Environmental and Experimental Biology (2011) 9: 105–113 *Original Paper*

Morphological and micromorphological characterization of some legume seeds from Gujarat, India

Dhara Gandhi, Susy Albert*, Neeta Pandya

Department of Botany, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara 390 002, Gujarat, India

*Corresponding author, E-mail: drsusyalbert@rediffmail.com, susyalbert63@gmail.com

Abstract

Light and scanning electron microscopical studies on seed morphological and micromorphological features of 17 legume species belonging to three genera (*Crotolaria*, *Alysicarpus* and *Indigofera*), of Faboideae, Fabaceae were examined and described. Seed characteristics, particularly exomorphic features, that are revealed through scanning electron microscopy can be used in resolving problems of systematics of species. Mature seeds of *Crotolaria* differ from those of *Alysicarpus* and *Indigofera* by its prominent kidney shape. Seeds of *Alysicarpus* and *Indigofera* are biconvex and shape varies from rectangular spherical oblong to ovoid. However, within the genera, the species differ in size, surface and hilum characteristics. The study showed that the seed coat ornamentation/spermoderm pattern can be helpful in identification of species.

Key words: hilum, legume, micromorphology, morphology, scanning electron microscopy, seed surface.
Abbreviations: SEM, scanning electron microscopy.

Introduction

Exomorphic features of seeds, in addition to vegetative and reproductive characters, have long been employed as important tools in various scientific studies. However, most of the light microscopic feature used are concerned with general shape and size rather than details of surface ornamentation.

Seed morphology has been shown to provide useful characteristics for the analysis of taxonomic relationships in a wide variety of plant families (Esau 1953; Shelter 1986; Takhtajan 1991; Buss et al. 2001, Zhang et al. 2005; Gontchaova et al. 2009). In addition to gross morphology of seeds, sculpturing details of outer seed coat are quite variable between different species and can be of systematic importance. (Chowdhury, Buth 1970; Gohary, Mohammed 2007). Seed characteristics, particularly exomorphic features revealed by means of scanning electron microscopy (SEM), have been used in resolving problems of systematics of species (Karihaloo, Malik 1994; Koul et al. 2000) and evolutionary relationships (Segarra, Mateu 2002). The importance of ultrastructural pattern analysis of the seed coat observed under the SEM has been well recognised as a reliable approach for assessing phenetic relationship and identification of species or taxa (Barthlott 1981; Tobe et al. 1987; Koul et al. 2000; Yoshizaki 2003; Javadi, Yamaguchi, 2004).

The Indian subcontinent is the centre of origin, endemism and diversity of a large number of cultivated legumes. The genus *Crotalaria* represents the largest legume taxa in India. *Crotalaria* species are important because of their accumulation of pyrrolizidine alkaloids. *Crotalaria juncea*, a widely cultivated fibre crop, known as Sunnhemp, Bombay hemp, Madras hemp, Rattle pods, etc. is not known in the wild, and is also used for its food and medicinal values by ethnic communities. Several other species of *Crotalaria* are economically important for fibre, forage/animal feed, green manure and for medicinal purpose (Wealth of India 1950; Ambasta et al. 1986; Pandey, Gupta 2003). *Indigofera* species are rich in organic and fatty acids, flavonoids such as carotenoids, and coumarins (Yinusa et al. 2007). *Indigofera tinctoria* is used to produce indigo dyes. Some other species of *Indigofera* are used for different purposes, for example seeds of *Indigofera articulate* are used for treatment of toothache. *Indigofera oblongifolia, Indigofera suffruticosa*, and *Indigofera aspalthoides* are used as anti-inflammatories for treatment of insect stings, snake bites and swellings, and *Indigofera arrecta* extract is used to relieve ulcer pain. *Alysicarpus* is another potential crop legume rich in protein. *Alysicarpus ovalifolius,* a protein-rich fodder, is a highly palatable feed for livestock grazing in rangelands. It is a valuable component of vegetation collected and traded as fodder in many regions. *Alysicarpus vaginalis* is known as soil improver, having a good fodder and forage value, and it is also used in treatment of cough. *Alysicarpus rugosus* seed containes higher amounts of crude protein and crude lipid when compared with most of the commonly consumed pulses (Siddhuraju et al. 1992).

Various seed morphological studies of leguminous taxa have been performed from time to time (Sharma et

al. 1977; Agarwal 1984; Buth, Narayan,1986; Sahai 1999; Murthy, Sanjappa 2002; Mallick, Sawhney 2003; Salimpour et al. 2007; Al-Ghamdi, Al-Zahrani, 2010). Seed and seed coat anatomy of some members of the *Crotolaria* (Buth, Narayan 1986) and *Indigofera* (Agrawal 1984) have been studied. However, in these studies, only a few species of those in the present study were examined.

