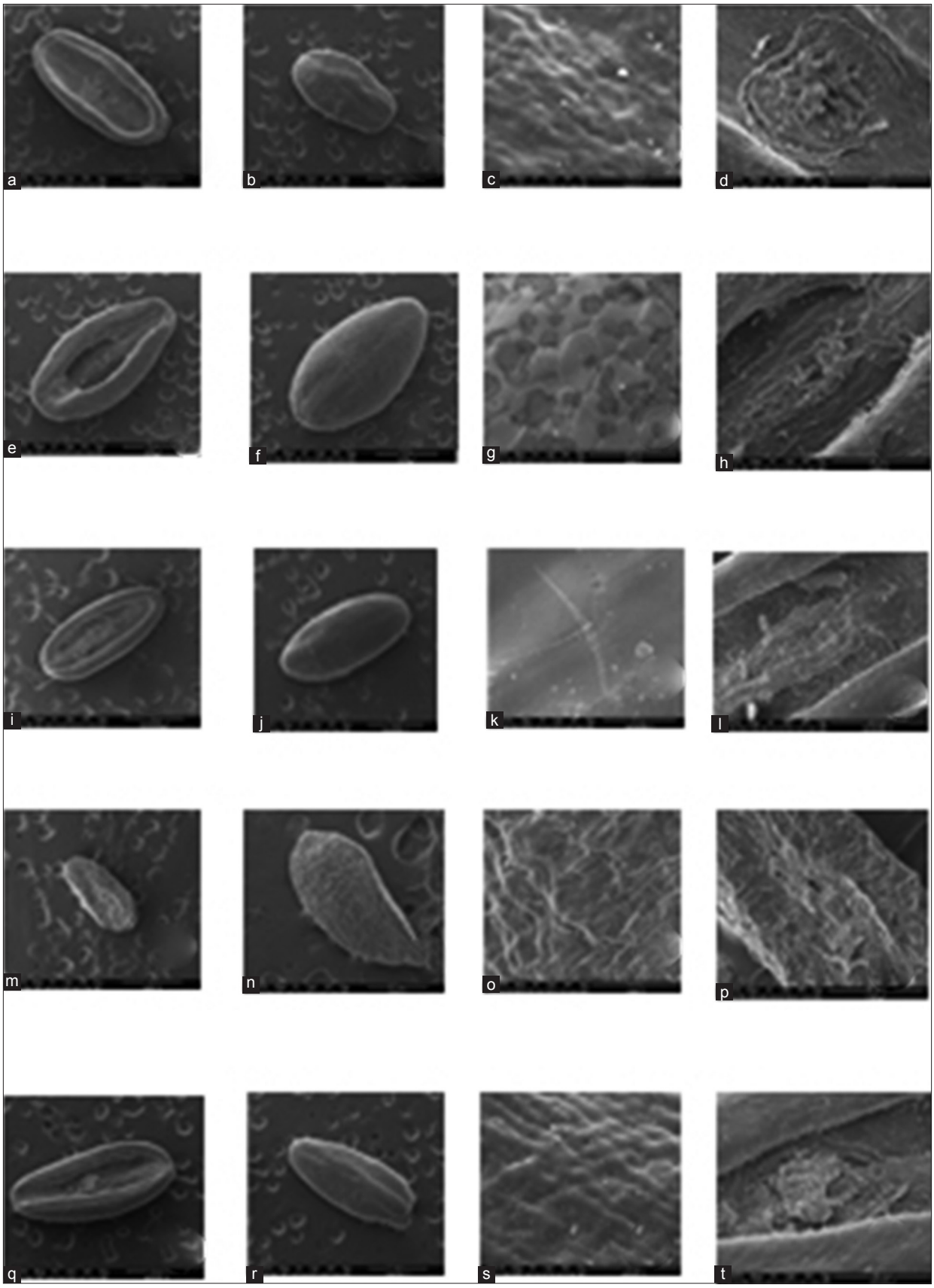

Figure 2: (a-d) *Plantago psyllium*; (e-h) *Plantago ovata;* (i-l) *Plantago lanceolata;* (m-p) *Plantago major*, (q-t) *Plantago arenaria*

