

# SCANNING ELECTRON MICROSCOPIC STUDIES ON SOME MEDICINALLY IMPORTANT SEEDS OF PAPILIONACEAE

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## Abstract

Macroscopic and scanning electron microscopic studies on the 15 species of medicinally useful seeds of Papilionaceae have been done. The seed and seed coat characters are very stable for identification, since the basic characters do not change under different geographical and climatic conditions. In the seed and seed coat characters, the family Papilionaceae is very diverse and the structures which have been observed by SEM have important diagnostic value for their identification.

## Introduction

The scanning electron microscope (SEM) has of late come to occupy an important place amongst the latest equipments in the study of vegetable drugs, because it highlights the morphological characters of the surface of different plant organs. Even details inside deep crevices or holes are clearly made out by SEM. Such data are extremely useful in identifying crude drugs.

Considerable information on the anatomy and morphology of these seeds is already available (Ambegaokar, 1976; Chowdhury & Buth, 1970; Corner, 1951, 1976), but the detailed information about the cuticular structure of these seeds, which has bearing on the identification of crude seed drugs, is lacking. Recently Farooqui *et al.* (1982) while studying three species of *Abrus* by LM and SEM did not mention the portion of seeds which are ineffectual for taxonomic diagnosis because it is only the central portion of the seed coat which situates below the hilum showing standard and constant seed coat structure; the other part of the seed coat shows great variation. Trivedi *et al.* (1982) have studied some important medicinal seeds of Caesalpiniaceae and Mimosaceae with the help of SEM and reported that in these families the seed coat ornamentations are very characteristic and useful in the identification of seeds.

In this paper seed surfaces of *Crotalaria juncea* Linn., *Trigonella foenum-graecum* Linn., *Melilotus indica* All., *Sesbania aegyptiaca* Pers., *S. aculeata* Pers., *Lens culinaris* Medik., *Abrus precatorius* Linn., *Cicer arietinum* Linn., *Lathyrus sativus* Linn., *Pisum sativum* Linn., *Vigna sinensis* (L.) Saviez Hassk., *Dolichos lablab* Linn., *Cajanus cajan* (L.) Millsp., *Dalbergia lanceolaria* Linn., and *Sophora tomentosa* Linn. from Papilionaceae have been investigated with the help of SEM.

## Material and Methods

Only mature and dry seeds are used for this investigation. Whole mounts of small seeds were made. In case of large seeds, sides of the seed coat below the hilum were carefully detached and mounted on circular standard aluminium stubs by double sided,



adhesive aluminium tape. Later, a thin layer of gold palladium was coated in a Polaron sputter coating unit, for the sake of uniformity. The part present below the hilum of each seed was scanned by Cambridge stereoscan-180 at constant potential difference of 20 K. V. and photographs were taken by normal 120 ASA films.

### Observations and results

*Crotalarira juncea* Linn.—Seeds are yellowish black with maximum axial length and breadth of  $6.0 \times 4.5$  mm. Radicular lobe is beak-like and pointed. Hilum is situated in the notch and oblong in shape. Lens is slightly raised and light yellowish in colour. Seed surface is smooth and shiny (Pl. 1, fig. 1) Seed coat surface is tuberculate (Pl. 1, fig. 16). Tubercles are blunt, dome-shaped and totally or partially covered by waxy deposition. The surface below the tubercles has fine reticulations.

*Trigonella foenum-graecum* Linn.—Seeds are rectangular and cylindrical in shape with hilar notch on one side of the seed. They are clayish yellow to grey in colour. The maximum axial length and breadth of the seeds is  $4.0 \times 3.0$  mm. Lens is like a small dark brown spot. Hilum is very small and situated in the notch. Aril is not so prominent (Pl. 1, fig. 2). The seed coat surface has tuberculate ornamentation (Pl. 1, fig. 17). Tubercles are blunt with smooth deposition on the surface. They are joined with each other by elongated processes which are drawn out in all directions; some of these extend from one tubercle and join adjacent ones.

*Melilotus indica* All.—The seeds are very small and yellowish or greenish yellow in colour. These are somewhat oval in shape with the maximum axial length and breadth of about  $2.0 \times 1.5$  mm. Hilum is very small and circular and about 0.2 mm in diameter. Aril is not prominent (Pl. 1, fig. 3). Seed coat surface is tuberculate and the tubercles are irregular and indistinct (Pl. 2, fig. 18). Heavy deposit of some substance(s) on its surface makes the tubercles indistinct and surface uneven.

*Sesbania aegyptiaca* Pers.—Seeds are small and cylindrical. The maximum axial length and breadth is about  $4.0 \times 2.0$  mm. They are greenish yellow in colour with an orange patch towards the hilum. Small black spots are also present all over the surface. Hilum is elliptical and very minute measuring about  $1.5 \times 0.5$  mm; aril is not prominent (Pl. 1, fig. 4). The seed coat has rugose pattern on the surface (Pl. 2, fig. 19), but the rugae are less prominent and have a deposition which makes them rough.