The present work has been undertaken to delinate specific variation of micromorphological characteristics in seeds of some legume species growing in association with grasses in the grasslands of the Baria and Godhra forest division in Gujarat.

Materials and methods

Seed characters of 17 species belonging to Fabaceae from three genera (*Crotolaria, Alysicarpus* and *Indigofera*) were studied using freshly collected mature seeds (Table 1). Plant specimens with mature pods were collected from different grasslands and forest areas of Baria and Godhra in Gujarat. The plant specimens were authentically identified at 'The Blatter Herbarium', St. Xavier's College, Mumbai. About 10 to 15 mature seeds of each taxon, procured by cleaning and manually separating from the pods, were used for the light and scanning electron microscopic studies. For light microscopic studies, mature, dry seeds were thoroughly cleaned with alcohol to avoid any alteration in the micromorphological features and examined for diagnostic features of shape, size, colour and size. About 10 seeds were examined for dimensional details.

Table 1. List of legume species studied

No.	Botanical name	Blatter Herbarium No.
1	*Alysicarpus bulgaumensis* Wt.	13880
2	*Alysicarpus monilifer* (L.) DC.	14351
3	*Alysicarpus procumbens* (Roxb.) Schindl	13869
4	*Alysicarpus vaginalis* (L.) DC.	–
5	*Indigofera cordifolia* B.Heyne ex Roth.	–
6	*Indigofera echinata* Willd.	19865
7	*Indigofera linifolia* (L.f.) Retz	19999
8	*Indigofera tinctoria* L.	20212
9	*Crotalaria albida* Roth.	16392
10	*Crotolaria calycina* Schrank	16434
11	*Crotolaria filipes* var. *trichophora* (Bth. ex. Baker) Cooke	16463
12	*Crotalaria linifolia* L. f.	14201
13	*Crotolaria mysorensis* Roth.	16596
14	*Crotolaria notonii* W. & A. Prodr.	17040
15	*Crotolaria orixensis* Rottler ex Willd.	17101
16	*Crotolaria retusa* L.	17261
17	*Crotolaria spectabilis* Roth.	17264

Micromorphological features and hilum characteristics were examined under SEM at the Metallurgy Department, Faculty of Technology and Engineering, The Maharaja Sayajirao University of Baroda, and photographed at different magnifications. Seed samples were washed with absolute alcohol or acetone for 1 to 2 min to remove any debris present. They were further subjected to ultrasonic cleaning by changing absolute alcohol repeatedly and then directly mounting over carbon conducting tape mounted on brass stubs. For evaluation of uniformity, seeds were placed on the stub with their dorsal, ventral and lateral side upwards so that characteristic features of all the different sides could be scanned and photographed using JEOL JEM - 5610 SEM. To achieve better resolution the accelerating voltage varied up to 15 kV.

Results and discussion

The study of epidermal surfaces revealed a number of important micro morphological characters, which exhibited interesting interspecific variation that was of significance for identification (Fig. 1 to 4). In the present work both light microscopic and scanning electron microscopic studies were used which complemented each other in obtaining a perfect differentiation between species.

Morphology of the seeds varied significantly in size, shape, colour, surface and hilum colour. Seeds of *Crotolaria* were characteristically kidney or bean shaped, compared to oblong to rectangular seeds of *Indigofera*.

Seed colour appeared to be of less diagnostic and system value. Presence of a cracked surface was a common feature noted in many of the legume seeds (Table 2). Seed coat pattern could be categorized into smooth, cracked and papillate. Except *Crotolaria spectabilis* and *Corotolaria albida,* all other species of *Corotolaria* had a smooth surface. Among the four different species of *Indigofera*, *Indigofera tinctoria* seeds were rectangular while all of the other species were ovoid. The hilar region is characteristic, as in all Papilionaceous seeds, with a very specialized organisation. Seeds of *Crotolaria* are characterised by a lateral notch formed between the radical tip and the cotyledon, which is the seat of hilum.

In all of the species of Indigofera, hilum was present in the center of the seed and was spherical in shape. A common feature observed by SEM was a pitted structure present on the surface. In *Alysicarpus,* the hilum was located slightly away from the center, towards the distal part of seed, which gave a dumbbell shaped appearance to the seed when viewed laterally. *Indigofera tinctoria* differed from *Indigofera linifolia* by having a smooth surface. *Indigofera cordifolia* and *Indigofera echinata* had a pitted surface.

