

## MICROMORPHOLOGICAL STUDIES OF SEVEN SPECIES OF THE GENUS *MEDICAGO* L., (FABACEAE) FROM PAKISTAN

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### Abstract

Using Scanning Electron Microscope a micromorphological study of leaf and seed coat surfaces of all the 7 species in the genus *Medicago* L., from Pakistan was carried out. The seed coat sculpturing revealed 3 main surface patterns viz., verrucate, papillate and rumeniate-rugose. Leaf epidermis was found to be covered with waxy material in all taxa, exhibiting verrucate to colliculate pattern with irregular arrangement of cells. Leaf trichomes are unicellular with granulate surfaces. A key based on micromorphological characters observed has been proposed to identify the species.

### Introduction

The genus *Medicago* L., (Fabaceae) comprises of some 50 species found in temperate and Mediterranean region (Hutchinson, 1964). Of these atleast 7 species are reported from Pakistan (Ali, 1977). Lesins & Lesins (1979) have produced an excellent monograph on taxonomic studies of the genus *Medicago* giving details of the general morphology. Gutterman & Heydecker (1973), Trivedi *et al.*, (1987 a,b; 1979, 1980) have studied leguminous seeds using seed coat structure as a secondary taxonomic tool but the leaf structure was not studied. In *M. sativa* and *M. denticulata* from India, the seed coat pattern has been described as tuberculate (Trivedi & Polhill, 1982).

There are differences of opinion regarding the partition of the genus into major taxa. Lesins & Lesins (1979), Small *et al.*, (1981) suggested that seed morphology itself can be used as a useful taxonomic tool in discriminating species. In the present study Scanning Electron Microscope was used for micromorphological characters of the seed coat and leaf surface as the taxonomic tool to identify species of the genus *Medicago* reported from Pakistan.

### Materials and Methods

Mature seeds and leaf samples of 7 species of the genus *Medicago* were taken from the herbarium specimens collected from different parts of Pakistan. The voucher specimens of the taxa studied are deposited in the herbarium of the Karachi University (KUH). Three samples of seeds and leaf epidermis in each case were

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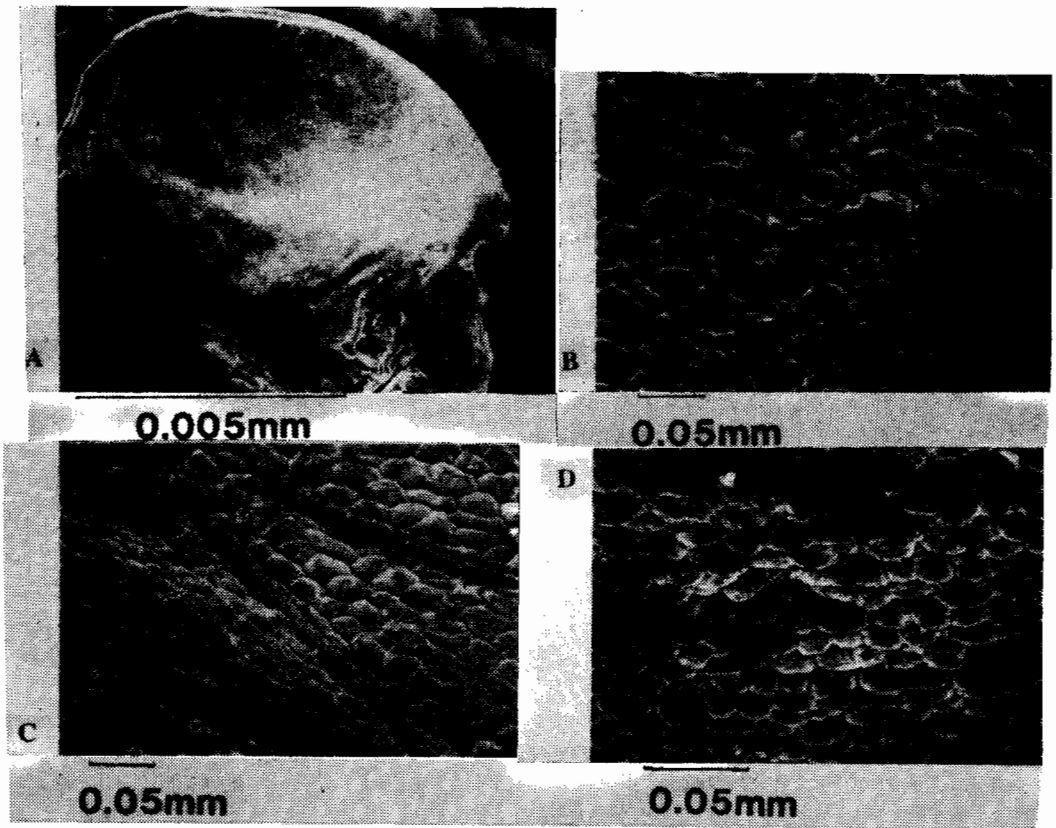