*Sesbania aculeata* Pers.—Seeds are small and black, flat and dumb-bell-shaped. The maximum axial length and breadth of the seed is about  $4.0 \times 2.0$  mm. Hilum is small, circular, almost like a spot, having a diameter of about 0.5 mm. Aril and rim arils are not prominent (Pl. 1, fig. 5). Seed coat shows rugose pattern on the surface (Pl. 2, fig. 20). The rugae are distinct, smooth and irregular in shape. Surface is without any deposition.

*Lens culinaris* Medik.—Seeds are round and the curvature of both the surfaces are convex. They are light brown or reddish brown in colour with small black spots all over the seed surface. The diameter of the seed is about 4-5 mm. Hilum is elongated and aril or rim aril is absent (Pl. 1, fig. 6). Hilum is situated almost at the level of the seed surface. Surface of the seed is slightly rough.

Seed coat has tubercles all over the surface (Pl. 2, fig. 21) which are smooth, octopus shaped, with a blunt apex and are arranged in regular rows. The lower surface is rough, consisting of irregular ridges and furrows, sometimes the ridges form reticulations. All tubercles have an equal amount of wax deposited on their outer surface,



*Abrus precatorius* Linn.—About 3/4th of the seed is scarlet red with 1/4th portion being black. They are oval to round in shape. The maximum length and breadth is about 7.0 × 6.0 mm. Seed surface is very smooth and shiny. Hilum is oval and has no prominent rim aril (Pl. 1, fig. 7). Seed coat is very smooth and it is interesting to note that at the boundary of red and black colours, wax is deposited (Pl. 2, fig. 22).

*Cicer arietinum* Linn.—Seeds are pointed towards the hilar side and yellowish brown in colour. The maximum axial length and breadth of the seed is about 8.0-6.0 mm. Hilum is oval and situated in a sunken pouch below the level of seed surface. Micropyle is prominent. Aril and rim arils are not prominent. The surface of the seed has prominent blisters and the general seed surface is rough (Pl. 1, fig. 8).

Seed coat ornamentation is of undulate type (Pl. 2, fig. 23). The undulations have crests and troughs. These troughs and crests show subsidiary minor projections and depressions. The cuticle also shows irregular striations on its surface. No deposit of wax is seen on the spermoderm surface.

*Lathyrus sativus* Linn.—Seeds are angular in shape and grey in colour with black spots all over the surface. The maximum axial length and breadth is about 5.0 × 4.8 mm seed surface is nearly smooth. Hilum is elongated and situated at the level of seed surface. Aril and rim arils are not prominent (Pl. 1, fig. 9).

Seed coat is tuberculate. Tubercles (Pl. 3, fig. 24) are irregular in shape and somewhat widely spaced, thus leaving larger depressions between them. The spines of these tubercles are blunt and have wax deposition. Elongated processes of wax on the sides of tubercles are thrown in all directions, though they never join with the processes of the adjacent tubercles.

*Pisum sativum* Linn.—Seeds are cream or light yellow in colour. They are almost rounded and varies from 7-8 mm in diameter. Hilum is oval and situated at the level of seed surface. Aril and rim arils are not prominent. Seed surface is slightly blistered (Pl. 1, fig. 10). Seed coat surface shows irregular undulations (Pl. 3, fig. 25). The troughs occupy more area than the crests. Pointed crests have dentate appearance and are generally arranged in circles forming rib-like structures. Such circular areas are surrounded by pointed crests which look like small craters. Very little wax is deposited on the seed surface.

*Vigna sinensis* (L) Saviex Hassk.—Seeds are kidney-shaped and variously coloured. Mostly the seeds have light brown and white patches but sometimes they are totally light brown in colour and their maximum axial length and breadth is about 10.0 × 5.0 mm. Hilum is quite large its length and breadth, i.e. about 5.0 × 2.5 mm. Hilum is oblong in shape and it is completely covered with white and hard aril. Seed surface is not very smooth, sometimes fine wrinkles are also present (Pl. 1, fig. 11). Seed coat has waxy deposition on its surface (Pl. 3, fig. 26). These depositions are not continuous but present in the form of thin flakes. Due to these depositions, the underlying seed coat ornamentations are indistinct. Critical observation shows that the pattern is of tuberculate type.

*Dolichos lablab* Linn.—Seeds are generally black but sometimes brown seeds can also be found. Seeds are large having maximum axial length and breadth of about 14.0 × 8.0 mm. Hilum is quite prominent, its length and breadth is about 13.0 × 2.0 mm and it is covered over with hard, whitish aril. Sometimes the aril at the level of seed surface peels away leaving a scar of aril which is called as rim aril. Seed surface is smooth but not shiny (Pl. 1, fig. 12).

