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Morphological and micromorphological studies of seeds of ten accessions of *Psophocarpus tetragonolobus* (L.) DC.

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Abstract

Seed morphological and micromorphological features of ten accessions of winged bean *Psophocarpus tetragonolobus* (L.) DC. were studied under light and scanning electron microscope. The comparative study of seeds showed marked differences among accessions in size, shape, colour, hilum size and surface ornamentation pattern. Polymorphism was evident also within each accession. Exomorphic features revealed under scanning electron microscopy suggested subspecific demarcations. The highest degree of seed polymorphism was found in the accession IC112417, and the lowest degree in accession EC38154. Accession EC27886 had comparatively large seeds, and accession IC95227 had small seeds. However, species cultivars differed in seed size, surface and hilum characteristics. The study showed that the seed coat ornamentation pattern can be helpful in identification of cultivars of this genus.

Key words: hilum, legume, microscopy, *Psophocarpus tetragonolobus*, scanning electron microscope..
Abbreviations: SEM, scanning electron microscope.

Introduction

Psophocarpus tetragonolobus (L.) DC., commonly known as winged bean, is attracting attention as a potentially valuable crop (Masefield 1978; National Research Council 1981). The entire plant is fit for human consumption from flowers and leaves to tuberous roots and seeds. The plant can be grown on soil types ranging from sandy to heavy clays.

Seed morphology provides 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; Gontcharova 2009). Gross morphology as well as seed coat characteristics have been successfully employed in the identification and classification of taxa (Erol et al. 2006; Kaplar Jafari et al. 2009; Farrington et al. 2008; Fawzi et al. 2010). In particular, scanning electron microscopy (SEM) can be used to detect minute taxonomically significant seed coat patterns, which might aid to define species characteristics (Taia 2004).

Genetic diversity for fruiting, i.e. pod shape, pod colour, pod size, as well as seed number, seed size, and colour has been described for winged bean (Chandel et al. 1978a; 1978b; 1981). Recently, seed morphology has been used to solve taxonomic issues for species in genera *Poecilantha* and *Indigofera* (Meireles, Tozzi 2008; Paulino et al. 2010). In addition, studies of *Tephrosia* have added new data of taxonomic importance (Teixeira de Queiroz et al. 2013)

The aim of the present study was to describe macromorphological and micromorphological characteristics of seeds of ten cultivars of winged bean and to assess the taxonomic significance of the characteristics in recognition of subgenera and species.

Materials and methods

Authentic seeds of ten accessions of *Psophocarpus tetragonolobus*, viz. EC38154, IC95226,IC95224, EC38825, EC38821B, EC38954, EC27886, IC95227, IC112417, and EC38955A were obtained from the Regional Station of the National Bureau of Plant Genetic Resources at Akola (Maharashtra, India). Disease-free vigorous seeds were sown at the experimental field of Crop Research Farm (23° 53' N and 83° 25' E), Department of Botany, The University of Burdwan, West Bengal, India. Suitable agronomic measures for proper growth and development of the crop were provided.

Dry seeds of each accession were observed with naked eye and also under dissecting microscope. The phenotypic characteristics viz. shape, colour, size were described in detail. Seed morphology and seed dimensions were recorded as an average of randomly chosen five replicates each of 20 seeds, representing each accession. To study the hilum region, seeds were also observed under stereo microscope (Zoom Star-IV, De Winter, Italy). For observation of the

surface ornamentation pattern, seeds were cleaned with absolute alcohol for 1 min to remove surface debris. Seeds were mounted directly on a stub with their dorsal, ventral, and lateral side upwards and the characteristic features of all sides were scanned and photographed using a Hitachi S-530 Scanning Electron Microscope (Hitachi Ltd., Tokyo Japan) at an accelerating potential of 20 to 25 kV. Seeds of each accession were studied for three generations. Seed dimensions were expressed as both minimum and maximum length × width ± SE.

