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Mohini Gupta
Lucknow University

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SEED COAT STRUCTURE IN SOME SPECIES OF TRIGONELLA

Mohini Gupta

Department of Botany,
Lucknow University,
Lucknow - 226 007, India
Phone No : 74794 (Office)

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Abstract

Seed coats of nine species of Trigonella, members of Trifolieae, were studied using light and scanning electron microscopy to assess seed coat characters taxonomically. In all species, the testa is of the papillose type except T.geminiflora in which it is of the multi-reticulate type. Based on some of the seed characters such as their shape, size, surface, position of hilum and the features of papillae, a key has been developed to identify different species of this genus. Further, in a highly evolved group like section Bucerates, it has been found that the surface is either papillose without mounds or multi-reticulate. This feature has to be confirmed in more species of this genus.

KEY WORDS: Trigonella, Papilionoideae (Fabaceae), SEM, hilum, spermoderm.

Introduction

Trigonella L. a genus of Papilionoideae, is represented by about 80 species (Polhill and Raven, 1981) distributed from the Mediterranean to central Europe, east to central Asia, west through Africa to the Canaries and with an isolated species in Australia, but the main center of diversity is the Mediterranean region; a few species are cultivated in India of which T. foenum-graecum is cultivated throughout for culinary and medicinal purposes.

Seed topography of four species of Trigonella (T. arabica, T. caerulea, T. foenum-graecum and T. pubescens) was described by Lersten and Gunn (1982) and of T. foenum-graecum by Trivedi and Bagchi (1982) and Bagchi and Tripathi (1986). In the present investigation seed coat characters of nine species, which were not previously studied, have been examined by using light and scanning electron microscopy to gain more information about this genus.

Materials and Methods

Table 1 shows the voucher number, place of collection and collector for the species used in this study. Seed data such as length, breadth, thickness and colour based on ten seeds were obtained using the LM. For SEM studies four seeds per species were selected randomly and mounted on brass stubs with double stick carpet tape and coated with gold-palladium in a sputter coater. Surfaces at mid-seed and near the hilum were examined to study the general pattern. For comparision of the pattern in different species, the portion immediately adjacent to the hilum was scanned in a JEOL - JSM - 35C SEM at an accelerating voltage of 15 kV and photographed on 120 mm (125 ASA) film.

Results

Seed characters such as size, colour, shape and surface are given in Table 2 using LM and SEM. Seeds illustrated using the SEM are finely tuberculated in T. besseriana

Table 1. Species with voucher number, place of collection and collector

1. T. besseriana Ser. (T. procumbens (Besser) Reichb) (34769, Australia, The N.I. Vavilov, All Union Institute of Plant Industry, 44, Herzenstreet, Leningrad, U.S.S.R.).
2. T. calliceras Fisch. (34805, German Dem. Rep. The N.I. Vavilov, All Union Institute of Plant Industry, 44, Herzenstreet, Leningrad, U.S.S.R.).
3. T. corniculata L. (Meerut, India).
4. T. geminiflora Bunge (S.n. 1931, V.5.A., U.S.S.R., Kazakh, Goloskokov, G.H.).
5. T. grandiflora Bunge (33283, Uzbek SSR. The N.I. Vavilov, All Union Institute of Plant Industry, 44, Herzenstreet, Leningrad, U.S.S.R.).
6. T. monspeliaca L. (S.n. 1890. 25.V.U.S.S.R., Daghestan, Petrousk, V. Lipsky, G.H.).
7. T. polyceratia L. (S.n. 1908 7/6, Spain : Castilla, Mirandade Ebro, H. Elias, G.H.).
8. T. radiata (L.) Boiss. (Nephromedia radiata, (L.) Kastel) (43299, Turkmen, SSR, The N.I. Vavilov, All Union Institute of Plant Industry, 44, Herzenstreet, Leningrad, U.S.S.R.).
9. T. stellata Forssk. (C. Heyn, Jerusalem, Israel).

Table 2. Seed characters in species of Trigonella

Species	Shape	Colour	Size* LxBxT (mm)	Surface LM+SEM	Spermoderm Near Hilum	Spermoderm Mid-seed	Hilum Position	Hilum Shape	Hilum Colour	Hilum Size*
1. T. besseriana	Sub-circular or oval or elliptical	Orangish yellow or greenish brown to brown	1.65±0.01x 1.52±0.05x 1.00±0.07	Finely tuber-culated, shiny	Papil-lose with mounds	Papil-lose with mounds	Central	Circular	White	0.15± 0.005
2. T. calliceras	Compressed, circular, elliptical or widely obovate	"	1.39±0.03x 1.54±0.02x 0.84±0.09	"	"	"	"	"	"	0.14± 0.01
3. T. corniculata	Compressed, ovate to oblong	Pale yellow to brown, streaked with black marks	0.95±0.01x 1.33±0.03x 0.49±0.03	Finely tuber-culated and dull	Papil-lose	Papil-lose	"	"	Brown	0.08± 0.01
4. T. geminiflora	Flattened, oblong or somewhat quadangular	Choco-late brown	1.65±0.06x 0.85±0.01x 0.35±0.03	Tuber-culated and dull	Multi-reti-culate	Multi-reti-culate	Chalazal	"	"	0.07± 0.01
5. T. grandiflora	Elliptic, oblong or somewhat quadangular	Yellowish green mottled with dark brown spots	1.81±0.06x 1.04±0.02x 0.82±0.05	Finely tuber-culated and dull	Papil-lose with mounds	Papil-lose with mounds	"	"	"	0.12± 0.006
6. T. monspeliaca	Quadangular or somewhat oblong	Brown to chocolate brown	1.12±0.05x 0.69±0.04x 0.61±0.02	Tuber-culated, dull	Papil-lose	Papil-lose	"	"	"	0.06± 0.15
7. T. polyceratia	Oblong to quadangular	Yellowish brown to brown	1.78±0.04x 0.94±0.01x 0.68±0.02	"	"	"	"	"	"	0.09± 0.17
8. T. radiata	Compressed, somewhat obovate	Greenish yellow to yellowish brown	1.97±0.03x 2.87±0.05x 0.87±0.04	Ridged, dull	"	"	"	"	"	0.145± 0.003
9. T. stellata	Compressed, elliptical obovate or oblong	Yellowish brown to brown	0.80±0.05x 1.06±0.04x 0.60±0.03	Tuber-culated, dull	"	"	"	"	"	0.08± 0.007