Fig.1. Scanning Electron micrographs of seed and leaf surfaces of *Medicago falcata*. A & B, seed surface; C, adaxial leaf surface; D, abaxial leaf surface.

examined by SEM. Specimens were also examined using the Stereozoom microscope. Seed samples were mounted on metal stubs with double sided cellophane tape, coated with ca. 30 nm of gold in a coating chamber and examined with a Jeol T20 Electron Microscope.

## Results

Seeds are estrophiolate and generally kidney shaped whereas oval, oblong and orbicular seed shapes were also observed. The seed surface appeared dull and rough under Light Microscope in *M. falcata*, *M. minima* and *M. orbicularis* and  $\pm$  glossy in *M. lupulina*, *M. laciniata*, *M. polymorpha* and *M. sativa*. Seed colour ranged from dark brown to light brown/golden. SEM studies of the seed coat showed verrucate, papillate to rugose-ruminate pattern. Leaf epidermal surface revealed verrucate to colliculate pattern under SEM. The arrangement of the epidermal cells was irregular and waxy material was observed in all the species. Trichomes were unicellular, cylindrical, sometimes flattened, densely to sparsely distributed. Anisocytic type of stomata were present on adaxial and abaxial leaf surfaces.

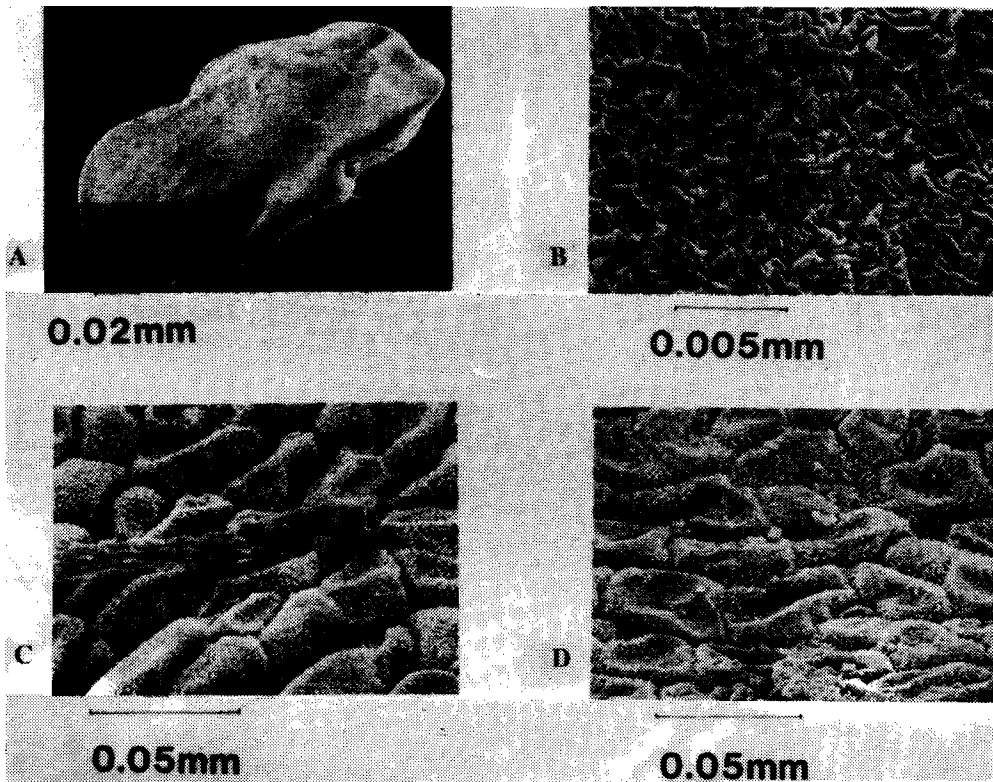


Fig.2. Scanning Electron micrographs of seed and leaf surfaces of *Medicago laciniata* var. *laciniata*. A & B, seed surface; C, adaxial leaf surface; D, abaxial leaf surface.