According to Skvortsov and Rusanovitch (1974) the spermaoderm characteristics are genetically determined and are the main source of intra- or interspecific variation. Lersten (1981) stated that the spermaoderm pattern reflects

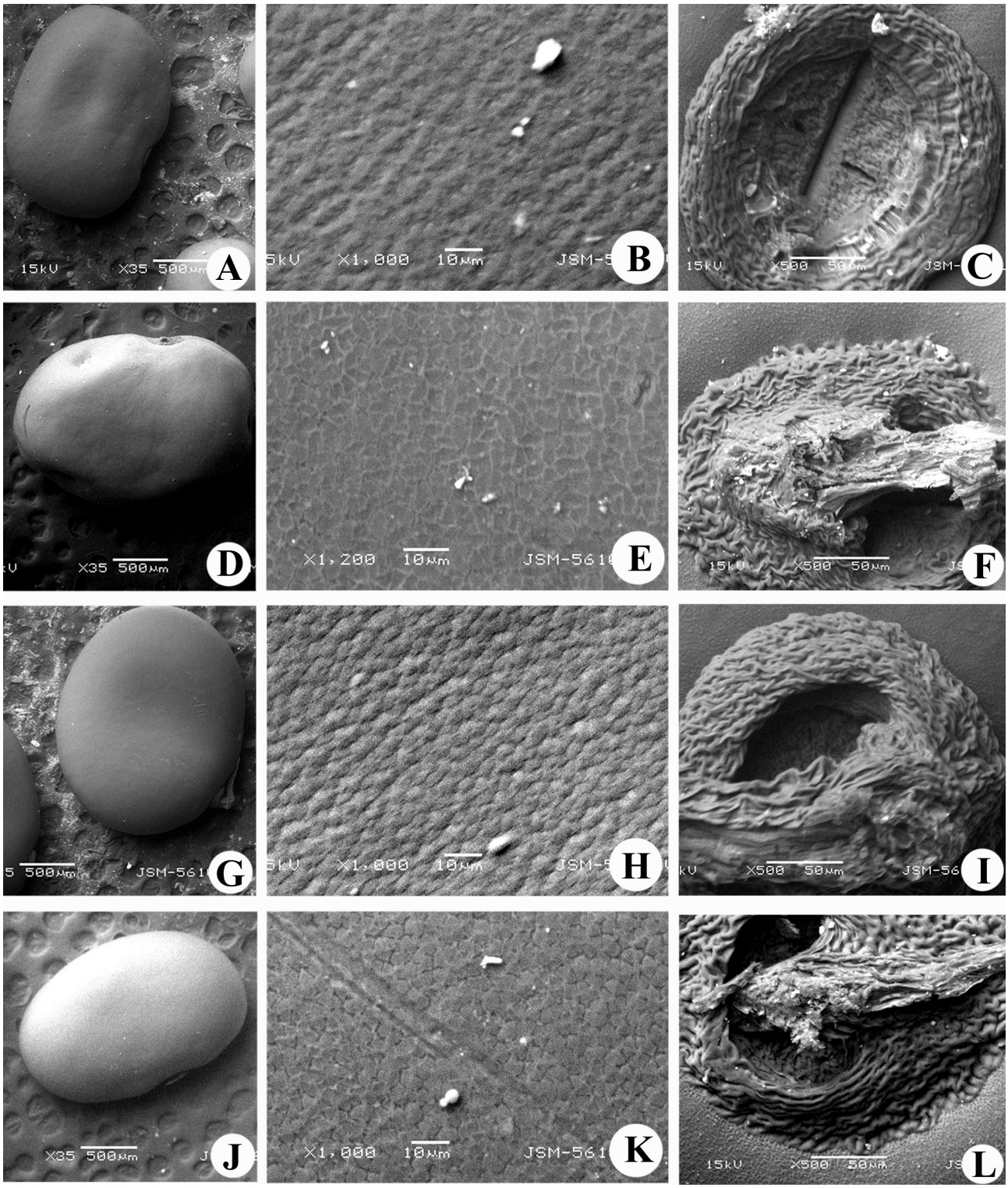

Fig. 1. Scanning electron microscopy study of legume seeds *Alysicarps bulgaminensis* (A, B, C), *Alysicarpus monilifer* (D, E, F), *Alysicarpus procumbens* (G, H, I), *Alysicarpus vaginalis* (J, K, L). A, D, G, J, micrographs of seeds; B, E, H, K, micrographs of seed surface; C, F, I, L, micrographs of hilum.