*Mean of 10 readings; Abbreviations; L=Length (Length is measured parallel to hilum); B=Breadth; T=Thickness; LM=Light Microscope; SEM=Scanning Electron Microscope

(Figs. 1,2), T. calliceras (Fig.3), T. corniculata and T. grandiflora, ridged in T. radiata (Fig.4) and tuberculated in the remaining species represented by T. polyceratia (Fig.5). Out of nine species, seeds are compressed only in T. calliceras (Fig.3), T. corniculata, T. radiata (Fig.4) and T. stellata.

Hilum and Micropyle:

The hilum is circular in all the species with a deltoid micropyle (Figs. 6-8). Hilar groove is distinct only in a few species. The hilum is centrally placed in T. besseriana (Fig.2), T. calliceras and T. corniculata or chalazal in the remaining species (Fig.6). A well-developed hilar rim is observed in T. calliceras (Fig.7) and in T. corniculata (Fig.8). Lens and micropyle are the same colour as the testa. Lens is clearly discernible and removed from the hilum in T. besseriana (Fig.2), T. calliceras and T. corniculata, indistinctly discernible in T. polyceratia (Fig.6) in which the discoloured area is touching the hilum and not discernible in the remaining species.

Testa:

The surface of the seeds in all the species is papillose except T. geminiflora in which it is multi-reticulate. Variations have been noticed in the shape of the papillae, their height and interconnections with other papillae. Therefore, the surface of each species is described separately as below:

1. T. besseriana: Papillae are less raised compactly arranged with mounds, somewhat irregular in shape with uneven deposition of wax on the cuticular striae on their apex. From the apex or on the side of the papillae elongated processes extend in all directions which may or may not join the adjacent papillae (Figs. 9-10).

2. T. calliceras: Papillae are raised with mounds and more or less circular in shape with smooth wax deposition on the apex. Elongated processes extend in all directions which generally join the neighbouring papillae (Fig.11).

3. T. corniculata : Papillae are low, ranging in varying shapes of pentangular to oblong and oval, inter-papillae regions are somewhat corrugated as if each papillae were seemingly surrounded by a reticulum. The apices of the papillae show slit-like or circular depressions in the center. The surface of the apex of the papillae shows uneven deposition of wax on the rugae formed by the cuticle (Fig.12).

4. T. geminiflora The pattern in this species is multi-reticulate. Thick ridges form the boundaries of the cells and the surfaces of the cells are covered by transverse striations forming a multi-reticulate pattern. Both thick ridges and transverse striations are undulated (Fig.13).

5. T. grandiflora: Papillae are less raised, loosely arranged with mounds variable in shapes. Wax is deposited unevenly on the papillae and mounds (Fig.14).

6. T. monspeliaca: Papillae are low or raised, elliptical, oblong or somewhat circular in shape. Wax is deposited in a uniform manner on the apex of the papillae or it is deposited on the transverse striations which form interconnections with the adjacent papillae or elongated processes extend out in all directions (Fig.15).

7. T. polyceratia: Papillae are low, widely spaced, oblong to somewhat circular in shape with smaller deposition of wax in the center. On the peripheral parts of the papillae, wax is heavily deposited on a reticulum by which the adjacent papillae may or may not be joined at some places (Figs.16,17).

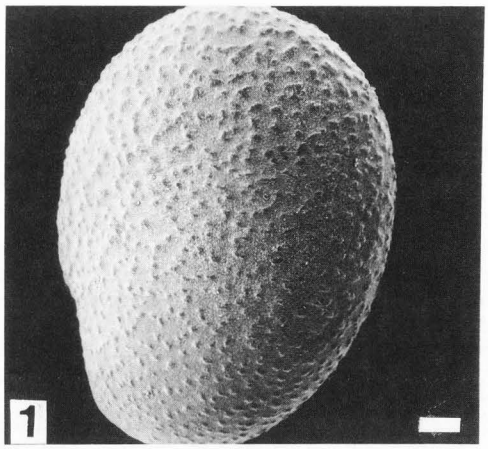
8. T. radiata: Papillae are low, widely spaced, oblong or circular in shape and are of varying sizes. Apex shows low to heavy deposition. Wax particles are also distributed randomly on the surface (Fig.18).