***Medicago falcata* L:** Seeds oblong, light/dark brown rough surface showing faint network patterns (Fig.1A). At high magnification the radial walls of each epidermal cells are raised into ridges around the central parts which are deeply sunken forming holes or few raised into shallow domes, exhibiting a papillate-reticulate pattern (Fig.1B).

Adaxial epidermal surface of leaves show colliculate pattern, cells are round to elongate and usually arranged in flat rows, some depressed in the centre and few raised conically. Trichomes are appressed, long, cylindrical and unicellular; basal part broad, branched, surface of the trichomes are sparsely granulated (Fig.1C). Trichomes are not observed on abaxial leaf surface. The epidermal cells are arranged in rows and are mostly round with shallow domes forming colliculate pattern (Fig.1D).

***Medicago laciniata* (Linn.) Mill. var. *laciniata*:** Seeds kidney shaped slightly flattened at convex edges, light to dark brown or golden brown in colour (Fig.2A). Seed sculpturing pattern is rugose-ruminant, the irregular elevations or ridges made up of wrinkles (Fig.2B).

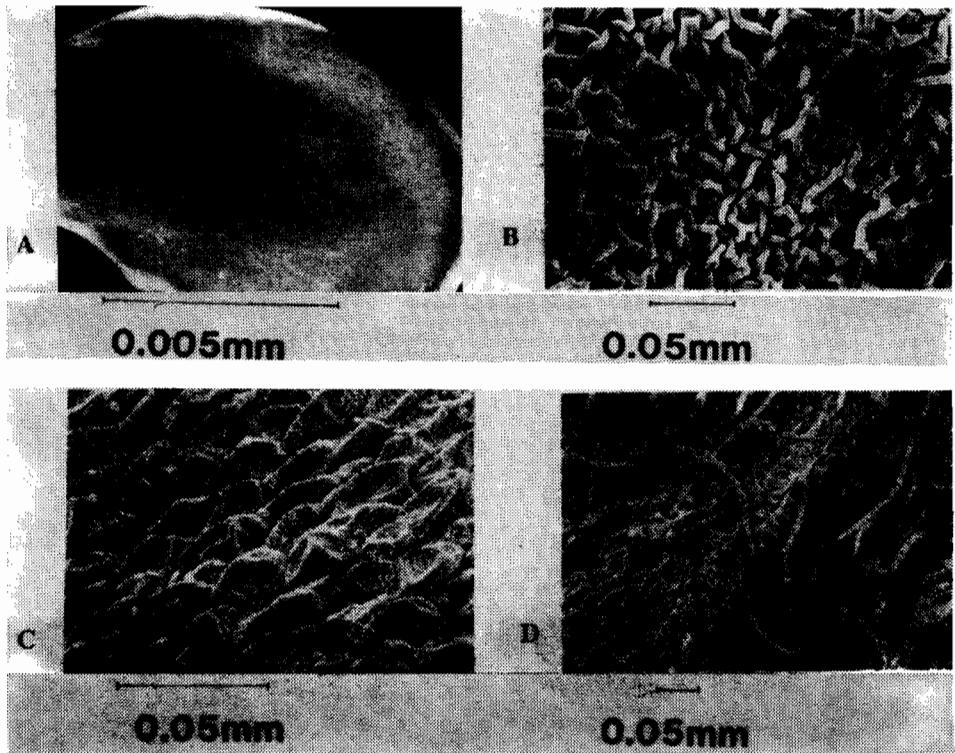


Fig.3. Scanning Electron micrographs of seed and leaf surfaces of *Medicago lupulina*. A & B, seed surface; C, adaxial leaf surface; D, abaxial leaf surface.

Adaxial and abaxial leaf surface is made up of irregularly arranged cells of different size and shape. Cell surface is covered with a waxy material of leathery appearance. Trichomes on the adaxial leaf surface are sparsely distributed, elongated unicellular and somewhat flattened covered with granules (Figs.2C & 2D). Trichomes are absent on the abaxial leaf surface.

***Medicago lupulina* Linn:** Seeds oval in shape and light brown in colour. Surface sculpturing is of somewhat ruminant type. The irregular prominence of ridges around the central part of the cells interconnect and form a reticulate pattern (Figs.3A & 3B).