Seed coat has reticulate pattern (Pl. 3, fig. 27). Reticular walls are the thickest amongst the studied seeds of Papilionaceae. Thickness of these walls is constant, their



surface is smooth, but some faint wrinkles can be seen on the sides. Spermoderm is without any deposition on its surface.

*Cajanus cajan* (L.) Millsp.—Seeds are almost round in shape having a beak-like projection towards the hilar end. The colour of the seed is variable but commonly the seeds are cream coloured with brown mottling on the surface. However, light to dark brown or even black seeds are also found. The seed surface is smooth. Hilum is about 3. × 2.0 mm in length and breadth. It is partially covered with whitish hard aril, which is raised above the level of seed surface (Pl. 1, fig. 13).

The seed coat has rugose type of ornamentation (Pl. 3, fig. 28). The rugae appear to have irregular thickenings and they are medium in length but their thickness varies. Below the level of large rugae smaller rugae are also present. Spermoderm does not have any deposition on its surface.

*Dalbergia lanceolaria* Linn.—Seeds are dark brown in colour. The maximum length and breadth is about 7.0 × 5.0 mm. Seeds are kidney-shaped. Hilum is situated in the notch, which is small and without prominent arillar lobes. Seeds are hard and somewhat flattened (Pl. 1, fig. 14).

Seed coat has reticulate type of ornamentation (Pl. 3, fig. 29). Below the larger reticula, smaller reticula are also present. These reticula form trigonal to polygonal areas. Thickening of the walls of the reticula is even and smooth. Seed coat lacks deposition on its surface.

*Sophora tomentosa* Linn.—Seeds are yellowish brown in colour and almost rounded in shape with maximum axial length and breadth of about 7.0 mm. Hilum is situated deep in the groove and there is a line connecting hilum with chalaza. Chalaza is distinct and aril is not prominent (Pl. 1, fig. 15).

Seeds coat is rough and seems to have rugose type of ornamentation (Pl. 3, fig. 30) but here most of the rugae are indistinct because of the deposition on the seed coat surface. The ridges and furrows, which may be due to underlying rugae, appear like sand dunes. Spermoderm also has granular and lumpy particles on its surface.

### Key—1

*Key for the identification of seeds of Papilionaceae based on macroscopic features : Papilionaceae seeds :* Compressed, asymmetrical at the transmedian plane. Chalaza at right angle to the hilum. The raphe is shorter than antiraphe. Hilum prominent with median groove and rim aril. Funicle short.

1. *Hilum small. concealed in the hilar notch.*

A. Seed reniform

a. Grey black seeds, MAL\* 6.0 mm—*Crotalaria juncea*.

2. *Hilum circular and well-defined.*

A. Seed oblong

a. Yellowish green and brown seeds, MAL 2.0 mm. *Melilotus indica*.

B. Seed cylindrical

a. Clayish yellow seeds, MAL 4.0 mm—*Trigonella foenum-graecum*.

b. Black seeds, MAL 4.0 mm—*Sesbania aculeata*.

\*MAL—Maximum axial length.

C. Seed oval.

a. scarlet & black seed, MAL 7.0 mm—*Abrus precatorius*

3. *Hilum elliptical and well-defined.*

A. Seed rounded.

a. Cream coloured with brown mottling or totally brown seeds, MAL 6.0 mm—*Cajanus cajan*.

b. Cream coloured seeds, MAL 8.0 mm—*Pisum sativum*.

c. Yellowish brown seeds, MAL 7.0 mm—*Sophora tomentosa*

B. Seed oblong

a. Partially brown and partially white seeds MAL 10.0 mm—*Vigna sinensis*.

b. Dark brown, MAL 7.0 mm—*Dalbergia lanceolaria*.

C. Seed cylindrical.

a. Greenish yellow seeds, MAL 4.0 mm—*Sesbania aegyptiaca*.

D. Seed angular.

a. Grey seeds with black spots, MAL 5.0 mm—*Lathyrus sativus*

4. *Hilum elongated and well-defined :*

A. Seed oblong.

a. Black to brown seeds, MAL 14.0 mm—*Dolichos lablab*

B. Seed angular.

a. Yellowish brown seeds, MAL 8.0 mm—*Cicer arietinum*.

C. Seed circular flat.

a. Brown seeds, MAL 5.0 mm—*Lens culinaris*.