Results

In general, seeds of *Psophocarpus tetragonolobus* were monochromatic, non-endospermic, ellipsoid to spheroid, hilum partially covered, raised above the level of seed surface and marked by a central lengthwise line or cleft. Seeds of *P. tetragonolobus* varied in shape, size, and surface characteristics for different cultivars. Seeds of each accession also varied in colour, shape, and size. According to characteristic features, seeds of the accessions were grouped into different categories (I, II, III etc.) based on observations under dissecting microscope. Table 1 illustrate diverse seed groups within each accession. Fig. 1 A, C, E, G, I, K, M, O, Q, S show hilum characteristics of different accessions viewed under stereo microscope and Fig. 1 B, D, F, H, J, L, N, P, R, T show the surface ornamentation pattern under scanning electron microscope.

Seeds of accession EC38154 were grouped into four categories (I to IV) with small, medium, and large light brown or brown coloured seeds; seed shape was spheroid or round; surface glossy either smooth or wrinkled. Dimensions were 0.7 to 1.0 ± 0.13 × 0.6 to 0.7 ± 0.11 cm. Testa ornamentation pattern was papillose. Hilum was linear.

Accession IC95226 was divided into eight categories (I to VIII) with small, medium and large light brown, yellowish brown to dark brown coloured seeds; seed shape was spheroid to round; surface glossy either smooth or wrinkled. Dimensions were 0.8 to 1.0 ± 0.10 × 0.6 to 0.8 ± 0.02 cm. Testa ornamentation pattern was granulose-papillose. Hilum was linear.

Accession IC95224 had nine categories (I to IX) with small, medium and large yellowish brown, yellow, dark brown and brown coloured seeds; seed shape was spheroid or round; surface smooth glossy or wrinkled. Dimensions were 0.8 to 1.0 ± 0.22 × 0.5 to 0.7 ± 0.21 cm. Testa ornamentation pattern was granulose with striations. Hilum was oval.

Accession EC38825 grouped into seven different categories (I to VII) of seeds: small to large light brown, yellowish brown, and dark brown; seed shape varied from spheroid to round; surface smooth or wrinkled. Dimensions were 0.7 to 1.0 ± 0.20 × 0.5 to 0.8 ± 0.03 cm. Testa ornamentation pattern was granulose with small pits. Hilum was linear.

Accession EC38821B exhibited five different categories (I to V), with medium to large brown, yellow and yellowish brown coloured seeds; seed shape varied from spheroid to round; surface smooth or wrinkled. Dimensions were 0.7 to 1.0 ± 0.03 × 0.6 to 0.7 ± 0.04 cm. Testa ornamentation pattern was tuberculate-aculeate. Hilum was linear.

Accession EC38954 had eight categories of seeds (I to VIII) from very small, medium to large yellowish brown, light brown and dark brown seeds, seed shape was round or spheroid either with smooth or wrinkled surface. Dimensions were 0.8 to 0.9 ± 0.11 × 0.5 to 0.8 ± 0.21 cm. Testa ornamentation pattern was large pitted. Hilum was elliptic.

Accession EC27886 showed eight categories of seeds (I to VIII) from small to large yellowish brown, brown and dark brown coloured spheroid and round smooth and wrinkled surface seeds. Dimensions were 0.7 to 1.2 ± 0.13 × 0.6 to 0.8 ± 0.12 cm. Testa ornamentation pattern was reticulate with finely interwoven. Hilum was elliptic.

Accession IC95227 had nine categories of seeds (I to IX) from small to large yellowish brown, blackish brown, and brown coloured spheroid and round either smooth or wrinkled surface. Dimensions were 0.7 to 1.2 ± 0.11 × 0.4 to 0.8 ± 0.12 cm. Testa ornamentation pattern was reticulate-foveolate. Hilum was elliptic.

Accession IC112417 showed ten categories of seeds (I to X) from small to large yellow, blackish brown and brown coloured spheroid or round with either smooth or wrinkled surface. Dimensions were 0.8 to 1.0 ± 0.01 × 0.5 to 0.8 ± 0.02 cm. Testa ornamentation pattern was granulose with ridges. Hilum was elliptic.

Accession EC38955A showed six categories of seeds (I to VI) from medium to large yellowish brown and dark brown, seed shape was spheroid or round, surface smooth or wrinkled. Dimensions were 0.6 to 1.1 ± 0.03 × 0.5 to 0.8 ± 0.11 cm. Testa ornamentation pattern was incomplete reticulate. Hilum was linear.

