J. Indian bot. Soc. 63 : 11—19, 1984.

SEED STRUCTURE IN SOME *INDIGOFERA* SPECIES[1]

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ABSTRACT

Morphology and anatomy of seeds have been studied in ten species of *Indigofera*. Mature seeds are biconvex and their shape varies from rectangular, square, oval to spherical. Seed colour ranges between light to dark brown with a smooth surface except in *I. linifolia* where it is rough. The outline of hilum is spherical and it is located more towards one end in *I. enneaphylla*, *I. gerardiana* and *I. pseudo-tinctoria* whereas in all other species its location is in the centre of the seed. The multi-layered seed coat comprises the outermost macrosclereid layer followed by an osteosclereid layer. A distinct *linea lucida* runs throughout the macrosclereid layer. The hilar region is typically papilionaceous in structure. Considerable amount of endosperm is present in the mature seeds. The shape of embryo varies from oval, triangular to square. The scanning electron microscopical (SEM) studies reveal smooth, rugose, reticulate or tuberculate ornamentation of spermoderm in different species.

INTRODUCTION

The genus *Indigofera* is placed in the tribe Indigofereae, subfamily Papilionoideae of the family Fabaceae (Polhill, 1981). Though *Indigofera* has been and is still of considerable economic importance, studies on morphology and anatomy of seeds are confined to only a very few species (Bergthell and Day, 1907; Deshpande and Untawale, 1971; Lersten, 1981). Further, no account is available where anatomical observations are supplemented with spermodermal structure based on scanning electron microscopic (SEM) studies. Such studies are important because the seed characters are relatively consistent for a plant species and may thus prove useful in distinguishing different species and also in grouping them under definite categories. The present paper thus deals with anatomical and spermodermal pattern in ten species of *Indigofera* (see Table I).

MATERIALS AND METHODS

Mature and dry seeds of ten species of *Indigofera* (Table I) were dehydrated in tertiary-butyl alcohol series and embedded in paraffin wax. Serial microtome sections cut between 6 to 10 μm thickness were stained in safranin-fast green combination. Seeds were measured with a scale and the measurements of macrosclereids and osteosclereids were taken with the help of an occular micrometer from the sections of mature seed coat. The colours of the seeds were matched with the standard colours given in the colour dictionary (Maerz and Ree Paul, 1950). For SEM studies the central portion of lateral sides of mature seeds were photographed.

1. The paper was awarded a Merit Certificate at the VI All India Botanical Conference at Bhubaneswar in Dec., 1983.

The author is extremely grateful to Professor B. S. Trivedi for valuable guidance and constant encouragement and to U. G. C. for the award of a Fellowship.

TABLE I

INDIGOFERA SPECIES STUDIED

S. no.	Name of the species	Places from where collected/obtained
1.	*I. anil* Linn.	Kyoto, Japan.
2.	*I. erecta* Thumb.	Rabat, Morocco.
3.	*I. enneaphylla* Linn.	Lucknow, U. P., India.
4.	*I. gerardiana* Wall.	Stalinabad, USSR.
5.	*I. hirsuta* Linn.	Goa, India.
6.	*I. linifolia* (Linn.f.) Ritz.	Lakhimpur Kheri, U.P. India.
7.	*I. prostrata* Willd.	Goa, India.
9.	*I. pseudo-tinctoria* Matsum.	Ashakhabad, USSR and Kasukaba, Japan.
9.	*I. suffruticosa* Mill.	Urban Service Dept,. Honkong.
10.	*I. tinctoria* Linn.	Mongher, Bihar, India.

OBSERVATIONS

Mature seed : Shape of the seed is nearly oval with a notch on one side in *I. enneaphylla* and *I. pseudo-tinctoria* (Fig. 1 I, K), rectangular in *I. erecta* (Fig. 10), almost square in *I. anil*, rectangular-oblong with round ends in *I. tinctroria* (Fig. 1 M), cylindrical-oblong with round ends in *I. gerardiana, I. hirsuta, I. prostrata* and *I. suffruticosa* (Fig. 1 Q) and spherical in *I. linifolia* (Fig. 1 S). The lateral sides of the seeds are biconvex.