## Key-2

*Key for the identification of seeds of Papilionaceae based on the seed coat characters as observed by SEM*

1. *Seed coat with rugose pattern on the surface.*

A. Depositions appear like sand dunes—*Sophora tomentosa*.

B. Rugae small with irregular thickening—*Sesbania aegyptiaca*.

C. Rugae with smooth surface and small vertically projected papillae—*Sesbania aculeata*.

D. Walls of the rugae are arched—*Cajanus cajan*.

2. *Seed coat with tuberculate pattern on the surface.*

A. Tubercles with smooth deposition.

a. Horizontally placed elongated processes—*Trigonella foenum-graecum*.

b. Without elongated processes.

i. Tubercles irregular in shape—*Melilotus indica*.

ii. Tubercles dome-shaped having fine reticulations in between—*Crotalaria juncea*.

B. Tubercles with wax deposition on the tips.

a. Tubercles octopus-shaped—*Lens culinaris*.

b. Tubercle tips are bent—*Lathyrus sativus*,



- C. Wax deposition in the form of loose flakes.
- a. Tubercles indistinct and depositions are in the form of loose, small flakes—*Vigna sinensis*.
3. Seed coat with regularly polygonal reticulate pattern on the surface.
- A. Reticula are thick-walled—*Dolichos lablab*.
  - B. Reticula are thin-walled—*Dalbergia lanceolaria*.
4. Seed coat with almost smooth surface.
- A. Surface with lumps of wax at the margin of red and black colour—*Abrus precatorius*.
5. Seed coat with undulated surface.
- A. Crests occupy more area—*Cicer arietinum*.
  - B. Troughs occupy more area—*Pisum sativum*.

## Conclusion

Seed coat ornamentation is a stable character for the identification of medicinal seeds. The most dependable part for such studies by Scanning Electron Microscope is the part of the seed coat, situated below the hilum. Gross seed coat ornamentation does not change under different geographical and climatic conditions.

Seed cuticle is usually responsible for seed coat ornamentation but sometimes underlying palisade layer of testa also plays a subsidiary role in this respect.

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## Explanation of Plates

### PLATE 1

1-15, Seed structures of : 1. *Crotalaria juncea*, 2. *Trigonella foenum-graecum*, 3. *Melilotus indica*, 4. *Sesbania aegyptiaca*, 5. *S. aculeata*, 6. *Lens culinaris*, 7. *Abrus precatorius*, 8. *Cicer arietinum*, 9. *Lathyrus sativus*, 10. *Pisum sativum*, 11. *Vigna sinensis*, 12. *Dolichos lablab*, 13. *Cajanus cajan*, 14. *Dalbergia lanceolaria*, 15. *Sophora tomentosa* (All  $\times 2$ ), 15-17 Seed coat surface ornamentation of 16. *Crotalaria juncea* ( $\times 1500$ ), 17. *Trigonella foenum-graecum* ( $\times 1500$ ).