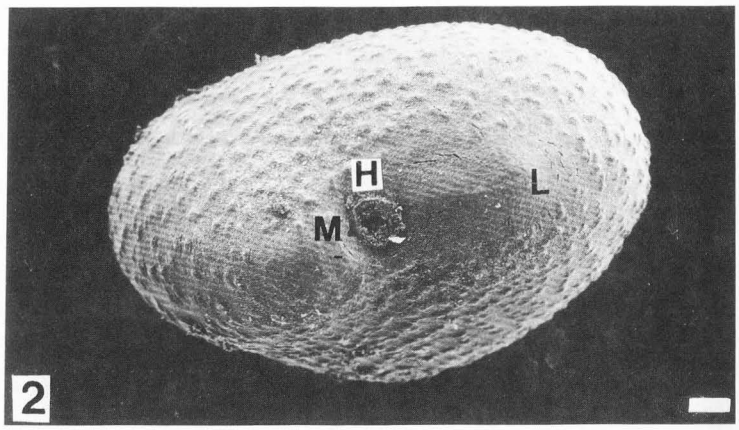
9. T. stellata: Papillae are low, oval or oblong or circular in shape. Most of the papillae are enclosed by a rugae. Wax is deposited on the apex of the papillae mostly on the cuticular transverse striations or as a uniform deposition (Fig.19).

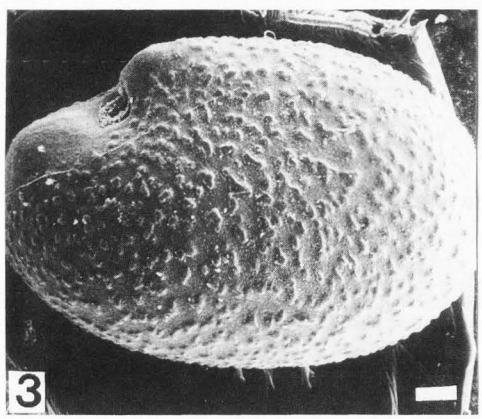
Discussion

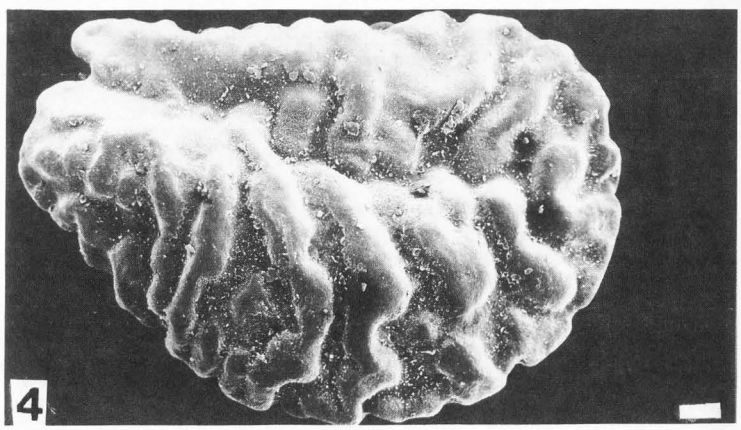
From the present investigation some striking features are revealed. Earlier workers (Lersten and Gunn, 1982; Trivedi and Bagchi, 1982; Bagchi and Tripathi, 1986) had reported only papillose type of testa ornamentation in few species of the genus Trigonella. However, in the present investigation a multi-reticulate pattern was found in T. geminiflora. Therefore, it can be concluded that patterns other than papillose also exist within this genus. Species of Trigonella are mainly identified by fruit morphology and by a few floral characters (Gupta,1984). In the present study, it has been established that in addition to fruit morphology and floral characters, the species of this genus can be identified by seed characters such as size, the hilum position, and the surface topography. Seeds of T. radiata are largest while those of T. stellata are smallest.

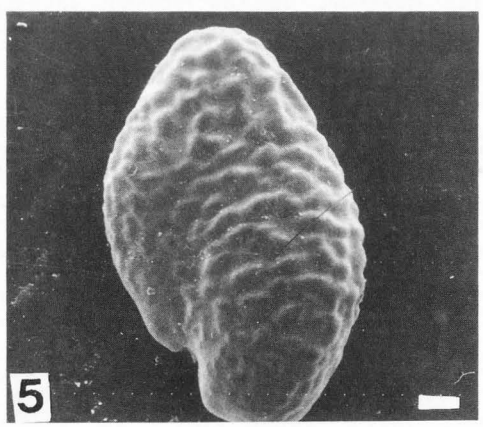
Small et al. (1981), on the basis of numerical taxonomical analysis of floral characters, have shown that species of Trigonella belonging to section Bucerates are quite distinct from the remaining Trigonella species. This fact was further confirmed by the author (Gupta,1982) while determining the petal venation within this genus. It was found that T. polyceratia, a member of Bucerates, possesses a medicagoid type of corolla and the largest number of anastomoses. Based on these two characters T. polyceratia was considered to be the most advanced species of the genus. Later Gupta (1984), while working on the floral anatomy of ten species of Trigonella, found that adnation of the bundles of different whorls