The colour of the seed is chocolate in *I. anil* and *I. suffruticosa*, brown in *I. erecta* and *I. tinctoria*, deep brown in *I. gerardiana, I. prostrata* and *I. pseudo-tinctoria*, chrom lemon or light-pinkish-peach in *I. enneaphylla*, dark brown or dark green or light orange mottled with black patches in *I. hirsuta* and dark brown in *I. linifolia*.

The seed surface is almost smooth with dull appearance in *I. anil, I. erecta* and *I. suffruticosa* while shining in six other species. In *I. linifolia* it is rough and dull. The hilum is spherical in outline in all the species and is centrally located (Fig. 1 N, P, R) except in *I. enneaphylla, I. gerardiana* and *I. pseudo-tinctoria* where it is placed towards one side (Fig. 1 J, L). The colour of the hilum is black in *I. anil*, yellow with a black coloured area in *I. enneaphylla*. white surrounded by a black coloured area in *I. erecta* and *I. pseudo-tinctoria*, deep brown with a light brown rim in *I. gerardiana*, black with dark-green rim in *I. hirsuta* and *I. suffruticosa*, brown with a black area in *I. tinifolia*, light brown with a dull white rim in *I. prostrata* and dark brown in *I. tinctoria* The size of seeds of different species have been recorded in Table II, and from the data in will be evident that the largest seeds are in *I. gerardiana* and smallest in *I. prostrata*.

Seed coat : The seed coat for the major part in the mature seed consists of 4 to 6 layers of cells including the two