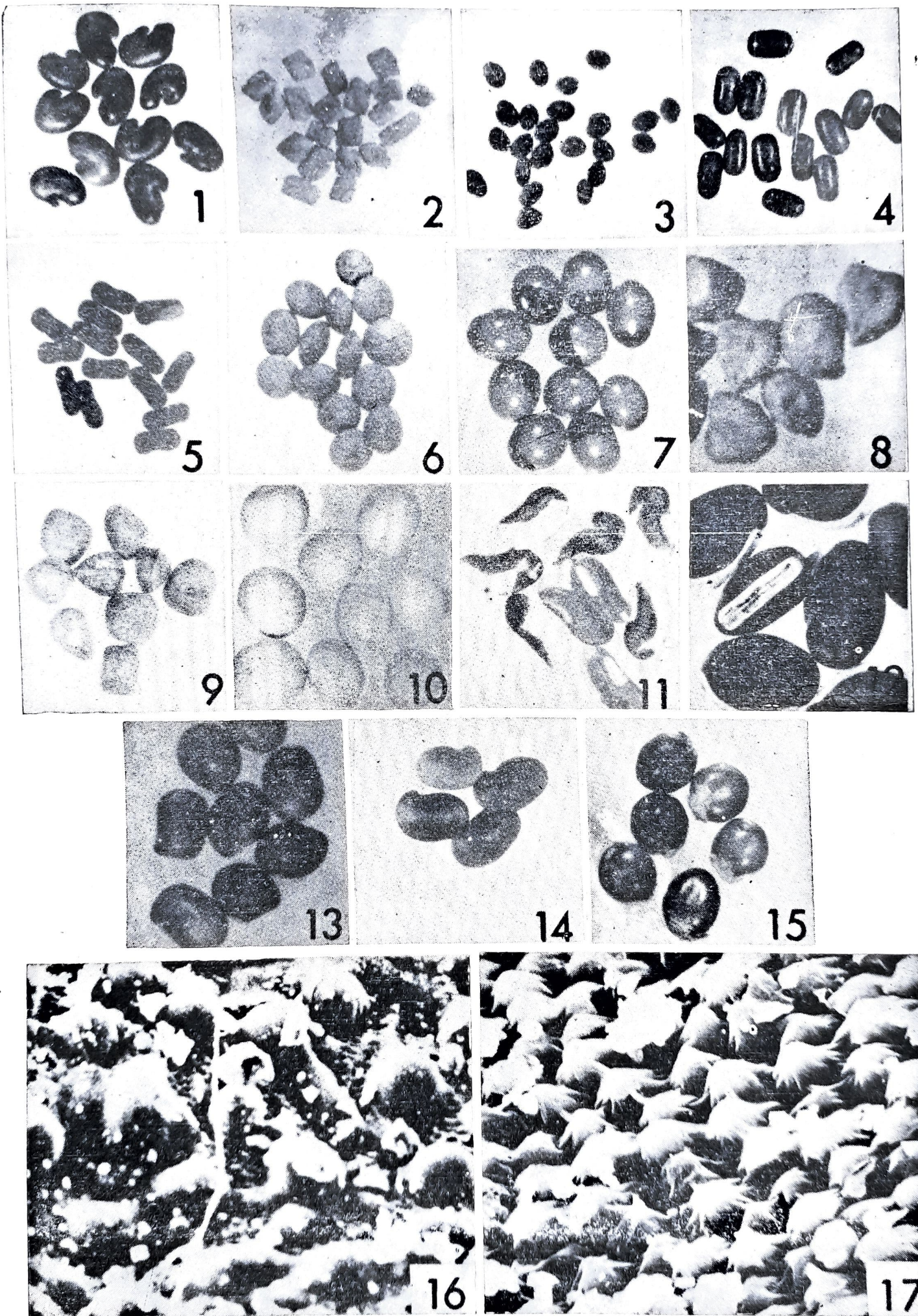
### PLATE 2

18-23. Seed coat surface ornamentation of 18. *Melilotus indica* ( $\times 4500$ ), 19. *Sesbania aegyptiaca* ( $\times 4500$ ), 20. *S. aculeata* ( $\times 4500$ ), 21. *Lens culinaris* ( $\times 4500$ ), 22. *Abrus precatorius* ( $\times 4500$ ), 23. *Cicer arietinum* ( $\times 1500$ ).