The adaxial leaf surface is without trichomes and leaf epidermal cells are elongated, arranged in rows, covered with waxy substance giving crustaceous appearance. The abaxial leaf epidermal surface show crustaceous cells with thin walls. Trichomes are long, densely distributed, multiseptate and flattened, covered with waxy substance (Figs.3C & 3D).

***Medicago minima* (Linn.) Grubb:** Seeds generally kidney shaped, laterally compressed, dark brown in colour. Seed sculpture under SEM exhibits rugose-ruminant pattern (Figs.4A & 4B). The radial cell walls appear compact and raised. Central portions deeply sunken and appear as deep furrows.

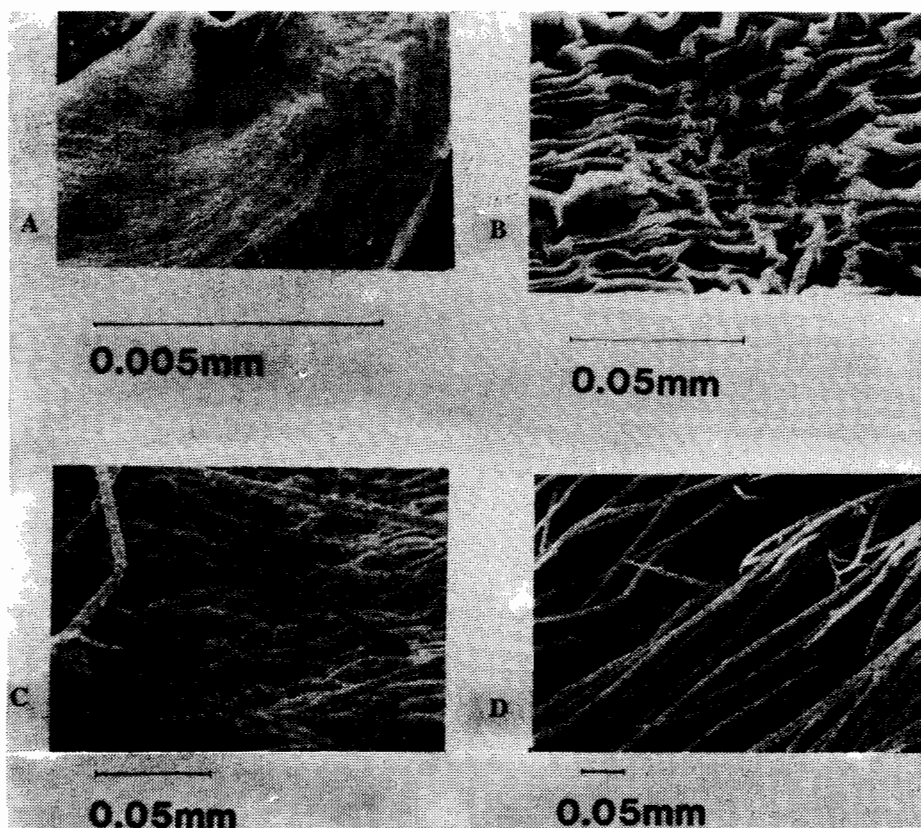


Fig.4. Scanning Electron micrographs of seed and leaf surfaces of *Medicago minima*. A & B, seed surface; C, adaxial leaf surface; D, abaxial leaf surface.

The epidermal cells of the adaxial leaf surface are spherical, irregularly arranged, forming colliculate pattern. Trichomes long, unicellular, cylindrical and granulated; the basal part is prominently bulbous surrounded by 5-7 epidermal cells forming a rim like structure (Fig.4C). The abaxial leaf surface is densely crowded with long, cylindrical, unicellular and densely granulated trichomes (Fig.4D).

***Medicago orbicularis* (Linn.) Bart.:** Seeds orbicular in shape, brown/dark brown in colour. Surface exhibits a verrucate type of sculpturing giving a warty appearance, warts are short and broad (Figs. 5A & 5B).

The adaxial leaf epidermal layer is covered with waxy material exhibiting a closely packed cell pattern. Cells are  $\pm$  spherical or of irregular shapes and slightly raised into shallow domes forming a colliculate pattern (Fig.5C). The abaxial leaf epidermal surface exhibits the same pattern as adaxial leaf surface except the cells are of irregular shape. Trichomes are sparsely distributed, elongated, appressed, cylindrical and covered with sparsely distributed papillae. The basal part of the trichomes is surrounded by the group of 5 or 6 cells (Fig.5D).