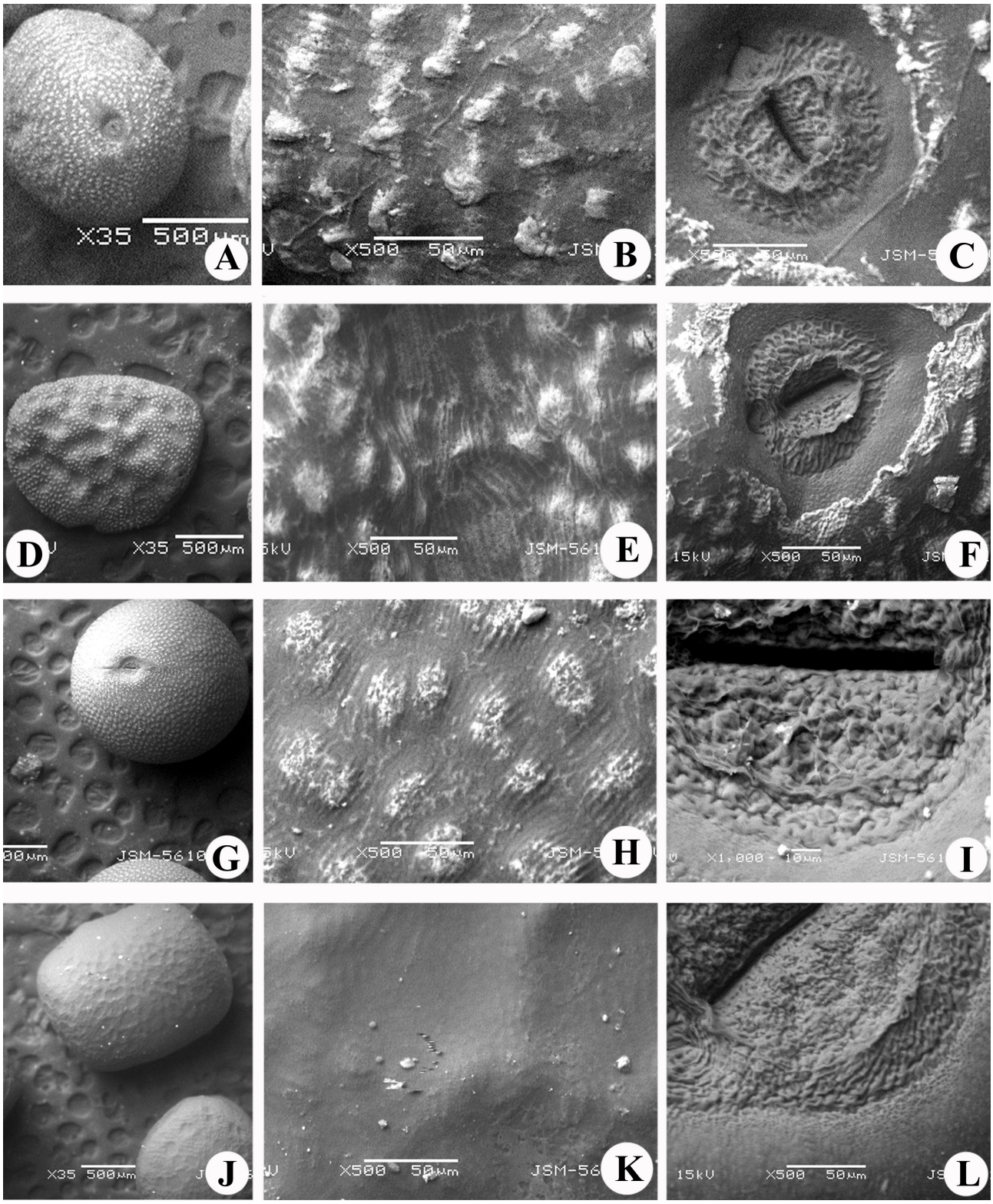

Fig. 2. Scanning electron microscopy study of legume seeds *Indigofera cordifolia* (A, B, C), *Indigofera echinata* (D, E, F), *Indigofera linifolia* (G, H, I), *Indigofera tinctoria* (J, K, L). A, D, G, J, micrographs of seeds; B, E, H, K, micrographs of seed surface; C, F, I, L, micrographs of hilum.