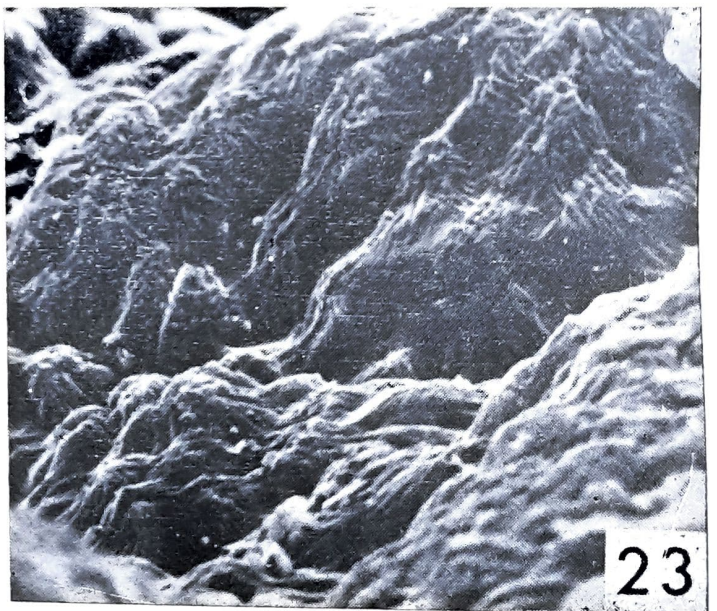
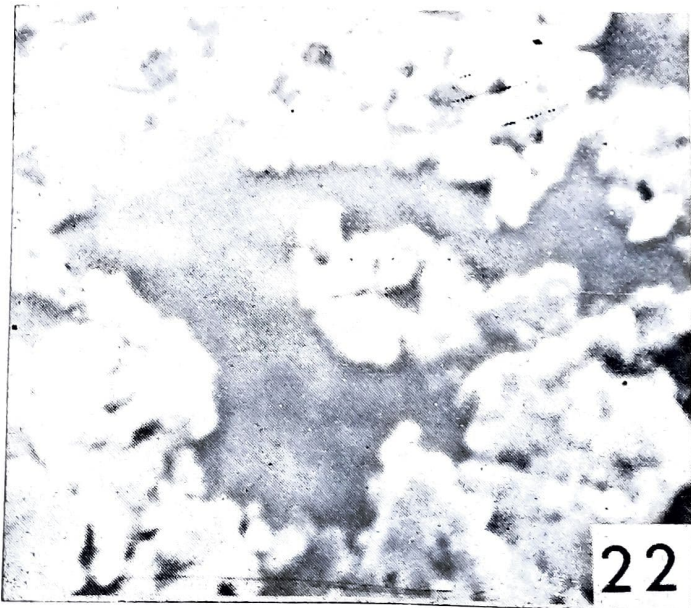
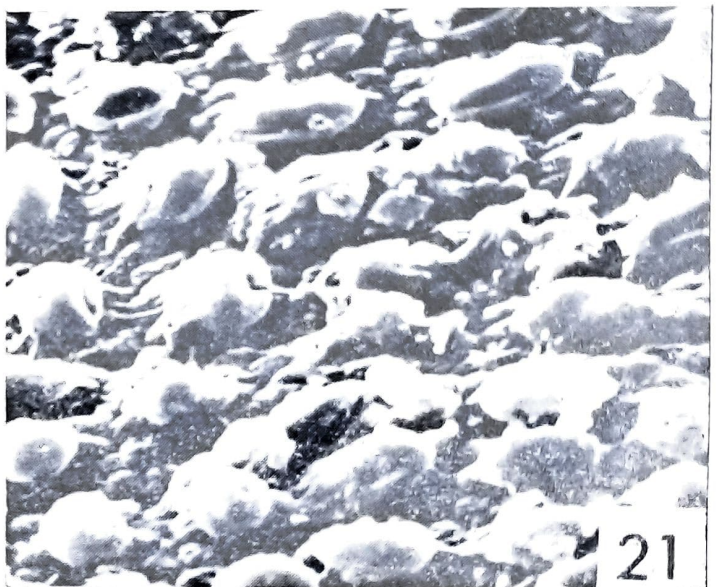
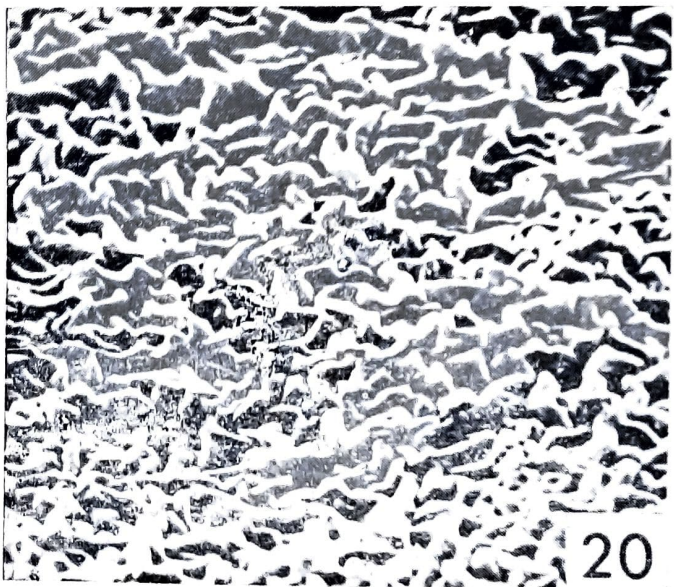
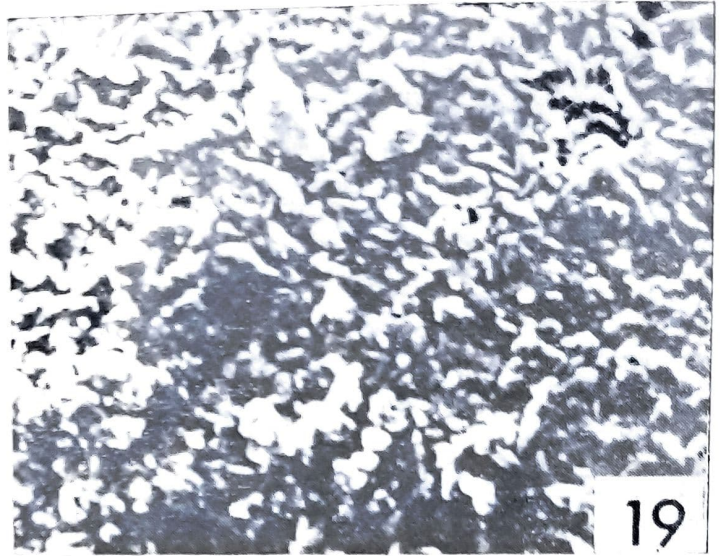
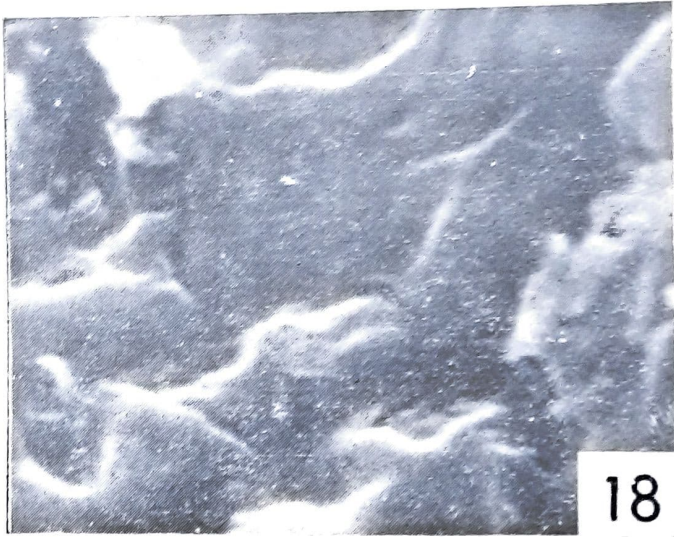
### PLATE 3