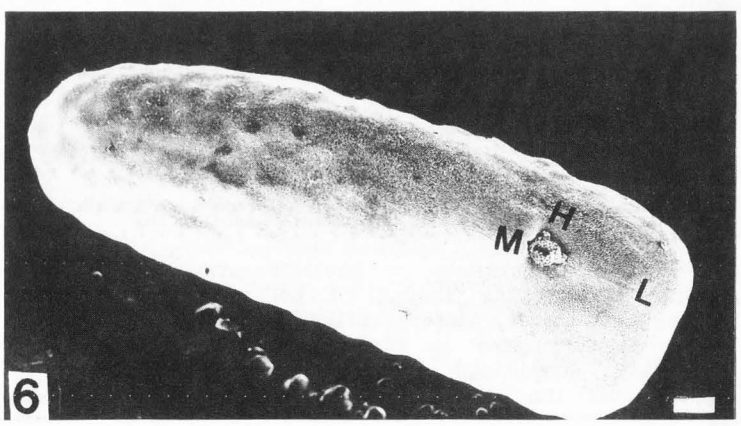
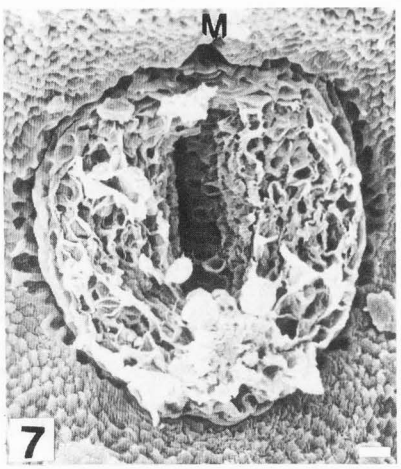
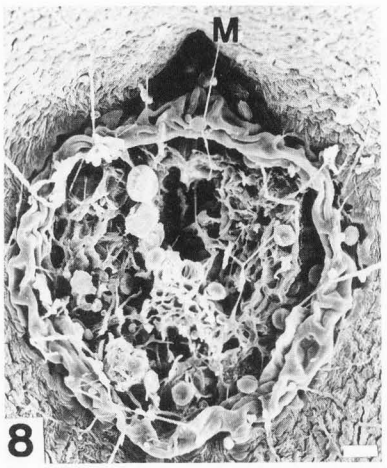
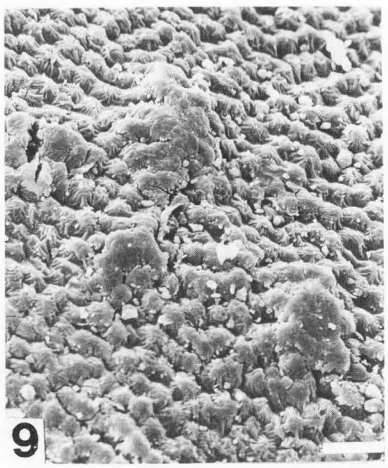
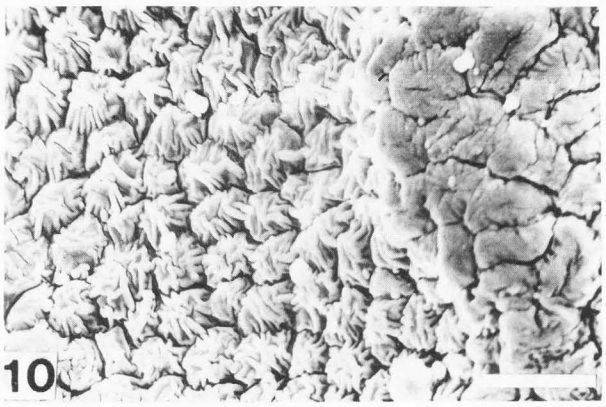


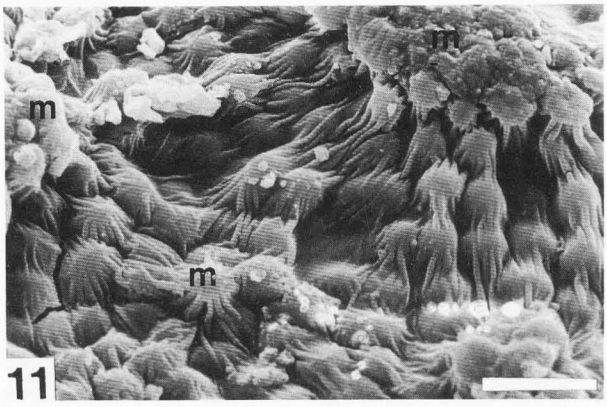
Figure 1. Seed of T. besseriana in side view showing fine tubercles on the surface. Bar = 100µm.
Figure 2. Seed of T. besseriana in surface view showing fine tubercles, micropyle (M), lens (L) and a circular central hilum (H). Bar = 100 µm.
Figure 3. Compressed seed of T. calliceras showing fine tubercles. Bar = 100 µm.
Figure 4. Seed of T. radiata showing ridges. Bar = 100 µm.
Figure 5. Seed of T. polyceratia in side view showing tubercles on the surface. Bar = 100 µm.
Figure 6. Seed of T. polyceratia in surface view showing a circular chalazal hilum (H), lens (L) and a micropyle (M). Bar = 100 µm.