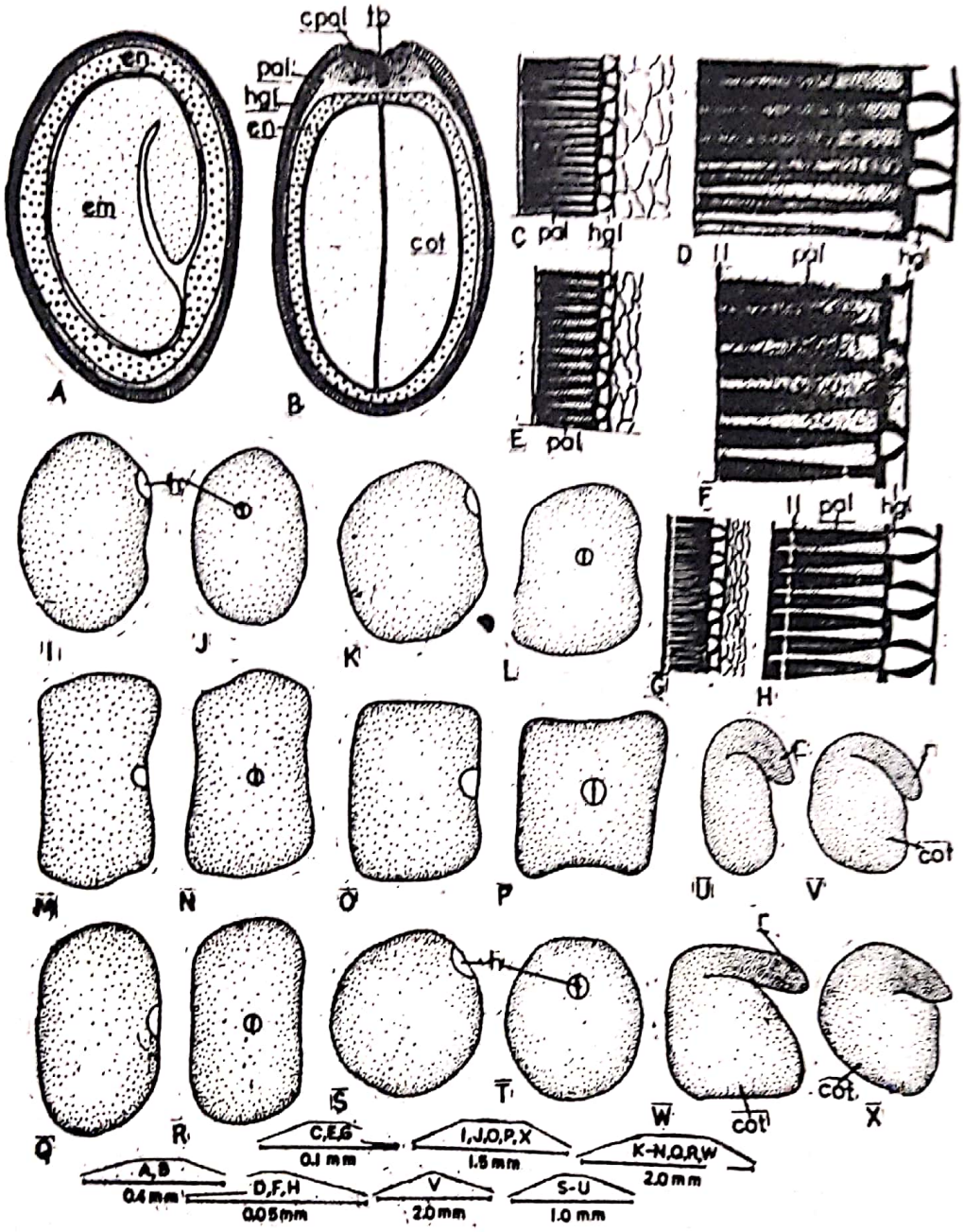

Fig. 1—A—X *Indigofera* species

A, Longi-section of seed of *I. prostrata*, B. Cross-section of seed of *I. prostrata* passing through hilar region. C, E, G. Longi-sections of part of seed coat of *I. hirsuta*, *I. tinctoria* and *I. linifolia* respectively. D, F, H. Magnified view of macrosclereids and 'hour-glass' cells of *I. hirsuta*, *I. tinctoria* and *I. linifolia* respectively. I, K, M, O, Q, S Lateral view of seeds of *I. enneaphylla*, *I. pseudo-tinctoria*, *I. tinctoria*, *I. erecta*, *I. hirsuta*, *I. linifolia* respectively. J, L, N, P, R, T. Seeds of *I. enneaphylla*, *I. pseudo-tinctoria*, *I. tinctoria*, *I. erecta*, *I, hirsuta*, *I. linifolia* respectively, showing hilum in front view. U, V, W, X. Lateral view of embryo of *I. prostrata*, *I. hirsuta*, *I. anil*, *I. enneaphylla* respectively.

(cot- coytledon, cpal- 'Counter-palisade' layer, em- embryo, en- endosperm, h- hilum, hgl- 'hour-glass' cells, ll- *linea lucida*, pal-palisade layer, r-radicle, th- 'tracheid-bar').

TABLE II

SEED MORPHOLOGY IN *INDIGOFERA* SPECIES

Name of species	Seed			Spermederum	Hilum		Seed size L X B X T (MM)
	Shape	Colour	Surface		Shape	Colour	
I. anil	Almost square	Chocolate	Dull, smooth	—	Spherical	Black	1.9×1.7×1.5
I. enneaphylla	Oval with a notch on one side	Light yellow or pinkish peach (chrome lemon or Jasmine Tition gold)	Bright shining, smooth	Smooth	Spherical	Yellow with a black rim	1.2×.9×.8
I. erecta	Rectangular	Brown (Burnt umber or Biskra date)	Dull, smooth	Smooth	Spherical	White with a black rim	1.75×1.4×.2
I. gerardiana	Rectangular	Deep brown (cocoa cattail)	Smooth, shining	Reticulate	Spherical	Deep brown with a light brown rim	3×2.1×2.5
I. hirsuta	Elongated oval	Dark brown or green with black patches (Mandalay of ivy green)	Slight shining and smooth	Rugose	Spherical	Black with dark green rim	2.3×1.5×1.1
I. linifolia	Spherical	Dark brown (Mandalay friar)	Dull, rough granulated	—	Spherical	Brown with black rim	1.4×1.4×.75
I. prostrata	Elongated oval	Deep brown or pinkish peach (Cocoa or Jasmine)	Shining, smooth	Rugose	Spherical	Light brown with dull white rim	1×.7×.4
I. pseudo-tinctoria	Almost oval with a notch on one side	Dark brown (Cocoa or Argas brown)	Shining, smooth	Tuberculate	Spherical	White with black rim	2×1.9×1.6
I. suffruticosa	Almost rectangular	Chocolate or deep green (Ivy green)	Dull, smooth	Reticulate	Spherical	Black with dark green rim	2.3×1.6×1.5
I. tinctoria	Almost rectangular	Brown (Inca Gold or Burnt umber)	Shining, smooth	Tuberculate	Spherical	Dark brown	2.1×1.2×1.3