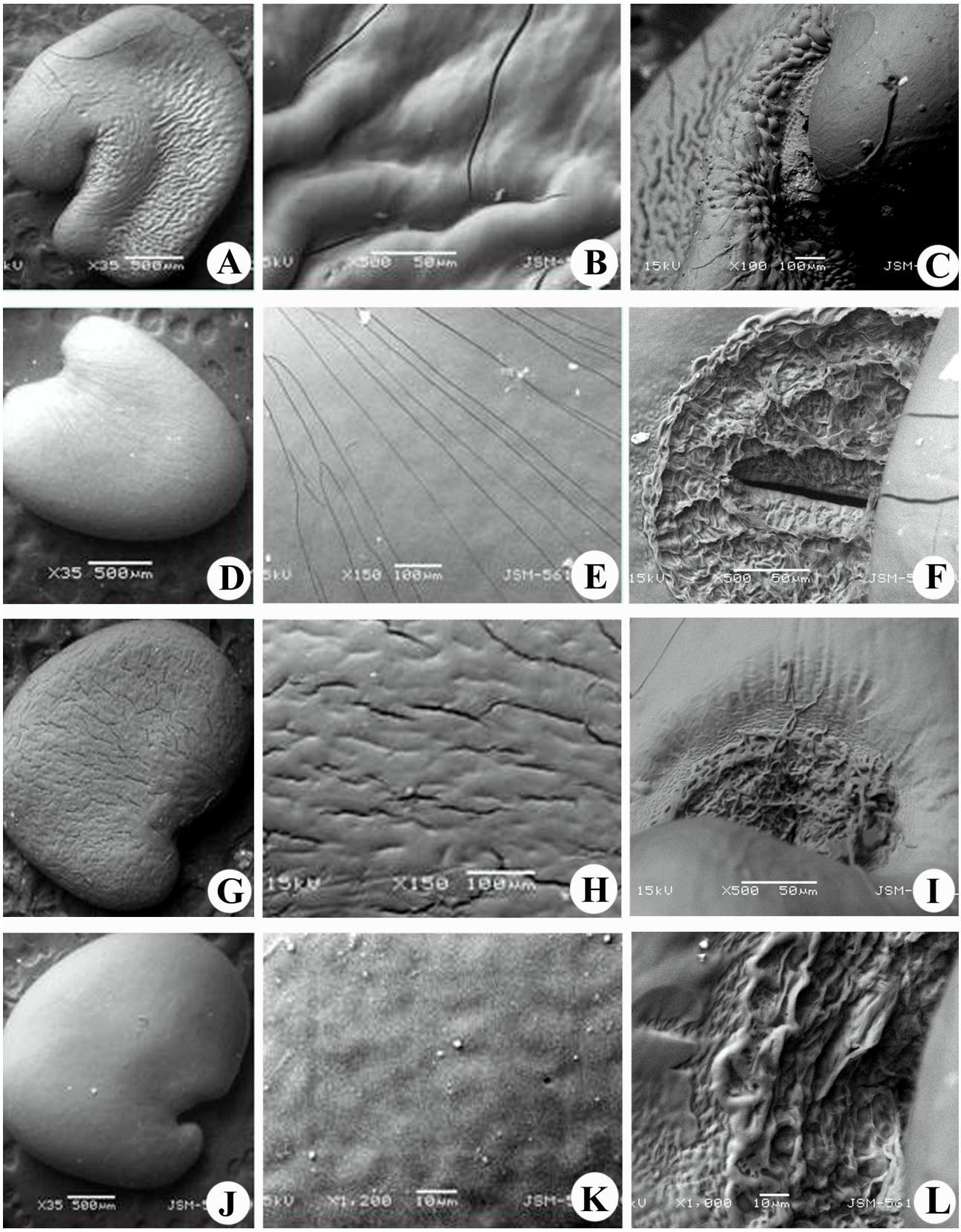

Fig. 3. Scanning electron microscopy study of legume seeds *Crotolaria albida* (A, B, C), *Crotolaria calycina* (D, E, F), *Crotolaria filipes* var. *trichophora* (G, H, I), *Crotolaria linifolia* (J, K, L). A, D, G, J, micrographs of seeds; B, E, H, K, micrographs of seed surface; C, F, I, L, micrographs of hilum.

