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SEED TESTA MORPHOLOGY OF 18 SPECIES OF RAFNIA THUNB. (FAM. FABACEAE, SUB-FAMILY PAPILIONOIDEAE)

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Abstract

Scanning electron microscopy was used to conduct a micromorphological study of testae of the seeds of 18 species of Rafnia Thunb. (Fam. Fabaceae, Sub-family Papilionoideae). The testa patterns revealed diagnostic taxonomic features which proved useful in the construction of a key for the identification of the various Rafnia species. Comparisons between air-dried freshly collected, and long-term stored herbarium specimens revealed that testa patterns remained consistent and valid for taxonomic purposes.

KEY WORDS: Rafnia, Fabaceae, Taxonomy, Testa Morphology, Scanning Electron Microscopy

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Introduction

Heywood (1969) predicted that scanning electron microscopy (SEM) of seeds would become a routine technique in the study of seed coat morphology, and in the last two decades SEM has been widely used to study testa surface patterns (e.g. Brisson and Peterson, 1976; La Sota et al 1979; Lersten, 1981; Bragg, 1983; Bridges and Bragg, 1983;). Most of these studies have been carried out on seeds of the sub-family Papilionoideae where the testae have been described from light microscope observations as smooth and featureless (Lersten, 1981). One exception was the description of testa surfaces of seeds of the Cruciferae by Murley (1951), in which she proposed a terminology of these features. The use of SEM has provided new information of taxonomic significance as it is more revealing than light microscopy for studying qualitative relationships. Initially only testa surface patterns were examined for their taxonomic usefulness although Bridges and Bragg (1983) also examined hilum shape and found it to be different for each of the species examined. In addition, Baker et al (1985) examined the pleurogram (testa groove) shapes of the seeds of twenty species of eight mimosoid genera, and concluded that the pleurogram was a more useful character than surface patterns.

Despite the extent of these investigations, no standard terminology of testa surface features has yet been agreed upon. Likewise, no standard location on the testa has been established for SEM observation. Lersten (1981), who carried out extensive surface observations on 30 different Papilionoid families, has proposed nine categories of testa surface patterns, eight of which are employed by Baker et al. (1985) in their description of testa surface patterns of North American Legumes.

The nine categories are as follows :

1. Levigate - smooth up to 1000x magnification.
2. Rugulate - irregularly roughened.
3. Substriate - short parallel ridges.
4. Simple-reticulate - meshwork of ridges enclosing single cells.
5. Multi-reticulate - primary plus secondary ridges.
6. Simple foveolate - single cell ends

isolated by grooves.

7. Multi-foveolate - unit of several cells surrounded by grooves.
8. Lophate - short ridges with irregular sides.
9. Papillose - single protruding epidermal cells.

Previous work (Brisson et al., 1976; La Sota et al, 1979; Lersten, 1981; Bragg, 1983; Bridges et al., 1983; Richardson et al., 1984; Baker et al., 1985) have shown that examination of the testa pattern can reveal useful definitive taxonomic features. The seeds of 18 of the 21 species of Rafnia were therefore examined by SEM in an effort to provide evidence in support of the findings of a systematic revision of this genus (Richardson, 1986). Rafnia Thunb. is a legume endemic to the southern and south-western Cape Province of South Africa. It does not have major economic importance.

Materials and methods

As most of the existing reference material used had been air-dried in the traditional manner for herbarium collections, SEM of the seed coats was carried out on air-dried material obtained from herbarium sheets. Where possible, results from old dried material were correlated with results obtained from freshly dried seeds. In addition, using Rafnia elliptica Thunb. seeds, a comparison was made between seeds which had been chemically fixed and critical point dried and seeds which had been air-dried only, to ascertain whether the use of different preparative methods gave rise to different surface morphology. As no distinct differences were observed, air-dried material was used for the remainder of the investigation.