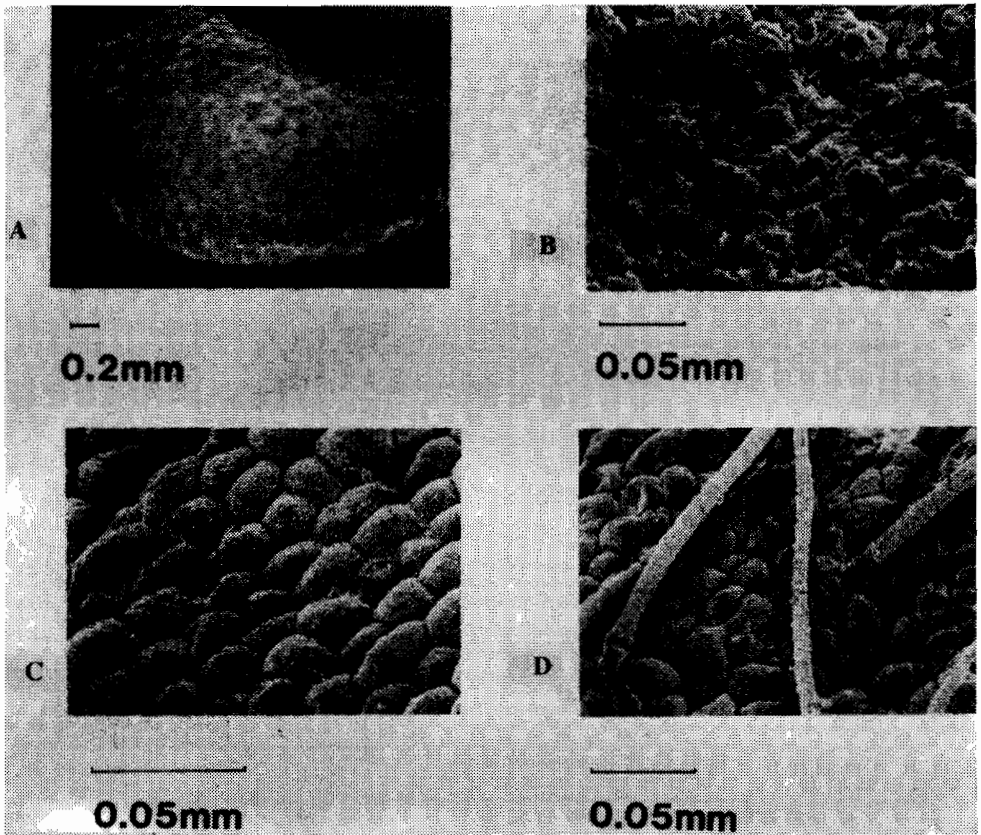


Fig.5. Scanning Electron micrographs of seed and leaf surfaces of *Medicago orbicularis*. A & B, seed surface; C, adaxial leaf surface; D, abaxial leaf surface.

***Medicago polymorpha* Linn.:** Seeds are kidney shaped, sub-glossy light/dark brown in colour. Seed sculpturing under SEM shows papillate pattern. The papillae are loosely arranged in somewhat concentric rows interconnecting with each other by fibrous ridges radiating from adjacent cells (Figs.6A & 6B).

The adaxial and abaxial leaf surfaces show sparse distribution of long, cylindrical, unicellular and densely granulated trichomes. The epidermal cells on adaxial leaf surface are thin walled, flat, arranged in rows (Fig.6C), whereas on abaxial leaf surface the cells are thick walled and exhibit  $\pm$  verrucate pattern. Cell surface showed waxy appearance (Fig.6D).

***Medicago sativa* Linn.:** Seeds are kidney shaped and brown/dark brown in colour. Seed coat pattern is  $\pm$  papillate. The raised cells exhibit a closely packed pattern. The radial walls of each cell elevate into a ridge interconnecting with each other forming a loose network (Figs.7A & 7B). The adaxial and abaxial leaf surfaces show verrucate pattern and are densely covered with waxy material giving spongy appearance. Trichomes are long, cylindrical, unicellular, granulate and sparsely distributed on the abaxial surface (Figs.7C & 7D).