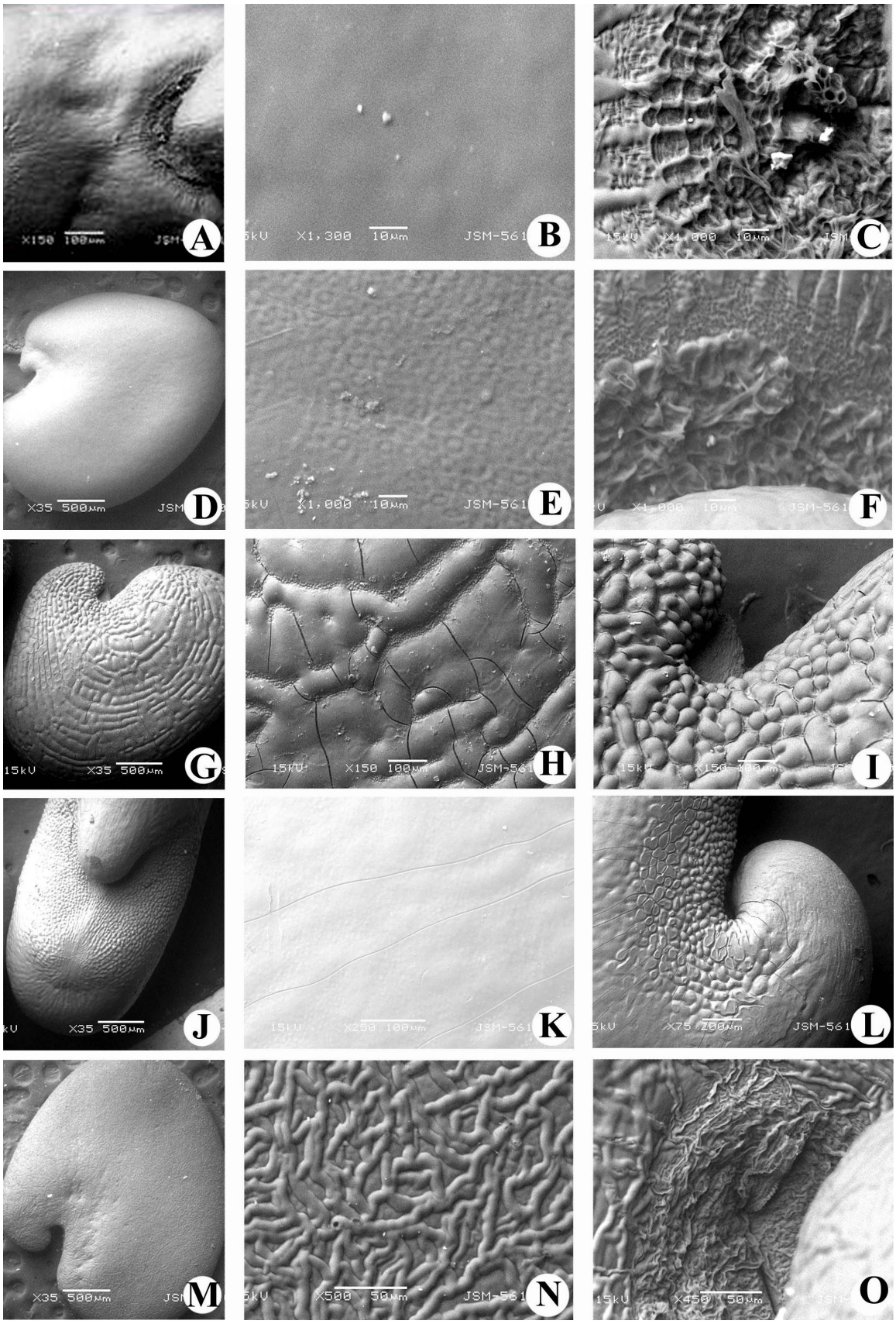

Fig. 4. Scanning electron microscopy study of legume seeds *Crotolaria mysorensis* (A, B, C), *Crotolaria notonii* (D, E, F), *Crotolaria orixensis* (G, H, I), *Crotolaria retusa* (J, K, L), *Crotolaria spectabilis* (M, N, O). A, D, G, J, M, micrographs of seeds; B, E, H, K, N, micrographs of seed surface; C, F, I, L, O, micrographs of hilum.