outer important layers (Fig. 1 C, E, G). The outermost layer is composed of lignified palisade-like cells, referred to as macrosclereids (Figs. 1D, F. H.). There exists uniformity in the structure of macrosclereids in all the ten species studied. The lignification on the radial walls of these cells is more towards the outer surface and getting reduced towards inner resulting in the broad lumen on the latter side (Fig. 1D, F, H). The lumen of these cells is filled with tanniniferous substances. The outer surface of the macrosclereids is covered by a cuticle in all the species. The *linea lucida* lies very close to the outer surface and runs uniformly throughout the macrosclereids in all the species (Fig. 1 C, E, D, F) except in *I. linifolia* where it lies slightly deeper (Fig. 1 G, H). The length and breadth of macrosclereids are given in Table III; evidently maximum length of these cells is in *I. suffruticosa* (53 microns) and the minimum is in *I. prostrata* (18 microns). However, the breadth of these cells ranges between 5 to 10 microns. The macrosclereids near the hilar region are longer in size in all the species.

Next to macrosclereid layer is the layer of thick-walled, biconcave osteosclereids having a truncate shape with a broad base narrowing somewhat towards the top (Fig. 1 C, D, E, F, G, H). Very conspicuous nearly triangular-shaped air spaces exist between these cells. Length and breadth of the osteosclereids in different species are given in Table III from which it is clear that the length ranges between 5 microns (*I. enneaphylla*) to 16 microns (*I. linifolia, I. suffruticosa.*) However, the minimum breadth of the

TABLE III

LENGTH AND BREADTH OF MACROSCLEREIDS AND OSTEOSCLEREIDS IN *INDIGOFERA* SPECIES.

Name of species	Macrosclereids		Osteosclereids		
	Length (microns)	Breadth (microns)	Length (microns)	Breadth (microns)	
				Base	Top
I. anil	42	10	13	19	13
I. enneaphylla	19	5	5	13	8
I. erecta	40	8	10	14	8
I. gerardiana	43	8	11	22	14
I. hirsuta	32	10	10	24	13
I. linifolia	29	8	16	16	10
I. prostrata	18	5	6	14	10
I. pseudo-tinctoria	42	8	13	19	10
I. suffruticosa	53	6	16	16	10
I. tinctoria	43	10	10	24	16

base and top regions is in *I. anil* (13 microns and 8 microns) and the maximum is in *I. tinctoria* (24 microns and 13 microns). The size of the osteosclereids occurring near the hilar region is considerably increased in all the species and in the hilar region these cells are completely missing (Fig. 1 B). Remaining two to four layers of the seed coat consist of highly vacuolated and degenerating parenchymatous cells (Fig. 1 C, E G).

The hilar region is very characteristic in having one more palisade-layer, the 'counter-palisade layer.' The structure and size of the cells of both the palisade layers are almost similar in the individual species except in *I. prostrata* and *I. tinctoria* where the cells of counter-palisade layer are slightly bigger than those of palisade layer. The *linea lucida* is also not found in counter-palisade layer. A long groove is present almost in the centre of the hilum and in this region a group of tracheid-like cells, the 'tracheid bar', is well-marked. In a cross-section, the pear-shaped 'tracheid-bar' is discernible directly under this groove (Fig. 1 B). The structure of the 'tracheid-bar' is similar in all the species. Rest of the hilar cells are thick-walled, stellate or rounded with abundant deposition of tanniniferous substances.