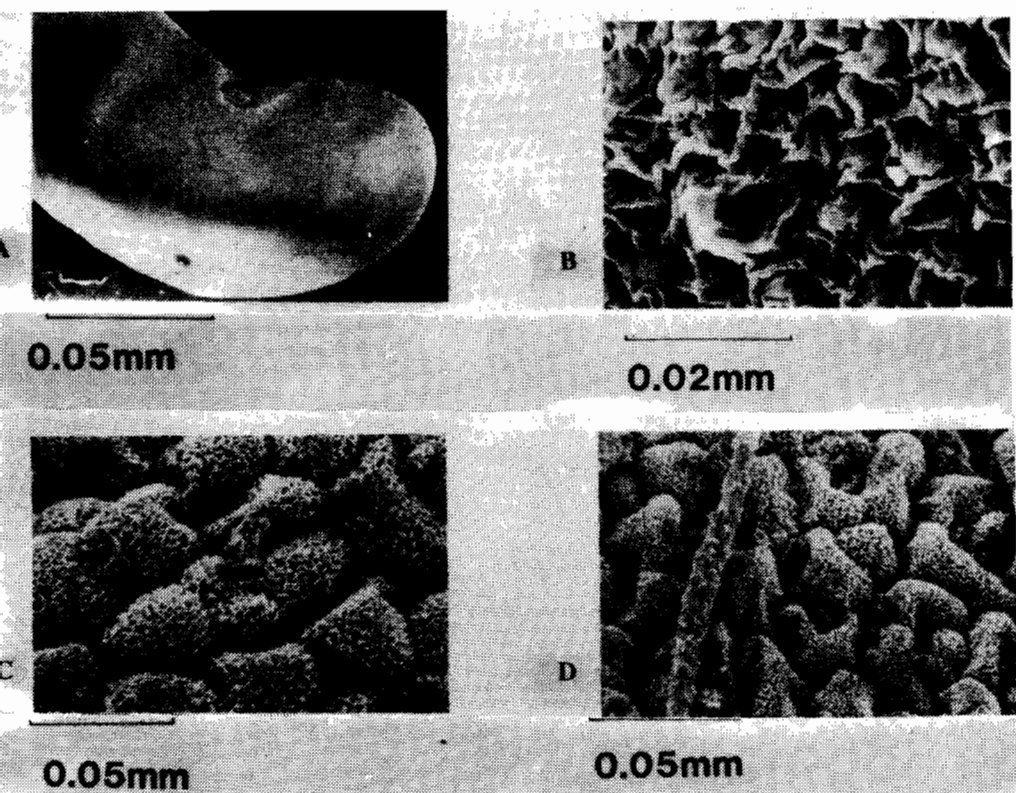


Fig.6. Scanning Electron micrographs of seed and leaf surfaces of *Medicago polymorpha*. A & B, seed surface; C, adaxial leaf surface; D, abaxial leaf surface.

## Discussion

Micromorphological investigation of seed surface and leaf epidermis showed a range of surface patterns in the taxa of *Medicago* L. Three main seed surface pattern (A) Verrucate, (B) Papillate and (C) Rugose-ruminate are seen. *M. orbicularis* show characteristic verrucate seed surface pattern, not observed in any other species. *M. sativa* and *M. polymorpha* show papillate seed surface pattern, whereas the seed coat pattern in *M. laciniata*, *M. lupulina* and *M. minima* is rugose-ruminate. In *M. falcata* the seed sculpturing resembles  $\pm$  papillate type where cells are raised into very shallow domes, and most of them are collapsed forming holes surrounded by elevated tangential walls. Trivedi & Bagchi (1982) found tuberculate seed coat pattern in *M. sativa* which in fact is papillate. The seed samples of *M. sativa* collected from different localities in Pakistan exhibit smooth to papillate and sometimes rugose surface pattern.

The leaf epidermal surfaces of *M. orbicularis*, *M. falcata* and *M. minima* show a colliculate pattern, whereas *M. lupulina*, *M. laciniata*, *M. polymorpha* and *M. sativa* exhibit different type of leaf epidermal pattern that varies from irregular cell