24-30, Seed coat surface ornamentation of 24, *Lathyrus sativus* ( $\times 1500$ ), 25. *Pisum sativum* ( $\times 4500$ ), 26. *Vigna sinensis* ( $\times 1500$ ), 27. *Dolichos lablab* ( $\times 1500$ ), 28. *Cajanus cajan* ( $\times 4500$ ), 29. *Dalbergia lanceolaria* ( $\times 4500$ ), 30. *Sophora tomentosa* ( $\times 1500$ ).

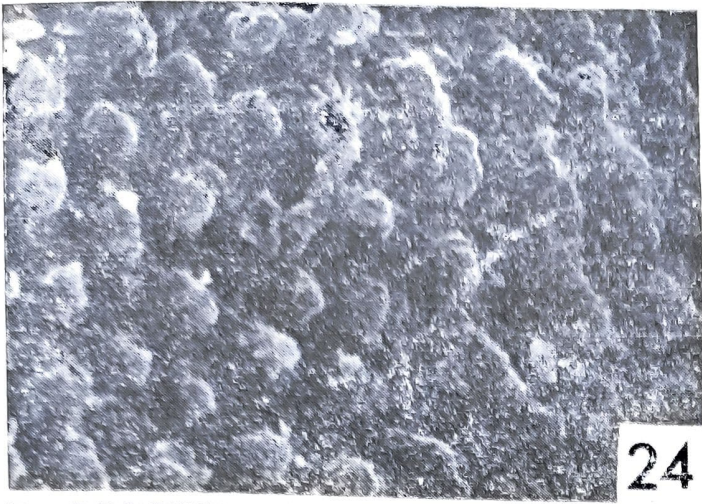




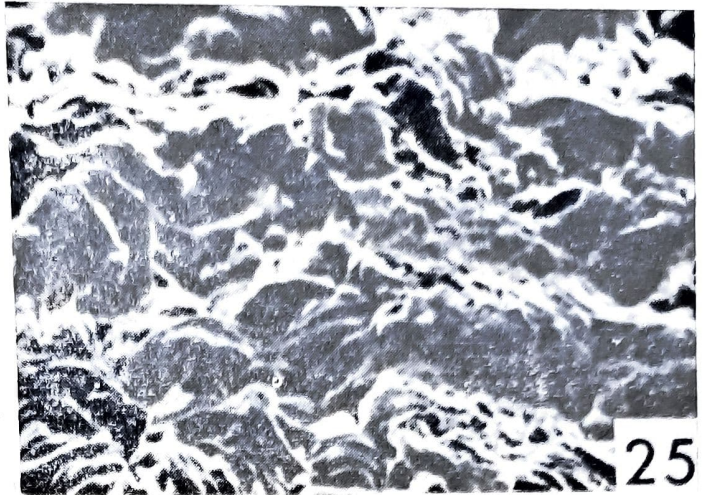