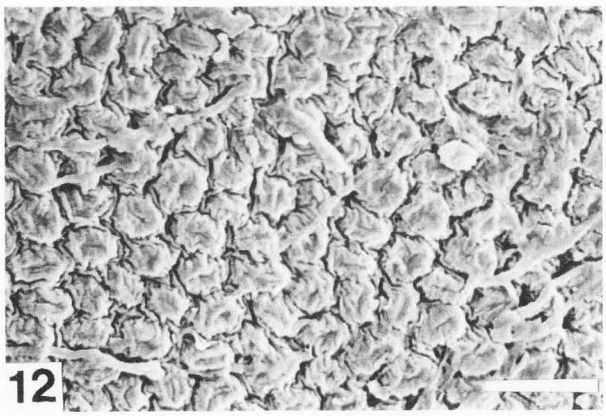












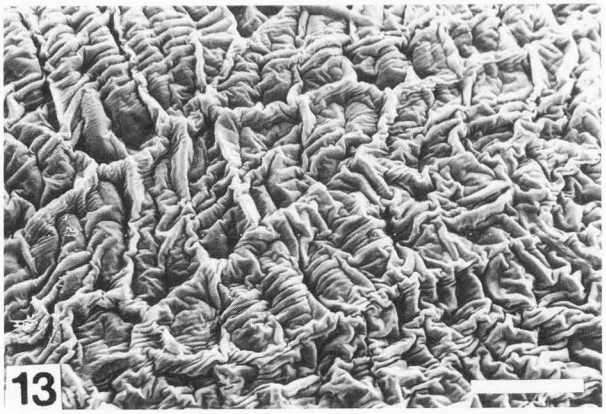
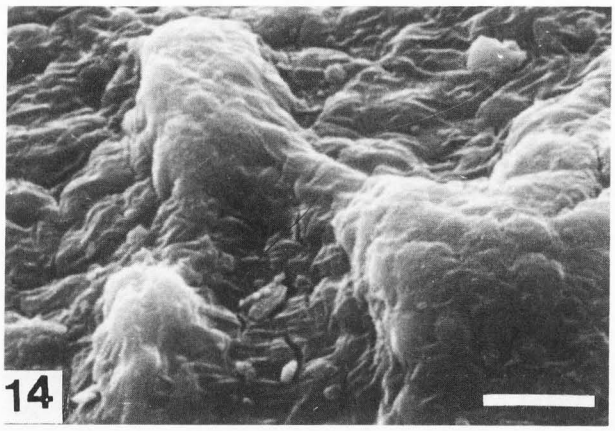
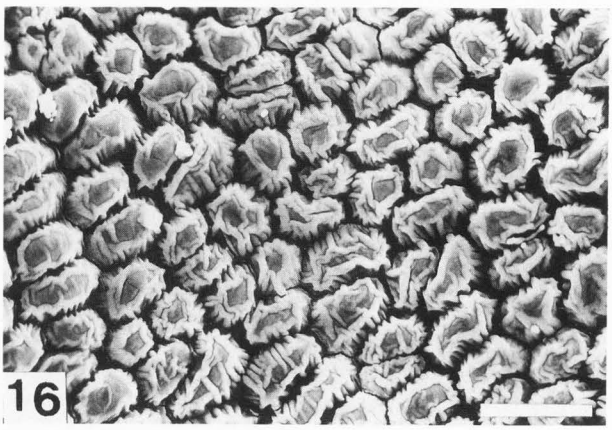
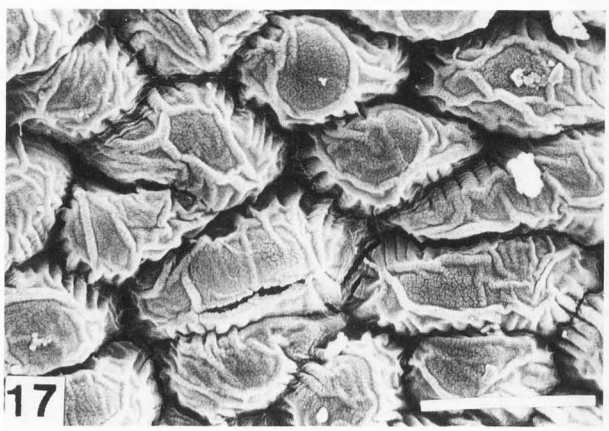


Figure 7. T. calliceras showing circular hilum with rim, a deltoid micropyle (M) and papillae around the hilum. Bar = 10 µm.
Figure 8. T. corniculata showing hilum with rim, and a deltoid micropyle (M). Bar = 10 µm.
Figure 9. T. besseriana showing papillae with mounds. Bar = 10 µm.
Figure 10. T. besseriana papillae enlarged. Bar = 10 µm.
Figure 11. T. calliceras showing jointed mounds and papillae (m = mound). Bar = 10 µm.
Figure 12. T. corniculata papillae showing slit-like or circular depression in the center. Bar = 10 µm.
Figure 13. T. geminiflora surface showing multi-reticulate pattern. Bar = 10 µm.







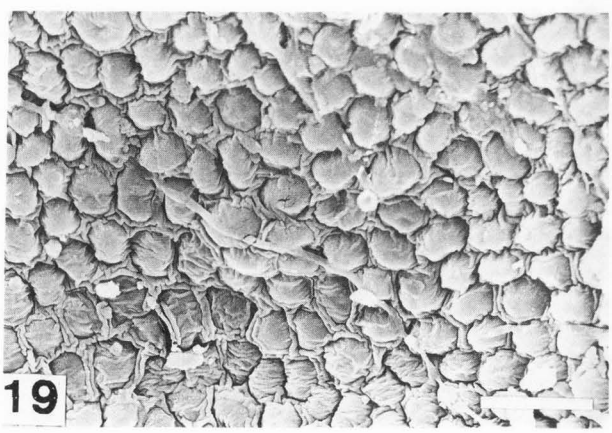


Figure 14. T. grandiflora - surface showing papillae with mounds. Bar = 10 µm.
Figure 15. T. monspeliaca - surface showing papillae. Bar = 10 µm.
Figures 16-17. T. polyceratia - surface showing papillae. Bar = 10 µm.
Figure 18. T. radiata - surface showing papillae. Bar = 10 µm.
Figure 19. T. stellata - surface showing papillae. Bar = 10 µm.