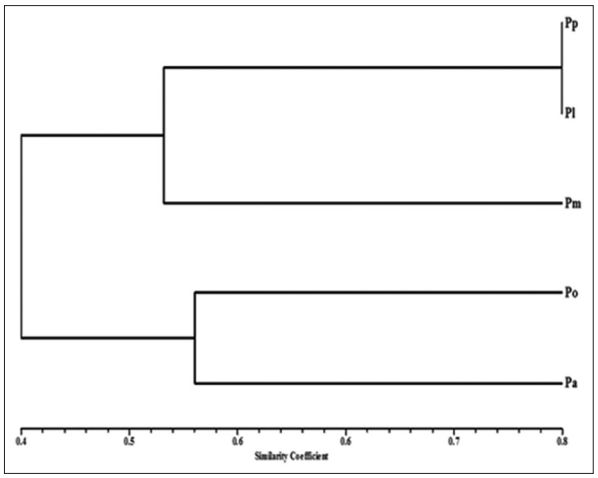

Figure 3: Dendrogram of five *Plantago* species based on similarity matrix (*Pp= P. psyllium, Pl= P. lanceolata, Pm= P. major, Po = P. ovata; Pa = P. arenaria)*

Table 2: Similarity matrix of five *Plantago* species based on seed phenology

Plant species	*P. psyllium*	*P. ovata*	*P. lanceolata*	*P. arenaria*
P. psyllium	1.00			
P. ovata	0.67	1.00		
P. lanceolata	0.15	0.00	1.00	
P. major	0.13	0.10	0.26	1.00
P. arenaria	0.00	0.26	0.53	0.44

4a. Narrow, bottle-shaped cavity with flattened edge
P. arenaria

4b. Broad, elliptic cavity with rounded edge
P. lanceolata

DISCUSSION

The study of fruit, seed, and leaf surface pattern using SEM can be useful taxonomic characteristics for different families and genera (Kumar *et al.*, 2012; Shavvon *et al.*, 2012; Akcin *et al.,* 2013). Shalabi and Abou-EL-Enain (2013) stated that *Plantago* is a problematic genus; most of the species are closely similar so discovering stable aspects of variation among them is not easy. According to Liu *et al.* (1992), seed morphology and seed coat structures exhibit specific characters by which some confused species can be clarified. For identification of plantago species characters such as seed surface pattern, seed color, and seed size were considered by earlier workers (Rezk, 1987; Kamel, 2003; Liu *et al.*, 1992; Shehata and Lautfy, 2006).

The reticulate to scalariform surface pattern is quite common among the considered species but in *P. major* tuberculate non-angular surface pattern which could be defining character for this species. The seed surface of *P. ovata* and *P. lanceolata* showed unique patches and striations, respectively, possible key characters for respective species. Earlier workers performed seed coat study in *Plantago* species but as per our concern, none of them reported the above-mentioned key characters for *P. ovata* and *P. lanceolata*. Our observations based on concave surface cavity were in consonance with the finding of Youngken (1935) in the seeds of *P. psyllium* and *P. arenaria.* Shehata and Loutfy (2006) reported that *P. arenaria* and *P. ovata* shares good relationship in terms of seed coat characteristics, contrary to them in our findings, variations were observed in seed coat appearance at micro-morphological level. Results of cluster analysis based on similarity matrix of seed phenology (Table 2, Figure 3) were showed agreement the finding of earlier workers as *P. ovata* and *P. arenaria*, *P. lanceolata,* and *P. major* belongs to same cluster and first to species have highest affinity with each other (Shehata and Loutfy, 2006).

Although the present study is not sufficient for seed identification on the basis of macro-morphological characters but can be helpful in species identification on the basis of micro-morphological seed coat characters. In conclusion, experimental findings again revalidate the scope of seed micro-morphological studies for the development of species specific marker keys and will be helpful in identification of economically important seeds at market and laboratory level.

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