Table 2. Characteristic features of the seeds

Species	Seed shape	Seed size (mm)			Colour	Texture	Surface features	Hilum shape	Hilum colour	Hilum feature
		L	W	T						
A. bulgaumensis	Oblong to ovoid	1.3	1.0	0.7	Green light brown	Smooth	Complex reticulate network	Oval	Pale green	Thick walled rim, with cells in folded manner
A. monilifer	Oblong	2.0	1.0	0.7	Brown	Smooth	Hexagonal to uneven areas with undulating walls	Oval	Dark brown	Uneven areas
A. procumbens	Ovoid	1.9	1.5	0.3	Green	Smooth	Granular undulated and knobbed	Round	Brown	Prominent matted rim
A. vaginalis	Oblong to ellipsoid	1.2	0.8	0.6	Green light brown	Smooth, shiny	Dispersed round rough uneven structures	Oval	Black	Sunken matted rim
I. cordifolia	Ovoid to orbicular	1.1	1.0	0.3	White dull green	Rough	Pitted- verrucate, rounded	Oval	Pale green	Round and distinctly sunken with matted rim
I. echinata	Orbicular to round	1.3	1.0	0.4	White dull green	Rough	Deeply pitted and verrucate, levigate with mound	Round	Pale green	Round and distinctly sunken with matted rim
I. linifolia	Ovoid to ellipsoid, spherical	1.2	1.2	1.2	Dark brown	Rough	Deeply pitted and verrucate, levigate surface with mound	Oval	Brown with thick rim	Oblong- hexagonal areas with edges having discontinuous lifted margins
I. tinctoria	Oblong-rectangular	1.6	1.3	0.9	Brown	Rough	Sunken with granular margin/tuberculate	Obovate	Black	Indistinctly sunken with cellular margin
C. albida	Kidney	2.6	2.1	0.5	Greenish black	Slightly rough, dull	Thick walled undulating lines	Sunken with cracks	Greenish brown	Surrounding surface is pitted
C. calycina	Kidney	1.6	1.4	0.4	Dark brown	Smooth, shiny	Smooth, long line cracks	Ovoid	Dark brown	Matted
C. filipes var. *trichophora*	Kidney	1.8	1.3	0.4	Greenish brown to brown	Smooth, shiny	Smooth, uneven cracks with minute depressions	Round	Brown	Irregular matted and sunken
C. linifolia	Kidney	2.0	1.3	0.4	Black	Smooth, shiny	Smooth, superficial pits seen	Round	Black	Matted reticulate
C. mysorensis	Kidney	2.8	1.7	1.1	Greenish yellow	Smooth, shiny	Smooth surface with superficial small papillate structures	Oval	Light brown	Matted slightly sunken hilum with uplifted square margined pits
C. notonii	Kidney	2.3	2.0	0.3	Grayish black	Smooth	Smooth, with feebly pitted surface	Sunken oval	Light brown	Matted structures
C. orixensis	Kidney	3.0	2.5	0.8	Whitish green	Rough	Smooth with prominent line depressions and cracks	Sunken	Green-light brown	Pitted
C. retusa	Kidney	1.5	3.0	0.9	Greenish light brown	Smooth, shiny	Smooth, with cracks	Sunken	White	Pitted
C. spectabilis	Kidney	2.9	2.3	0.3	Black	Rough	Reticulate matted with network of interwoven rugae	Round	Brown	Rim posses smooth matted type structures

epidermal configuration and cuticular deposition as influenced by seed expansion. Gutterman and Heydecker (1973) demonstrated that day length affects seed coat structure while Sharma et al. (1977) concluded that edaphic factors are responsible for the difference. In studies on *Indigofera pseudo-tinctoria,* Agrawal (1984) concluded the spermoderm pattern was similar but that the density of the ornamentation varied. Such a difference appears to be due to varying amounts of surface deposition. Our observations of seed surface patterns in *Indigofera linifolia* and *Indigofera tinctoria* confirmed the findings of Agrawal (1984) and Murthy (2002).

The present study supports the use of seed coat patterns as features for species identification. The seeds display diversity in shape, dimensions and seed coat surface. The SEM study revealed seed coat remarkable topographic diversity among different species, to be characteristic of each species. This kind of study with more species may help to open a frame work of our knowledge about interspecific relationships in the genus. The present study provided some useful characters of seed for infrageneric classification and also for delimiting species. Light microscopic features supplemented with SEM proved to be a great tool to achieve more accurate seed identification, as previously suggested by Brisson and Peterson (1976). This method can be used as a routine technique in the study of spermoderm morphology (Heywood 1971; Barthlott 1984).

Acknowledgements

We thank Dr. M.N. Patel, Head of Faculty of Technology and Engineering, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat for providing facilities and support in the SEM studies; The Conservator of Forests, Working Plan Circle, Forest Department, Vadodara, Gujarat state for providing financial support; Dr. (Mrs.) U.C. Bapat for providing access to herbarium specimens to confirm the identity of the specimen.

References

Agarwal S. 1984. Seed structure in some *Indigofera* species. *J. Indian Bot. Soc.* 63: 11–19.

Al-Ghamdi F.A., Al-Zahrani R.M. 2010 Seed morphology of some species of *Tephrosia* Pers. (Fabaceae) from Saudi Arabia. Identification of species and systematic significance. *Feddes Repertorium* 121: 59–65.

Ambasta S.P., Ramachandran K., Kashyapa K., Chand R. (eds). 1986. Useful plants of India. Publications and Information Directorate. Council Scientific and Industrial Research, New Delhi, pp. 146–147.

Barthlott W. 1981. Epidermal and seed surface characters of plants: systematic applicability and some evolutionary aspects. *Nordic J. Bot.* 1: 345–355.

Barthlott W. 1984. Microstructural features of seed surfaces. In Heywood V.H., Moore D.M. (eds.), *Current Concepts in Plant Taxonomy*. Academic Press, London. 488 p.

Buss C.C., Lammers T.G., Wise R.R. 2001. Seed coat morphology and its systematic implications in *Cyanea* and other genera of Lobelioideae (Campanulaceae). *Amer. J. Bot.* 88: 1301–1308.

Buth G.M., Narayan A. 1986. Seed and seed coat anatomy of some members of *Crotolaria* (Papilionaceae). *J. Indian Bot. Soc.* 66: 317–324.