Seeds were selected for being free of obvious surface damage, cleaned of superficial dirt with a jet of compressed air, mounted on brass stubs with glue, sputter coated with gold using a Polaron E5100 sputter coater, and observed and photographed using a JEOL JSM U3 and a JEOL JSM 840 scanning electron microscope. All examinations were carried out at a magnification of 1000x, near the hilum, as Lersten (1981) had provided evidence that patterns are usually most conspicuous in that region. Where unrestricted numbers of seeds were available at least 6 undamaged specimens were examined in order to obtain representative observations. In some cases, however, where only a limited number of seeds were available (R. dichotoma, R.ericifolia, R. schlechteriana), a minimum of 2 undamaged seeds were examined. The hila of some species were also examined using SEM following the suggestion by Bragg (1983) that this may be another useful taxonomic feature. As Rafnia seeds are highly predated it was difficult to obtain seeds undamaged to the extent that the hila remained sufficiently intact for observation. Consequently the hila of only 3 species were examined.

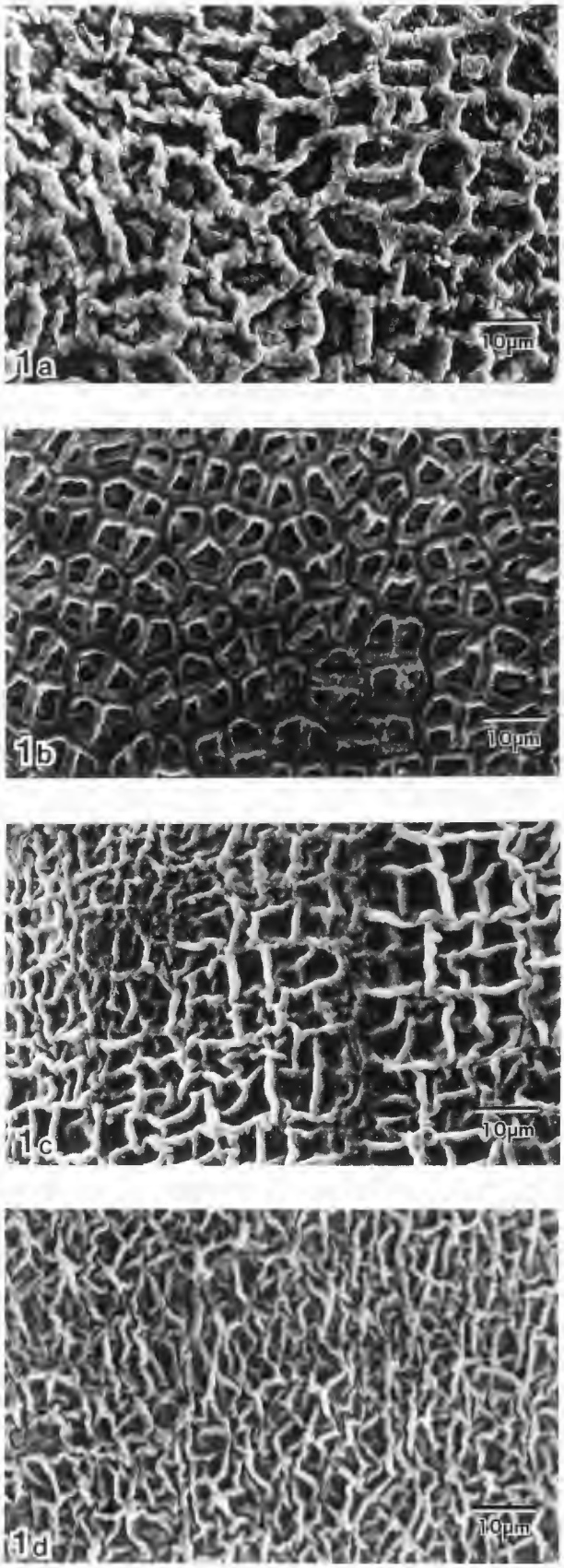
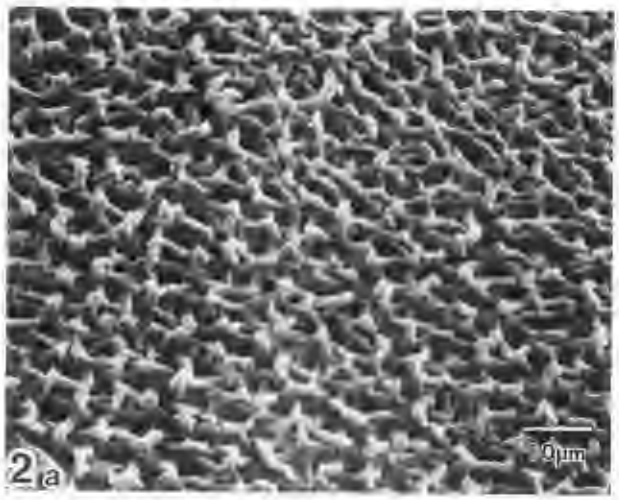
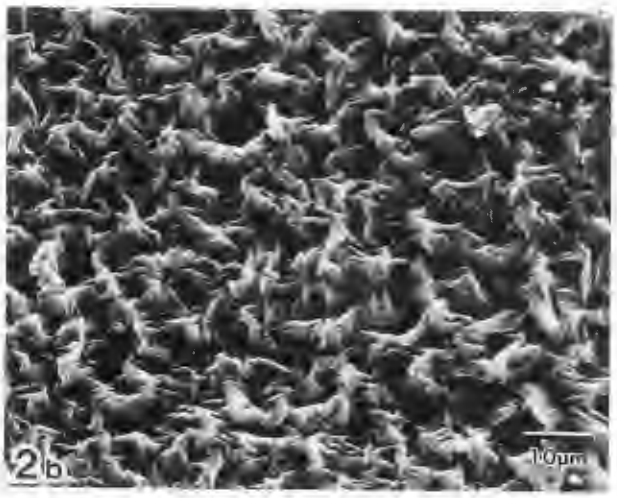
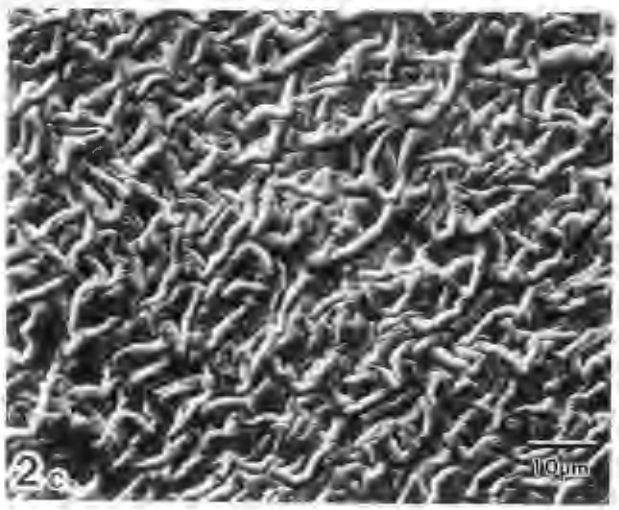


Figure 1. Variations of reticulate testa surface pattern: Simple reticulate with thick walls in R. ovata (1a) and R. schlechteriana (1b), simple reticulate with thin walls in R. capensis (1c) and multi-reticulate in R. diffusa (1d).