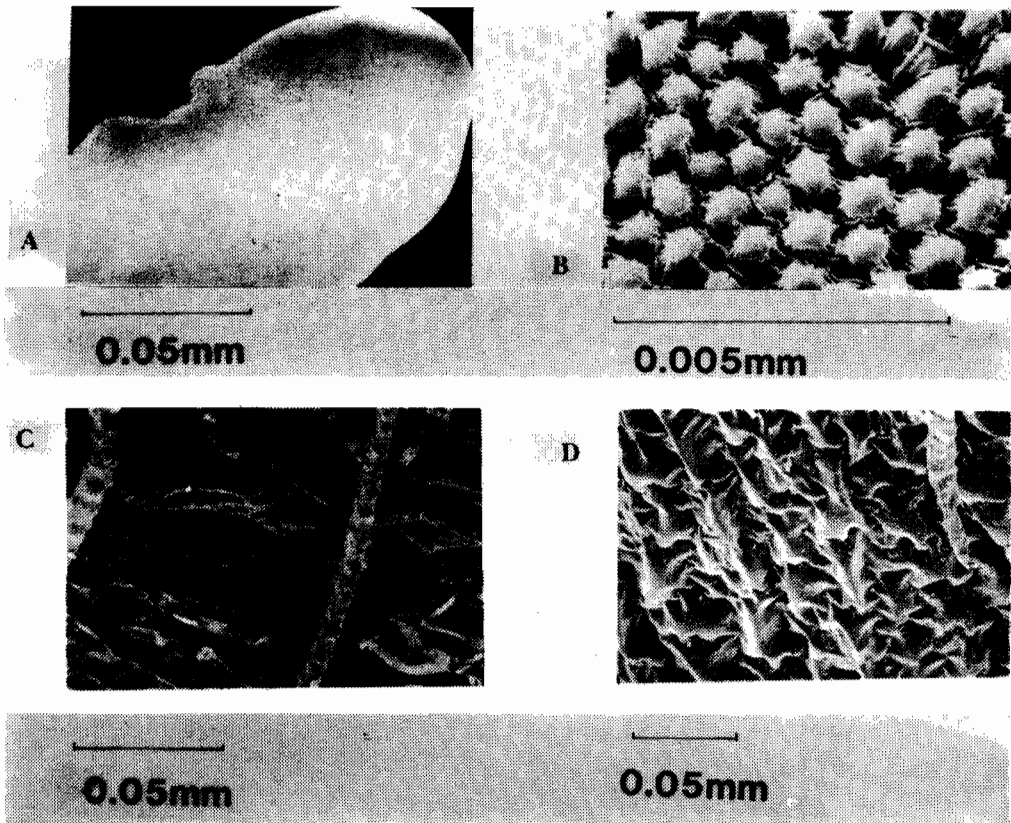


Fig.7. Scanning Electron micrographs of seed and leaf surfaces of *Medicago sativa*. A & B, seed surface; C, adaxial leaf surface; D, abaxial leaf surface.

arrangement to verrucate pattern. In *M. orbicularis*, leaf epidermal layer is covered with waxy material and the cells are of irregular shape and arrangement, trichomes are only found on abaxial surfaces. Leaf surface of *M. falcata* does not show any waxy material and the trichomes are present on adaxial surface only, although this species exhibit a papillate pattern on its seed coat surface, but it is quite distinct as the cells are depressed in the centres forming holes. In *M. minima* densely distributed trichomes on both adaxial and abaxial leaf surfaces are seen. Leaf epidermal surfaces of *M. polymorpha* and *M. sativa* show verrucate pattern. In *M. polymorpha* the abaxial leaf surface only show regular verrucate pattern, whereas the adaxial leaf surface exhibit depressed and thin walled cells. In *M. sativa* verrucate seed coat pattern can be seen, more-over the adaxial epidermal cell surface is covered with waxy material giving spongy appearance which is characteristic of this species. Sparsely distributed trichomes are present on both adaxial and abaxial leaf epidermal surfaces of these two species. *M. lupulina* and *M. laciniata* show irregular cells arrangement giving + colliculate appearance, most of the cells are flat or have depressed centres, usually covered with waxy material.



Present study followed the infrageneric classification of the genus proposed by Lesins & Lesins (1979). According to this classification the genus has been divided into 4 subgenera where *M. orbicularis* is placed in subgenus *orbicularia* (section *Orbicularis*) in having characteristic orbicular seed with verrucate surface. This pattern is not observed in other species of the genus. The placement of *M. lupulina* in subgenus *Lupularia* (section *Suffruticosae*) could be confirmed due to oval shaped seed with ruminant surface and multiseptate flattened trichomes.

In the subgenus *Medicago* (section *Falcago*), *M. sativa* and *M. falcata* could be distinguished as *M. sativa* which shows verrucate pattern in both abaxial and adaxial surface of leaf while *M. falcata* shows colliculate pattern. The papillated seed surface and the sparsely granulated unicellular trichomes are common characters in both the species.