Chowdhury K.A., Buth G.M. 1970 Seed coat structure and anatomy of Indian Pulses. *J. Linn. Soc. Bot.* 63: 169–179.

Esau K. 1953. *Anatomy of Seed Plants*. John Wiley and Sons, New York.

Gohary I., Mohammed A.H. 2007. Seed morphology of *Acacia* in Egypt and its taxonomic significance. *Int. J Agric. Biol.* 9: 435–438.

Gontcharova S.B., Gontcharova A.A., Yakubov V.V., Kondo K. 2009. Seed surface morphology in some representatives of the genus *Rhodiola* sect. *Rhodiola* (Crassulacea) in Russian Far East. *Flora* 204: 17–24.

Heydecker W. 1973. Germination of an idea the priming of seeds. *University of Nottingham School of Agriculture Report* 1973/1974. 50-67.

Heywood V.H. 1971. The characteristics of the scanning electron microcopes and their importance in biological studies. In Heywood V.H. (ed) *Scanning Electron Microscopy: Systematic and Evolutionary Applications*. Academic Press, London.

Javadi F., Yamaguchi H. 2004. A note on seed coat and plumule morphological variation in the genus *Cicer* (Fabaceae). *Sci. Rep. Grad. Sch. Agric. Biol. Sci.* 56: 7–16.

Karihaloo J.L., Malik, S.K. 1994. Systematic relationships among some *Solanum* L. sect. *melongana* L. Evidence from seed characters. *Indian J. Plant Genet. Resour.* 7: 13–21.

Koul K.K., Ranjna N., Raina S. N. 2000. Seed coat microsculpturing in *Brassica* and allied genera (Subtribe Brassicinae, Raphanine, Moricandiinae). *Ann. Bot.* 86: 385–397.

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

Mallick D.K., Sawhney S. 2003. Seed coat ornamentation in wild and cultivated lentil taxa. *Phytomorphology* 53: 187–195.

Murthy G.V.S., Sanjappa M. 2002. SEM studies on seed morphology of *Indigofera* L. (Fabaceae) and its taxonomic utility. *Rheedea* 12: 21–51.

Pandey A., Gupta R. 2003. Fibre yielding plants of India: genetic resources, perspective for collection and utilization. *Nat. Prod. Radiance* 2:194–204.

Sahai K. 1999. Structural diversity in the lens of the seeds of some *Cassia* L. (Caesalpinioideae) species and its taxonomic significance. Phytomorphology. 49: 203–208.

Salimpour F., Mostafavi G., Sharifnia F. 2007. Micromorphologic study of the seed of the genus *Trifolium*, section *Lotoidea*, in Iran. *Pak. J. Biol. Sci.* 10: 378–382.

Segarra J.G., Mateu I. 2002. Seed morphology of *Linaria* species from Eastern Spain: identification of species and taxonomic implications. *Bot. J. Linn. Soc.* 135: 375–389.

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

Shelter S.J. 1986. Seed morphology in North American Campanulaceae. *Ann. Missouri. Bot. Gardens* 73: 653–688.

Siddhuraju P., Viajayakumari K., Janardhanan K. 1992. The biochemical composition and nutritional potential of the tribal pulse, *Alysicarpus rugosus* (Wild.) DC. *Food Chem.* 45: 251–255.

Skvortsov A.K., Rusanovitch I.I. 1974. Scanning electron microscopy of the seed-coat surface in *Epilobium* species. *Bot. Notes* 127: 392–401.

Takhtajan A. 1991. *Evolutionary Trends in Flowering Plants.* Columbia Univ. Press, New York.

Tobe H., Wagner W.L., Chin H.C. 1987. A systematic and evolutionary study of *Oenothera* (Onagraceae): Seed coat anatomy. *Bot. Gaz.* 148: 235–257.

Wealth of India. 1950. *The Wealth of India – Raw Materials.* Vol. II. Publications and Information Directorate, Council of Scientific and Industrial Research, New Delhi, pp. 372–383.

Yoshizaki M. 2003. Millets in prehistoric remain: Paleobotany on barnyard millets and azuki beans in Japan. In Yamaguchi H., Kawase M. (eds) *Natural History of Millets*. Hokaido University Press, Sapporo.

Yinusa I., Ndukwe I.G., Amupitan J.O. 2007. Phytochemical and antimicrobial screening of aerial part of *Indigofera pulchra*. *Chem. Class Journal, CSN Zaria*. pp. 162.

Zhang Z.-Y., Yang D.-Z., Lu A.-M., Knapp S. 2005. Seed morphology of the tribe Hyoscyameae (Soloanaceae). *Taxon* 54: 71–83.

Received 20 September 2011; received in revised form 24 October 2011; accepted 10 November 2011