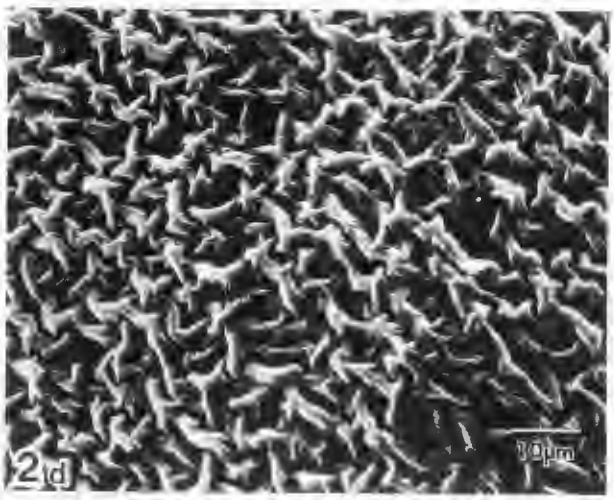


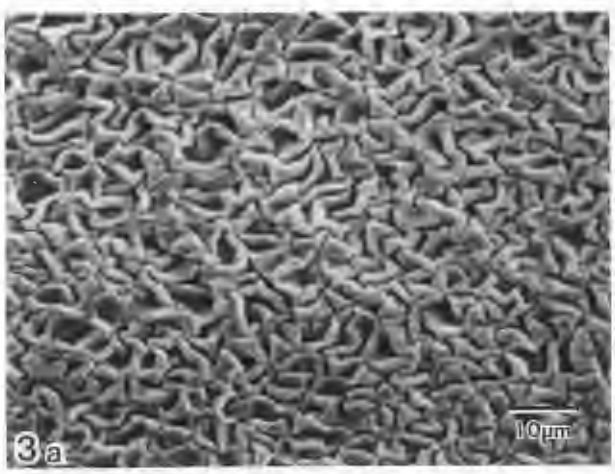
Figure 2. Rugulate testa surface patterns: Simple rugulate in R. racemosa (2a), R. swartbergensis (2b), R. meyeri (2c), R. lancea (2d) and R. angulata (2e).

Results

Four of the nine categories proposed by Lersten (1981) were found among the 18 Rafnia species studied. Seeds of R. ovata (Berg.) Schinz, R. schlechteriana Schinz, R. capensis (L.) Schinz, and R. diffusa Thunb. exhibited a reticulate pattern (Fig. 1). The testa pattern on the seeds of R. ovata (Fig. 1a) and R. schlechteriana (Fig. 1b) was reticulate with thick walls, R. capensis (Fig. 1c) was reticulate with thin walls, and R. diffusa (Fig. 1d) was multi-reticulate with thin walls.

Seeds of many of the species examined showed a generally rugulate pattern but were still individually distinguishable if comparisons of SEM micrographs were made. This applied to R. racemosa Eckl. & Zeyh. (Fig. 2a), R. swartbergensis Richardson and Vincent ined. (Fig. 2b), R. meyeri Schinz (Fig. 2c), R. lancea (Thunb.) DC, (Fig. 2d) and R. angulata Thunb. (Fig. 2e). Rafnia thunbergii Harv. (Fig. 3a) had a rectangular compressed rugulate pattern and R. dichotoma Eckl. & Zeyh. (Fig. 3b) had a striate-rugulate pattern. Rafnia vlokii Richardson and Vincent ined. (Fig. 4a), R. amplexicaulis (L.) Thunb. (Fig. 4b) and R. perfoliata (Thunb.) E. Mey. (Fig. 4c) had a tuberculate pattern. The latter three species were

differentiated from each other by the occurrence and shape of these tubercles (La Sota et al,1979). Rafnia vlokii had tubercles which appeared to be continuous and the pattern was therefore confluent. In R. amplexicaulis and R. perfoliata the tubercles were separated and their shape distinguished the two species. Rafnia amplexicaulis had limpet-shaped tubercles, while in the case of R. perfoliata the tubercles were humped. Finally, R. elliptica Thunb., R. crassifolia Harv., R. triflora (L) Thunb. and R. ericifolia Salter exhibited very distinct testa patterns. Rafnia elliptica (Figs. 5a & b) had an foveolate pattern of cells composed of central depressions with raised circular borders, irrespective of age of seeds used. The pattern on the testa of R. crassifolia (Fig. 5c) was papillose and no central depressions were observed on the papillae. Rafnia triflora (Fig. 5d) had a lophate pattern composed of short ridges with irregular sides. A densely involute pattern was observed on the testa of R. ericifolia (Fig. 5e).