Endosperm : Seed of all the species possess considerable amount of endosperm surrounding the embryo (Fig. 1 A, B), though Deshpande and Untawale (1971) have reported complete absence of endosperm in the mature seeds of *I. enneaphylla*. Seeds of *Indigofera* species are well-known to possess water soluble mucilage (galactomannan) which is located in the endosperm (Farooqui, 1975).

Mature embryo : The shape of embryo along the long axis is somewhat squarish in *I. anil* (Fig. 1 W), elongated oval with a notch on one side in *I. prostrata* and *I. tinctoria* (Fig. 1 U) and nearly triangular in *I. enneaphylla* (Fig. 1 X). However, in remaining six species it is almost oval with a notch on one side (Fig. 1 V). The hypocotyl-root-axis is quite long and curved. The two cotyledons are thick and fleshy (Fig. 1 A, B).

Spermoderm pattern : The spermoderm pattern found in the eight species presently studied can be grouped in the following four categories :

a) *Smooth :* The seed surface is almost smooth in *I. erecta* and *I. enneaphylla*. In the former species polygonal areas can be demarcated all over (Fig. 2 A), whereas it is not so in latter. The surface has flakes of waxy depositions.

b) *Rugose :* This type of pattern is seen in *I. hirsuta* and *I. prostrata*. The surface exhibits haphazardly entangled rugae. The rugae have almost uniform thickenings but are of varying length (Fig. 2 B).

c) *Reticulate :* Spermoderm has complex network of interwoven rugae. The elongation of reticulae is more in the same direction. Thickness of rugae is nearly uniform but at places they give beaded appearance e. g. *I. gerardiana* and *I. suffruticosa* (Fig. 2 C).

d) *Tuberculate :* The tubercules are arranged in regular rows and are dome-shaped in *I. tinctoria* (Fig. 2 F) but in *I. pseudo-tinctoria* the surface depicts undulations with troughs and crests. In Japanese samples of *I. pseudo-tinctoria* seeds, the crests seem to be occupied by distinct tubercles which appear as mounds (Fig. 2 E), whereas in USSR samples the tubercles are not so well-