In the subgenus (section *Leptospirae*) *M. laciniata*, *M. minima* and *M. polymorpha* have been considered to be closely related (Lesins & Lesins, 1979). In *M. laciniata* trichomes are absent in abaxial surface of the leaf while in *M. minima* and *M. polymorpha* show trichomes on both surface. Seeds of *M. minima* are laterally compressed. In *M. polymorpha* seeds show the papillate type of surface pattern. The present investigation also correlates the chemotaxonomic studies of the genus (Aziz *et al.*, 1993).

#### Key to the species of *Medicago* L

Key to the species of *Medicago* L. based on micromorphological characters (seed and leaf surface).

- 1 Seed orbicular; seed coat sculpturing verrucate ----- *M. orbicularis*
- Seed kidney shaped to oval, sometimes oblong; seed coat sculpturing rugose-ruminant, reticulate to papillate ----- 2
- 2 Trichomes present on both surfaces of leaf ----- 3
- Trichomes present on one surface of leaf ----- 4
- 3 Trichomes abundant, sparsely granulated; epidermal cells are thickwalled on adaxial leaf surface and thin walled on abaxial leaf surface ----- *M. minima*
- Trichomes very few, densely granulated; epidermal cells are thin walled on adaxial leaf surface and thick walled on abaxial leaf surface ----- *M. polymorpha*
- 4 Trichomes present on abaxial leaf surface ----- 5
- Trichomes present on adaxial leaf surface ----- 6
5. Seeds kidney shaped; epidermal leaf surface show verrucate pattern, cells giving spongy appearance ----- *M. sativa*
- Seeds oval; epidermal leaf surface show irregular cells arrangement; cells giving crustaceous appearance ----- *M. lupulina*
- 6 Seeds oblong; seed coat surface sculpturing is papillate reticulate Trichomes cylindrical appressed and with broad basal part ----- *M. falcata*
- Seeds kidney shaped; seed coat surface sculpturing is rugoseruminant. Trichomes somewhat flattened, without broad basal part ----- *M. laciniata*

## Appendix 1. List of specimens studied.

Species	Locality	Collector	No.
<i>Medicago sativa</i> L.	c. 40-45 miles from Juglote on way to Astore	S. Omer & M. Qaiser	2303
<i>M. sativa</i> L.	Chitral Dist. c.45 Km from Chitral on way to Booni	A. Ghafoor & S. Omer	2985
<i>M. sativa</i> L.	c. 10 miles from Nasirabad on way to Larkana	Nazim, S. Abedin & M. Qaiser	122
<i>M. sativa</i> L.	Near Bangla Hussain Shah, between Shahpur and Sahiwal	A. Ghafoor & Tahir Ali	3842
<i>M. sativa</i> L.	Chautair, Loralai Dist.	Tahir Ali & Tufail Ahmed	1271
<i>M. sativa</i> L.	c. 8 miles from Mahmori on way to Kaghan	M. Qaiser & A. Ghafoor	5236
<i>M. sativa</i> L.	Bot Garden P.C.S.I.R., Karachi.	S. Abedin	5756
<i>M. falcata</i> L.	Gilgit, Rama Rest House	S. Omer & M. Qaiser	2341
<i>M. lupulina</i> L.	Murree	S. Abedin	2868
<i>M. lupulina</i> L.	c.30 Km to Ziarat, Kach road	S. Khatoon, E. Ahmed & Mola Baksh	548
<i>M. lupulina</i> L.	Shahezan Hills, Parachinar	S. Nazimuddin & S. Abedin	112
<i>M. lupulina</i> L.	Utror, Swat	S. Abedin	8442
<i>M. orbicularis</i> (L.) Bart.	Muzaffarabad Dist. Neelam valley, between Shadi and Nari Noor	M. Qaiser & R.Y. Hashmi	8086
<i>M. laciniata</i> (L.) Mill. var. <i>laciniata</i>	Parachinar	M. Qaiser & S. Abedin	5904
<i>M. minima</i> (L.) Grufb	c.2 miles from Mingora towards Medayan	S.I. Ali	38798
<i>M. minima</i> (L.) Grufb	National Agricultural Research Centre, Chak Shahzad, Islamabad.	R.Y. Hashmi	155
<i>M. polymorpha</i> L.	Aainabad c. 65 Km from Sost, on way to Aliabad	S.I. Ali <i>et al.</i> ,	3451
<i>M. polymorpha</i> L.	c.1 mile before Dauletpur Hyderabad rd.	S.A. Farooqi	509
<i>M. polymorpha</i> L.	Teejahban 12 miles from Hushab on way to Turbat	S. Abedin, A. Hussain	6019

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