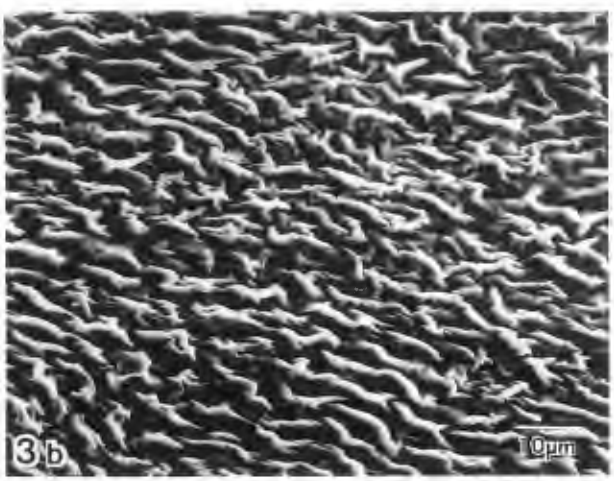
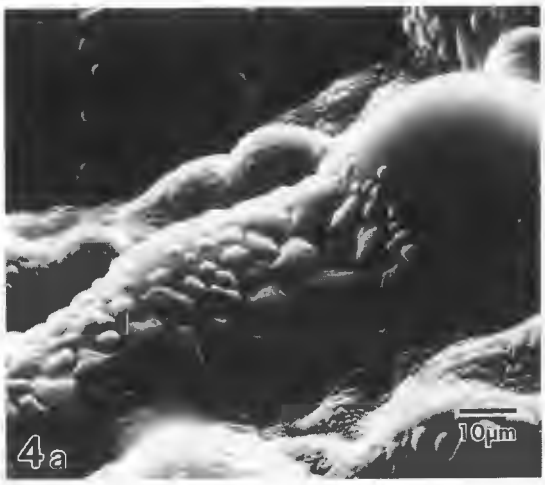


Figure 3. Modified rugulate testa surface patterns: Rectangular-compressed in R. thunbergii (3a) and striate rugulate in R. dichotoma (3b).