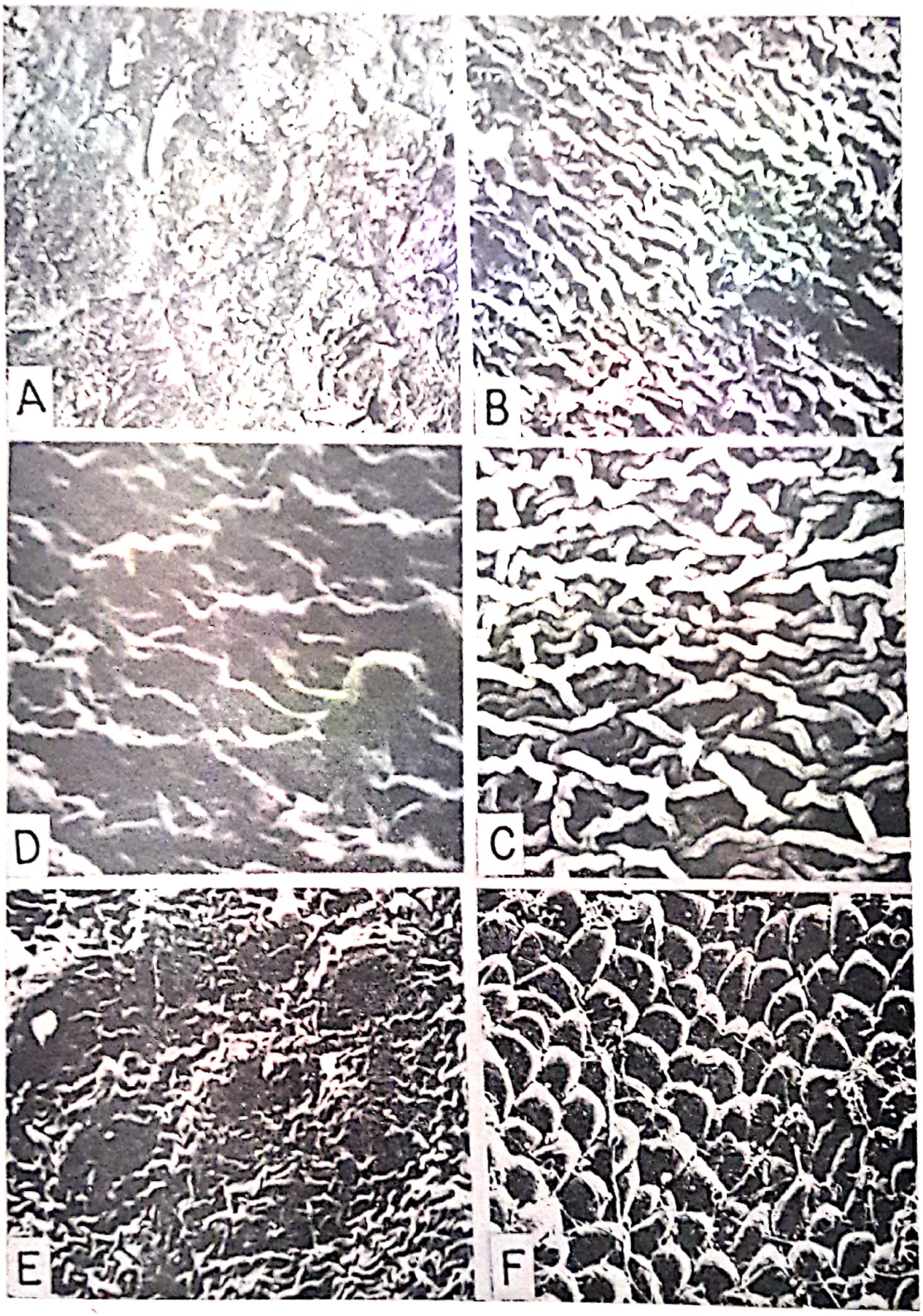

Fig. 2 A-F Spermoderm pattern in *Indigofera* species. A. *I. erecta*, B. *I. prostrata*, C. *I. suffruticosa*, D. *I. pseudo-tinctoria* (U.S.S.R.), E. *I. pseudo-tinctoria* (Japan), F. *I. tinctoria*. (A–E ×4000; F ×200.)

marked (Fig. 2 D) and have indistinct mounds.

DISCUSSION

Morphology of the seeds varies slightly in size, shape, colour, surface, texture and hilum colour but the shape of hilum is similar in all the species studied. The seed coat is about seven cell-layers thick and is derived from the outer integument only. The outermost macrosclereid layer is composed of radially elongated, highly lignified palisade-like cells covered with a cuticular layer. Thickening in the macrosclereids in all the species is more towards outer surface getting thinner towards the inner side thus resulting in a narrower lumen towards the peripheral surface. A distinct *linea lucida* runs throughout the macrosclereid layer near its peripheral region. However, Deshpande and Untawale (1971) have not reported this feature in *Indigofera enneaphylla*. *Linea lucida* is considered to be an important characteristic feature of papilionaceous seed coats (Netolitzky, 1926; Corner 1976; Gunn, 1981).

The osteosclereids are truncate with a broad base narrowing upwards. Conspicuous triangular air spaces occur in between them. Similar type of osteosclereids has been reported in *Indigofera arrecta* by Bergthell and Day (1907). However , in *I. enneaphylla* these are reported to be nearly 'I-shaped' (Deshpande and Untawale, 1971). During the present study the osteosclereids are found to be arranged in a single row, though Deshpande and Untawale (1971) observed these cells to be arranged in two rows, an observation which might have been recorded from oblique sections.

The conspicuous hilar region is characterised by the presence of 'counter-palisade' layer, palisade layer and 'tracheid-bar', a characteristic feature of the members of Papilionoideae (Netolitzky, 1926; Corner, 1951; Gunn, 1981). The cells of 'counter-palisade' layer are devoid of *linea lucida* whereas it is distinctly seen in the main palisade-like cells of this region.

The endosperm tissue in all the species is tough and hyaline with indistinct cellular structure. Same type of endosperm tissue has been reported in *Crotalaria* species (Wellendorf, 1964).