(an advanced character) was maximum in T. foenum-graecum followed by T. polyceratia. Based on this character T. foenum-graecum was considered to be the most advanced species followed by T. polyceratia. For the present study of the testa topography of nine species of this genus, T.geminiflora, T. monspeliaca and T. polyceratia belong to the Bucerates group. The testa topography of T. geminiflora is quite distinctive from the remaining species due to the presence of multi-reticulate pattern. In T. monspeliaca and T. polyceratia testa topography is papillose without mounds but this pattern is also found in other species which are the members of other sections. Lersten (1981) has shown that Trifolieae shows a progressive shift from papillose with mounds to loss of mounds. It thus seems possible that in a highly evolved group like Bucerates all species may have papillose ornamentation without mounds. This feature has to be confirmed in more species of the genus.

Based on some of the characters of seeds, such as size, shape, position of hilum and popillae, the following key has been prepared to identify the species of Trigonella studied.

Key for identification of the species of Trigonella.

1. Testa multi-reticulate..... T. geminiflora.
2. Testa papillose.
   A. Testa papillose with mounds.
      i. Hilum central.
      a. Seeds not compressed, papillae less raised and mounds not interconnected..... T. besseriana.
      b. Seeds compressed, papillae raised and mounds interconnected..... T. calliceras.
      ii. Hilum chalazal..... T. grandiflora.
   B. Testa papillose without mounds.
      i. Hilum central..... T. corniculata.
      ii. Hilum chalazal.
      a. Seeds not compressed.
      (i). Papillae compactly arranged, elliptical, oblong or circular..... T. monspeliaca.
      (ii). Papillae loosely arranged oblong to somewhat circular..... T. polyceratia.
      b. Seeds compressed.
      i. Seeds less than 1 mm in length and tuberculated..... T. stellata.
      ii. Seeds about 2 mm in length and ridged..... T. radiata.

Acknowledgements

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Discussion with Reviewers

L.H. Bragg: You described the papillae as low, raised or less raised. What did you use for a standard of comparison basis? How is this determined?

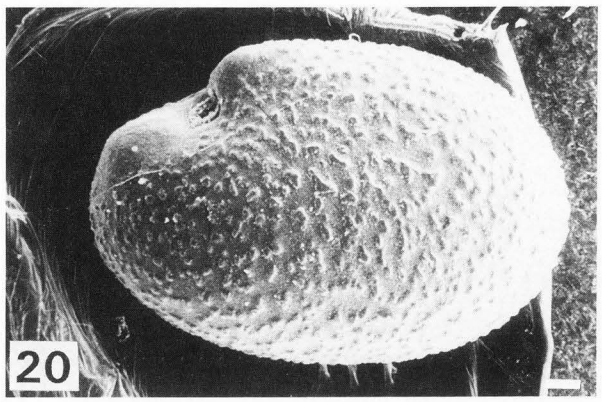
Author: Papillae were determined arbitrarily as low, raised or less raised depending upon their visual appearance in the micrograph.

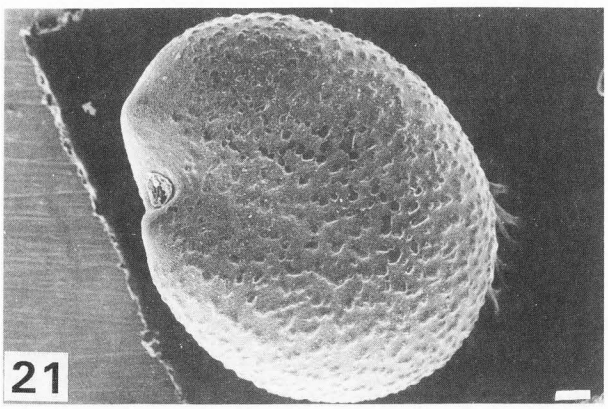
(1) **Low papillae**: Papillae are appearing as if they are in the level of the seed surface. Only apices of the papillae are seen in the surface view (Figs. 12, 16, 17).

(2) **Raised papillae**: Papillae are appearing as knob-like projections coming out on the surface with prominent deep grooves in between. Stalks of the papillae are also seen and mounds are generally found to be associated with the raised papillae (Fig. 11).

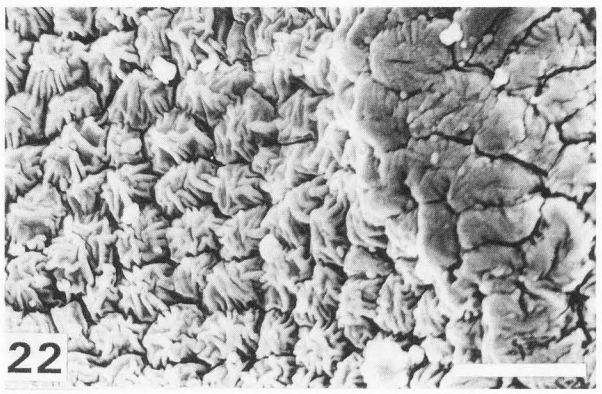
(3) **Less Raised**: If the papillae are separated by shallow grooves they are considered as less raised. Stalks are also not appearing as prominent as they are in the raised ones (Figs. 9, 10).