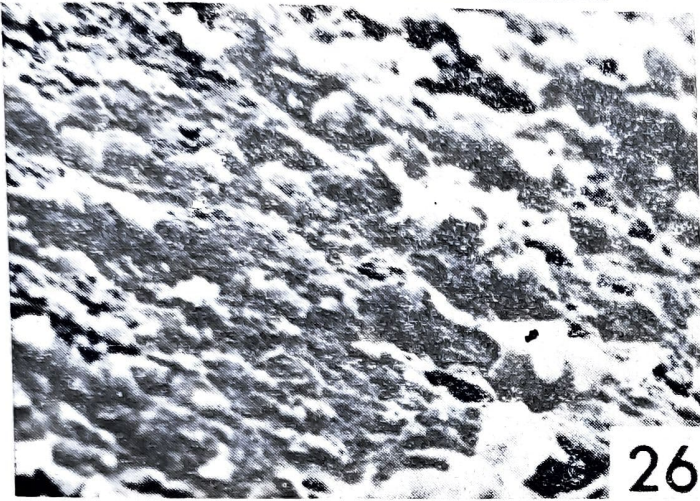




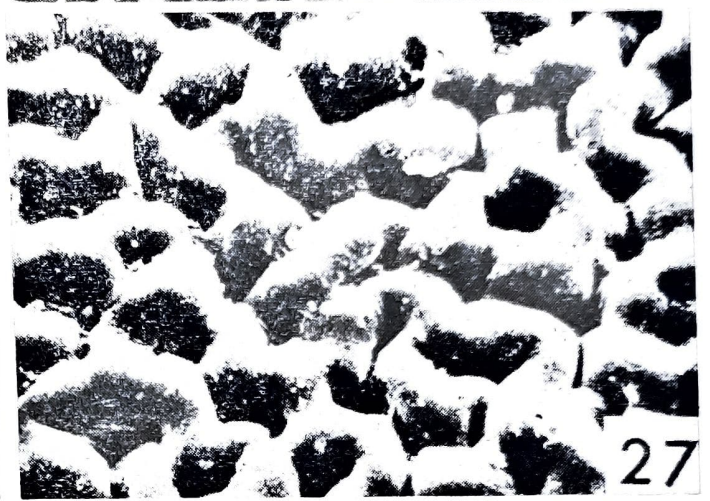
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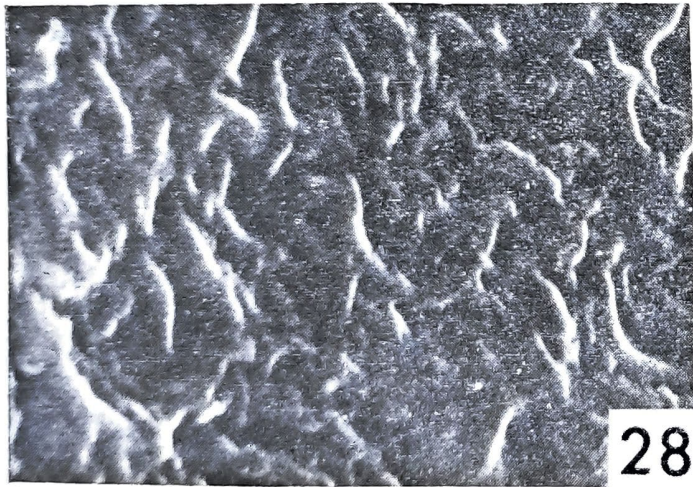
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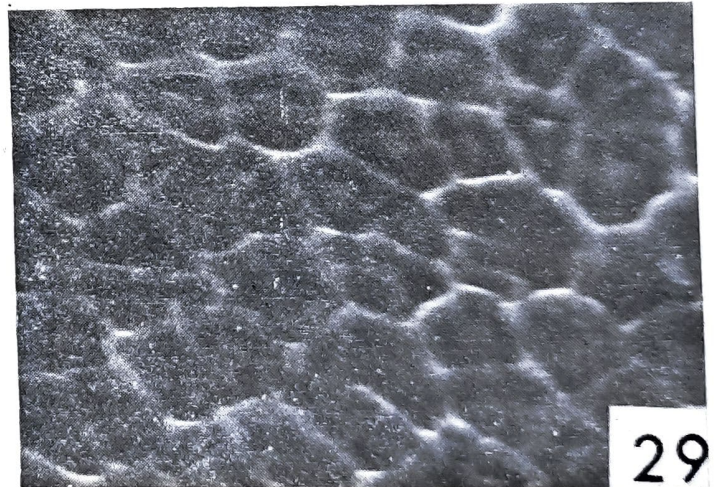
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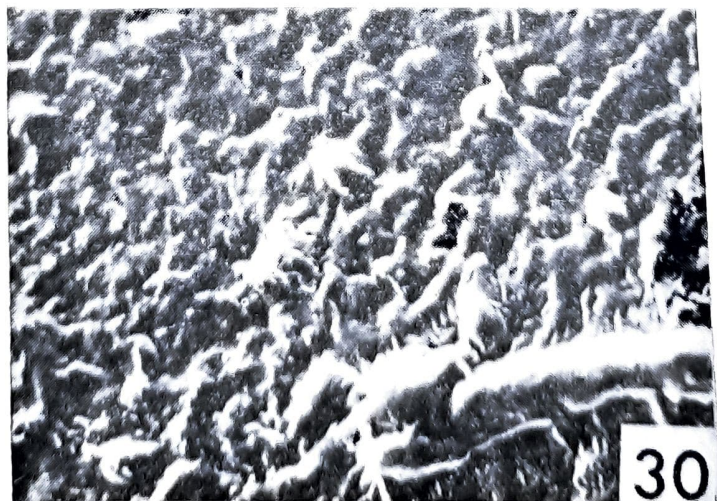
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