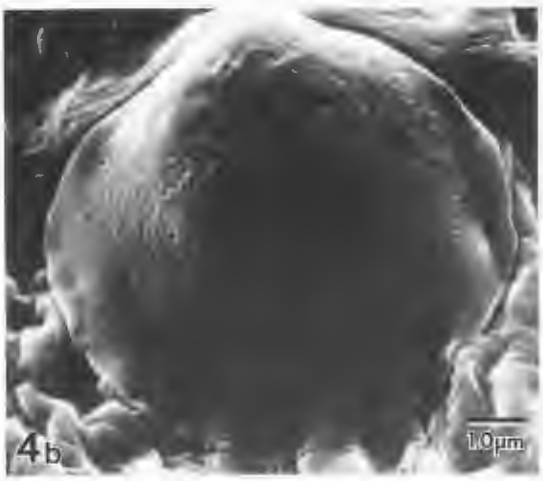
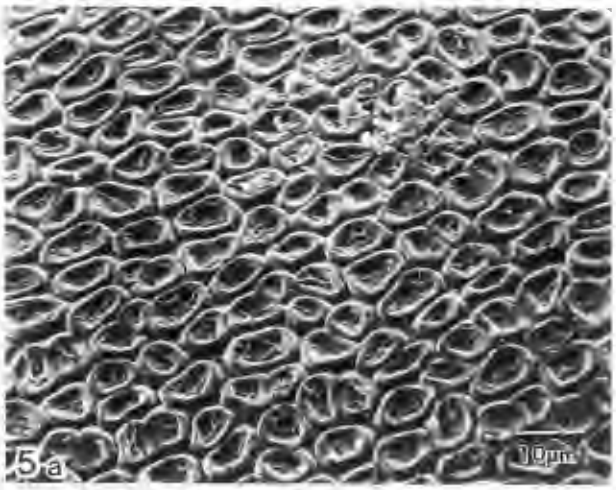
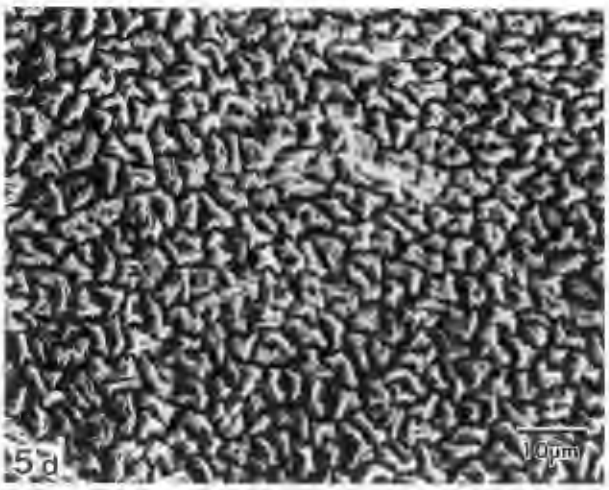
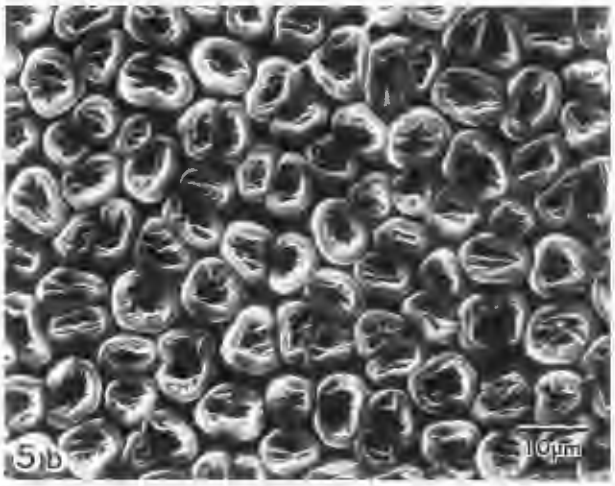


Figure 4. Variations of tuberculate testa surface patterns: Confluent tuberculate in R. vlokii (4a), limpet-shaped tuberculate in R. amplexicaulis (4b) and humped tuberculate in R. perfoliata (4c).







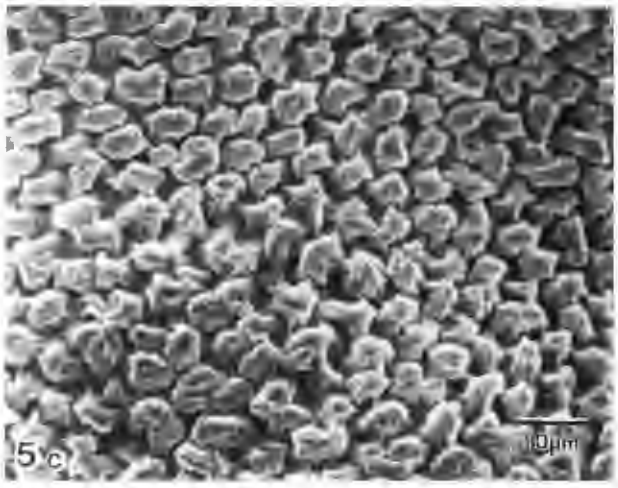


Figure 5. Other testa surface patterns: Simple foveolate in both old (5a) and fresh (5b) seeds of R. elliptica, papillose in R. crassifolia (5c), lophate in R. triflora (5d) and densely-involute in R. ericifolia (5e).