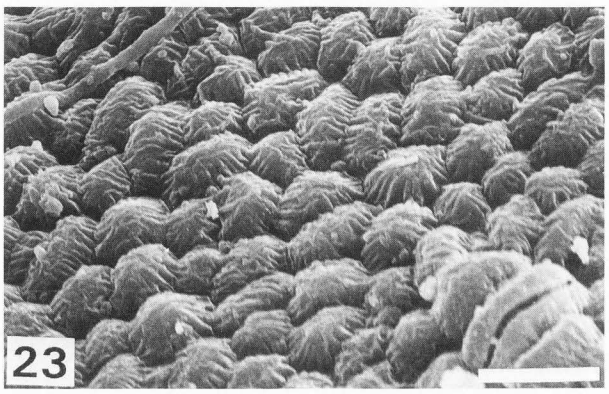
L.H. Bragg: You described the papillae as having wax deposited on them evenly or unevenly. How did you determine that this substance was indeed wax? Did you use a chemical test?





Figures 20-21. T. calliceras: full seed before (**Fig. 20**) and after (**Fig. 21**) chloroform treatment. Bar = 10 μm.





Figures 22-23. T. besseriana: papillar surface before (**Fig. 22**) and after (**Fig. 23**) chloroform treatment. Bar = 10 μm.

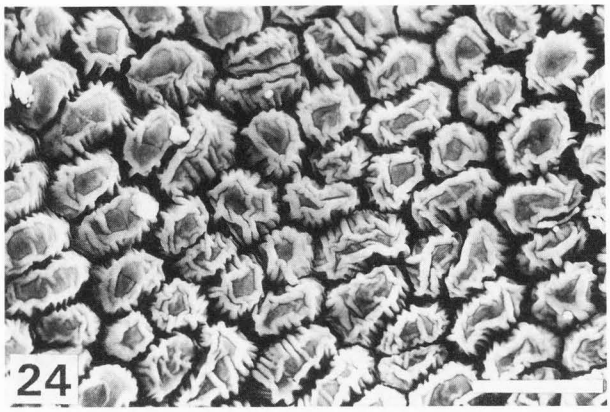
Did you subject the seeds to any solvent and then reexamine the seeds?

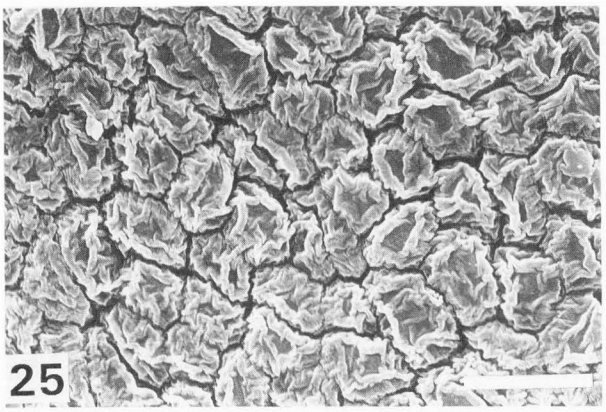
W.J.Wolf: What is the evidence for wax deposits on the surface of some of the seeds? Do hexane and similar substances remove them? If so, examination of seeds before and after removing the wax may reveal more of the structural details of the underlying cells. In studies of soybean seed coats we found that hexane did not remove deposits that we suspected might be waxes (Scanning Electron Microscopy **1981**;III, 531-534).

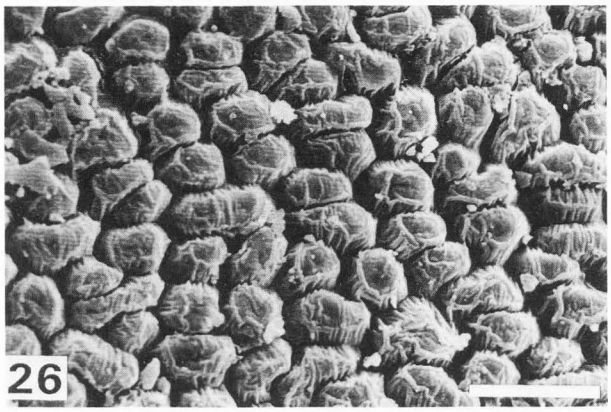
Author: The observations to indicate that the wax is deposited evenly or unevenly were based mainly on Trivedi et al. (1978) observations that tuberculate spermoderms show heavy deposits of wax on the apices of the tubercles. Initially, I had not used any chemical test to confirm that this substance was indeed wax. Recently, to confirm the waxy nature of the substance, seeds of four species (T. besseriana, T. calliceras, T. polyceratia and T. radiata) were boiled for 2 minutes in chloroform (Rangaswamy and Nand Kumar, 1985) to dissolve the epicuticular wax. The chloroform treated seeds were air dried and reexamined by SEM. After chloroform treatment it has been noticed that in the seeds of all the four species there was a considerable reduction in the amount of wax present on the surface of the seeds as well as on the surface of the papillae (Figs. 20-29). Thus, these observations clearly show that the wax is deposited on the seed surface, although its amount may vary from species to species (Figs. 20-29).

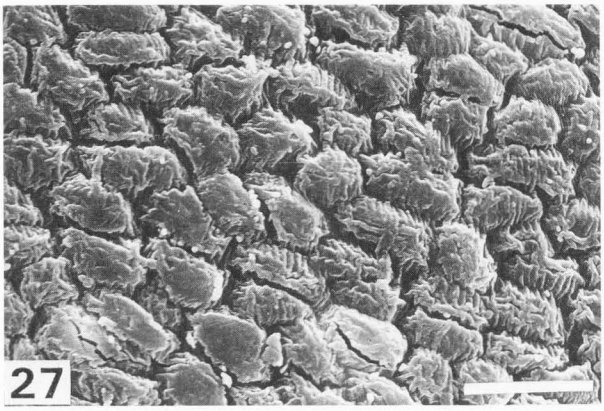
L.H.Bragg: Are the tubercles you mention for Fig. 1 associated with the mounds that are mentioned for other seeds? What, if any, is the relationship?