The scanning electron microscopy reveals four types of spermoderm ornamentation in species presently studied; (1) Smooth, (2) Rugose, (3) Reticulate and (4) Tuberculate. Lersten (1981) has reported occurrence of levigate surface with mounds in *Indigofera cordifolia*, reticulate in *I. subulata* and rugulate with mounds in *I. pseudo-tinctoria*. The present observation on *I. pseudo-tinctoria* confirms the finding of Lersten.

According to Skvortsov and Rusanovitch (1973), the spermoderm is genetically affected and is the main cause of intra-or inter-specific variations. Lersten (1981) has stated that seed surface pattern reflects epidermal configuration and cuticular deposition as influenced by seed expansion.

The seeds of *I. pseudo-tinctoria* obtained from two sources namely, U. S. S. R. and Japan during the present study revealed same spermoderm pattern (undulating-tuberculate type), but the tubercles are distinct and regularly arranged on crests in Japanese sample while in USSR sample the tubercles do not appear to be so distinct and regularly arranged. Such a difference appears to be due to varying amount of surface deposition . Sharma *et al.* (1977) have concluded that such differences in *Phaseolus sublobatus* are due to eda-

phic factors rather than climatic. However, Gutterman and Heydecker (1973) have demonstrated that day length also affects seed coat structure in *Ononis secula*.

As observed during the present investigation, seed surface patterns provide a useful basis for distinguishing the species and grouping them within a genus, but more species need to be investigated for providing key to identification as the genus *Indigofera* consists of about seven hundred species (Polhill, 1981).

REFERENCES

Bergthell, C. and D. L. Day 1907. On the causes of 'hardness' in the seeds of *Indigofera erecta*. *Ann. Bot.* **21** : 51-61.

Corner, E. J. H. 1951. The leguminous seed. *Phytomorphology* **1** : 117-134.

Corner, E. J. H. 1976. '*The seeds of Dicotyledons*' Vols. I & II. Cambridge Univ. Press, Cambridge.

Deshpande, P. K. and A. G. Untawale 1971. Development of seed and fruit in *Indigofera enneaphylla* L. *Bot. Gaz.* **132** : 96-112.

Farooqi, M. I. H. 1975. A search for new Sources of industrial seed gums. *Tech. Report* PL-480, *Natl. Bot. Gdns.*, Lucknow.

Gunn, C. R. 1981. Seeds of Leguminosae. In : '*Advances of Legume Systematics*. Eds. R. M. Polhill and P. H. Raven. pp. 913-925. Roya' bot. Gdn. Kew.

Gutterman, Y. and W. Heydecker 1973. Studies of the surfaces of desert plant seeds 1. Effect of day length upon maturation of the seed coat of *Ononis secula* Guss. *Ann. Bot.* **37** : 1049-1050.

Lersten, N. R. 1981. Testa topography in Leguminosae subfamily Papilionoideae *Proc. Iowa Acad. Sci.* **88** : 180-191.

Maerz, A. and M. Ree Paul 1950. *A dictionary of color* 2nd Ed. McGrew Hill Book Co. Inc., New York.

Netolitzky, F. 1926. '*Anatomie der Angiospermen Samen*' Linsbaeur Handb. Pfl. Anat. Vol. 10, Berlin.

Polhill, R. M. 1981. 'Papilionoideae'. In: *Advances in Legume Systematics* (eds. R. M. Polhill, and P. H. Raven) pp. 191-208, Royal bot. Gdns., Kew.

Sharma, S. K., C. R. Babu, B. M. Johri and A. Hepworth 1977. SEM studies on seed coat pattern in *Phaseolus mungo*, *P. radiatus-sublobatus*. *Phytomorphology* **27** : 106-111.

Skvortsov, A. K. and I. I. Rusanovitch 1974. Scanning electron microscopy of the seed coat surface in *Epilobium* species. *Bot. Nat.* **127** : 292-401.

Wellendorf, M. 1964. Morphology and anatomy of *Crotalaria* seeds. *Lloydia* **27** : 251-253.