Discussion

The testa surfaces do exhibit different patterns which facilitate differentiation among the species examined; the differences in some cases being species specific and of diagnostic value (Dahlgren, 1970). Much confusion, for example, has existed in the circumscription of R. vlokii and R. elliptica but, as can be seen in Figs. 4a and 5a & b, testa patterns of these two species reveal sufficient significant differences to confirm that they are two different species. A similar situation arises with R. amplexicaulis (Fig. 4b) and R. perfoliata (Fig. 4c). While the testa surfaces of both species appear tuberculate, the tubercles of R. amplexicaulis are flat and limpet-shaped, whereas in R. perfoliata they are humped. As these (R. amplexicaulis and R. perfoliata) were the only two species examined which showed presence of discreet tubercles, their presence in these species also suggests putative phyletic affinity.

Specimen age appears to have little effect on the testa pattern. Evidence in support of this can be seen from Figs. 5a and b. Fig. 5a is of a seed

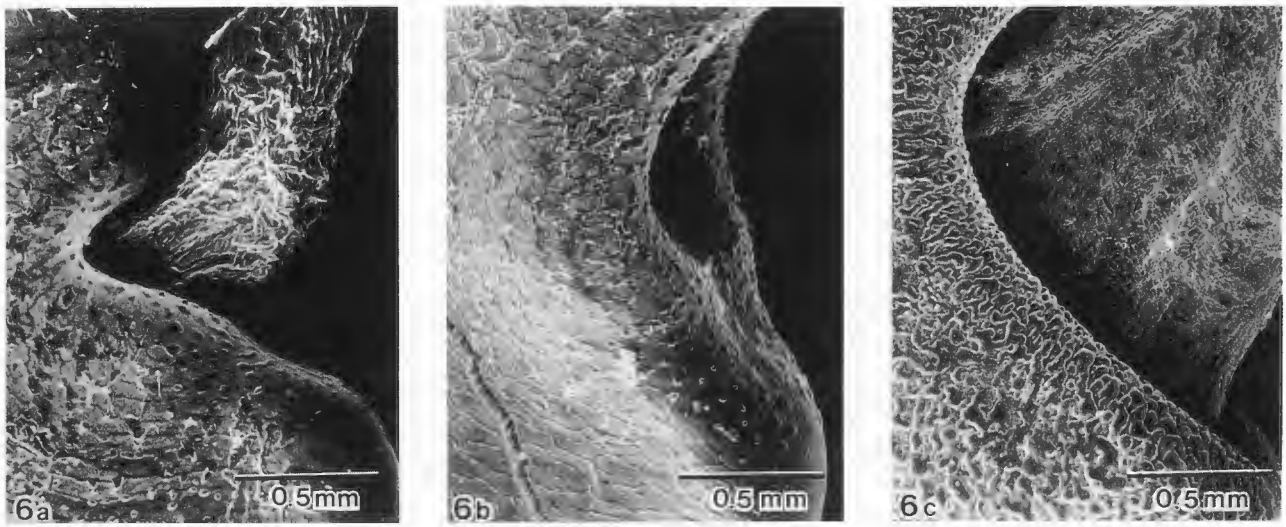


Figure 6. Acute-shaped hilum of R. perfoliata (6a) and flattened hilum of R. amplexicaulis (6b). The hilum of R. vlokii (6c) shows an intermediate shape.

of R. elliptica, taken from an old herbarium sheet (Gillet 1581, STE) while Fig. 5b is of a recently-dried seed of R. elliptica (Richardson 172, RUH).

The testa patterns of seeds of the same species from different geographical areas are also comparable. The only species of Rafnia which has a wide distribution is R. elliptica. Taking this as an example, therefore, Figs. 5a and 5b show the surfaces of seed material collected in the S.W. Cape, and Grahamstown districts respectively. The seeds have similar testa patterns. This is a clear indication that external environmental conditions have little or no effect on the surface morphology.