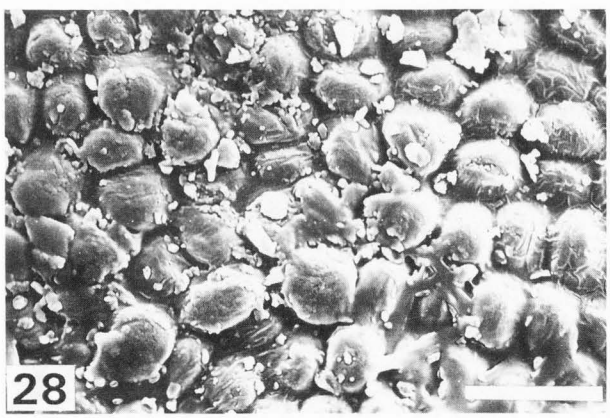
Author: Tubercles shown in Fig. 1 are noticed in four species (T. besseriana, T. calliceras, T. corniculata and T. grandiflora) and in all the species except T. corniculata the tubercles are associated with mounds. Jha and Pandey (1989) reported tuberculated testa in five species of Melilotus (M. indica, M. messanensis, M. neopolitana, M. segetalis and M. wolgica) but mounds were noticed only in M. messanensis and M. neopolitana. Lersten and Gunn (1982) also reported infrequently tuberculated testa in Ononis and Trigonella and they found mounds only in two out of six species in Ononis and in three out of the four species of Trigonella examined by them. Thus, from these studies it can be concluded that tuberculated testa may or may not be associated with mounds. More species should be studied to generalize this statement.

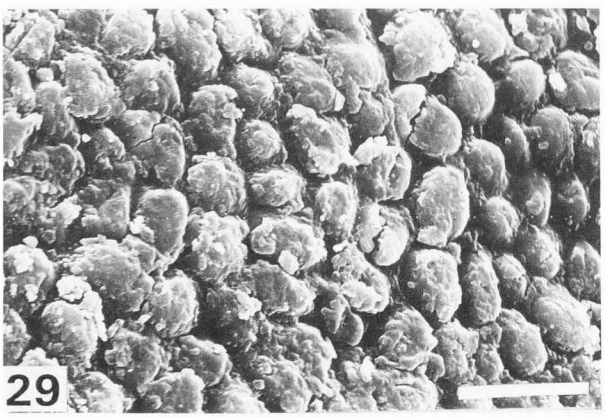












Figures 24-25. T. polyceratia: papillae near the hilum before (**Fig. 24**) and after (**Fig. 25**) chloroform treatment. Bars = 10 μm.

Figures 26-27. T. polyceratia: papillae at the mid-seed region before (**Fig. 26**) and after (**Fig. 27**) chloroform treatment to show the deposition of wax. Bars = 10 μm.

Figures 28-29. T. radiata: papillae before (**Fig. 28**) and after (**Fig. 29**) chloroform treatment. Bars = 10 μm.

Reviewer III: In a recent paper [Small E, Brookes B, and Lassen P (1989). Circumscription of the genus Medicago (Leguminosae) by seed characters. Can. J. Bot. 68: 613-629], pointing out the value of scanning electron microscopic seed characters in discriminating Medicago from allies including Trigonella, it is suggested that the correct nomenclature for four of the species of 'Trigonella' that the author examined really should be Medicago. Please comment.

Author: In the above study by Small et al. (1989) the topography of the seeds of different species was studied at a very low magnification, which is not optimal for taxonomic studies. It has also been shown by Lersten (1981) in a study made on 30 of the 32 tribes of the Papilionoideae that in most (85%) of the seeds examined a discernible pattern is seen around the hilum and he categorized testa patterns into nine categories based on a standard magnification of 1000x. In the present paper I have mainly stressed the structure and position of the hilum and testa patterns for specific differentiation.

In an earlier paper, Small et al. (1987) have proposed that a number of 'medicagoid' species of Trigonella possessing a shared syndrome of more than a dozen correlated flower characters associated with an explosive mode of pollination be reclassified in the genus Medicago and also pointed out that the four species, out of nine species which I have worked out are really Medicago. I am giving below the list of the four reclassified species of Trigonella.

1. Trigonella geminiflora Bunge = Medicago monantha (C. Meyer) Trautv.
2. T. monspeliaca L. = M. monspeliaca (L.) Trautv.
3. T. polyceratia L. = M. polyceratia (L.) Trautv.
4. T. radiata (L.) Boiss = M. radiata L.

From the testa topography it is difficult to say that these species should be kept in Trigonella or Medicago but I can only suggest that fruit character, which is a very distinct character, should be used to specify the generic limits of Trigonella and Medicago.

Additional References

Jha SS, Pandey AK (1989) Seed coat structure in Melilotus (Fabaceae). Phytomorphology 39 (2,3): 221-229.

Rangaswamy NS, Nand Kumar L (1985) Correlative studies on seed coat structure, chemical composition, and impermeability in the legume Rhynchosia minima. Bot. Gaz. 146 (4): 501-509.

Small E, Lassen P, Brookes BS (1987) An expanded circumscription of Medicago (Leguminosae, Trifolieae) based on explosive flower tripping. Willdenowia, 16: 415-437.

Trivedi BS, Bagchi GD, Bajpai U (1978) Spermoderm patterns in some taxa of Vicieae (Papilionatae-Leguminosae). Phytomorphology, 28 (4): 405-410.