SEM studies of the hila of R. perfoliata (Fig. 6a) and R.amplexicaulis (Fig. 6b) also reveal distinct differences in the shape of this structure; the hilum of R. perfoliata being much more acute than that of R. amplexicaulis. The hilum of R. vlokii (Fig. 6c) is again different from the former two species being intermediate between the two. Further studies of the hilum of Rafnia might also prove to be of systematic value.

Conclusions

This study has demonstrated that features of seed testa surfaces formed an additional useful, and in some cases definitive, aid in the identification of 18 of the 21 known species of Rafnia. An extension of this study to include an SEM study of hilum shape might provide additional data from seeds useful in the delimitation and identification of species of Rafnia.

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Key to Rafnia species using testa morphology

Many species can be separated by differences in testa morphology. The following key, using SEM identified features, is based on testa surface differences as observed in the region of the hilum. The terminology is adapted from Murley (1951) and Lersten (1981).

1. Testa reticulate	2
Testa rugulate	4
2. Testa reticulate with thick walls	R. ovata
	R. schlechteriana
Testa reticulate with thin walls	3
3. Testa simple reticulate (square-elongate) with thin walls	R. capensis
Testa multi-reticulate	R. diffusa
4. Testa simple rugulate	R. racemosa
	R. swartbergensis
	R. meyeri
	R. lancea
	R. angulata
Testa compound rugulate	5
5. Testa rugulate (rectangular compressed)	R. thunbergii
Testa elongate	6
6. Testa striate-rugulate	R. dichotoma
Testa foveolate or tuberculate	7
7. Testa tuberculate	8
Testa foveolate (papillose, lophate or involute)	10
8. Tubercles discreet bodies	9
Tubercles confluent	R. vlokii
9. Tubercles limpet-shaped	R. amplexicaulis
Tubercles humped	R. perfoliata
10. Testa simple foveolate	R. elliptica
Testa papillose, lophate or involute	11
11. Testa papillose	R. crassifolia
Testa lophate or involute	12
12. Testa lophate	R. triflora
Testa densely involute	R. ericifolia

Key to names

1. reticulate	= net-like
2. rugulate	= wrinkled
3. simple foveolate	= single cell ends isolated by grooves
4. tuberculate	= presence of tubercle-like surface structures
5. papillose	= single cells, protruding without concavities
6. lophate	= short ridges with irregular sides
7. involute	= highly infolded
8. confluent	= running into each other so as to form one surface

Discussion with Reviewers

L.H. Bragg: Is there a relationship between the testa morphology and the palisade cells which are found just below the surface, or is the testa morphology independent of palisade cell occurrence?
Authors: No sections were cut through the seeds so it is not possible to state authoritatively from our observations that palisade cell arrangement has any influence on testa surface features. However, Lersten (1981) and Baker et al. (1985) have referred to sub-surface cell structure in their definitions of some testa surface patterns.

L.H. Bragg: In Figure 6b the lower left corner shows a crack which exposes the palisade cells. Are these cracks common on the seeds of this and the other included species, or is this due to mechanical injury of that particular specimen?
Authors: A re-examination of all our micrographs of seeds of this species, and a examination of further seeds, revealed no extensive cracking, thus suggesting that this crack resulted from mechanical injury.

L.H. Bragg: You have described the area around the hilum for each species in Figures 4 a-c. In Figures 6 a-c, the surface pattern away from the hilum is different from that near the hilum. Which is more representative of the testa morphology for each of the species?
Authors: Lersten (1981) recommended that observations of testa surface patterns be carried out near the hilum. Our experience with testa of Rafnia confirmed that observations made in this area are most consistently reliable.

W.J. Wolf: The differences between R. lancea (Thunb.) DC (Figure 2d) and R. dichotoma Eckl. & Seyh. (Figure 3b) appear to be very subtle. Have you looked at a number of seeds from each of these species and have you been able to identify them on the basis of their SEM micrographs?
Authors: Yes. Although only a limited number of good R. dichotoma seeds were available for this study we are confident that our identification of these species on the basis of scanning electron microscopy of the seed testa surface features is valid.