Discussion

Morphological and micromorphological investigation of seeds showed a range of structure and surface ornamentation patterns in the taxa of *P. tetragonolobus*. Most of the seeds had a hard smooth surface with a few having a hard wrinkled surface; linear to elliptic hyla; lens shaped deltoid, halos complete, funicular margin thick, cotyledon interface flap. The features observed in our study were consistent with these found in the subfamily Papilionoideae MacDonald (Idu, Omoruyi 2002). In general, comparatively large seeds were found for accession EC27886 (0.7 to 1.2 × 0.6 to 0.8 cm), while comparatively small seeds were observed for accession IC95227 (0.7 to 1.2 × 0.4 to 0.8 cm). Accession IC112417 showed the highest degree of seed polymorphism, but the lowest degree of

Table 1. Morphological characteristics of diverse seed groups for each accession of *Psophocarpus tetragonolobus*

Accession	Category	Size	Colour	Shape	Surface
EC38154	Group I	Large	Light brown	Round	Smooth, glossy
	Group II	Medium	Light brown	Round	Smooth, glossy
	Group III	Medium	Light brown	Round	Wrinkled, glossy
	Group IV	Small	Brown	Spheroid	Wrinkled, glossy
IC95226	Group I	Large	Light brown	Spheroid	Smooth, glossy
	Group II	Medium	Dark brown	Round	Smooth, glossy
	Group III	Small	Dark brown	Round	Smooth, glossy
	Group IV	Medium	Dark brown	Spheroid	Wrinkled, glossy
	Group V	Small	Dark brown	Spheroid	Wrinkled, glossy
	Group VI	Large	Yellowish brown	Round	Smooth, glossy
	Group VII	Small	Yellowish brown	Round	Smooth, glossy
IC95224	Group I	Large	Yellowish brown	Spheroid	Smooth, glossy
	Group II	Large	Yellowish brown	Round	Smooth, glossy
	Group III	Medium	Yellowish brown	Round	Smooth, glossy
	Group IV	Medium	Yellow	Round	Smooth
	Group V	Large	Dark brown	Spheroid	Wrinkled, glossy
	Group VI	Large	Dark brown	Round	Smooth, glossy
	Group VII	Medium	Brown	Round	Smooth, glossy
	Group VIII	Medium	Dark brown	Round	Smooth, glossy
	Group IX	Large	Brown	Spheroid	Wrinkled
EC38825	Group I	Large	Dark brown	Spheroid	Smooth, glossy
	Group II	Large	Light brown	Spheroid	Smooth, glossy
	Group III	Medium	Dark brown	Round	Smooth, glossy
	Group IV	Small	Light brown	Round	Smooth, glossy
	Group V	Large	Yellowish brown	Spheroid	Smooth, glossy
	Group VI	Medium	Yellowish brown	Round	Smooth
	Group VII	Medium	Light brown	Spheroid	Wrinkled
EC38821B	Group I	Large	Brown	Round	Smooth, glossy
	Group II	Medium	Brown	Round	Smooth, glossy
	Group III	Medium	Yellow	Round	Smooth, glossy
	Group IV	Medium	Yellow brown	Round	Smooth, glossy
	Group V	Medium	Yellow brown	Spheroid	Wrinkled
EC38954	Group I	Large	Dark brown	Spheroid	Smooth, glossy
	Group II	Medium	Light brown	Round	Smooth, glossy
	Group III	Large	Light brown	Spheroid	Smooth, glossy
	Group IV	Medium	Dark brown	Round	Smooth, glossy
	Group V	Medium	Light brown	Spheroid	Wrinkled
	Group VI	Medium	Yellowish brown	Spheroid	Smooth
	Group VII	Small	Yellowish brown	Spheroid	Smooth
	Group VIII	Very small	Yellowish brown	Spheroid	Wrinkled
EC27886	Group I	Large	Brown	Spheroid	Smooth, glossy
	Group II	Large	Dark brown	Spheroid	Smooth, glossy
	Group III	Medium	Brown	Spheroid	Smooth, glossy
	Group IV	Medium	Yellowish brown	Spheroid	Smooth
	Group V	Medium	Dark brown	Spheroid	Smooth, glossy
	Group VI	Medium	Dark brown	Round	Smooth, glossy
	Group VII	Small	Dark brown	Spheroid	Smooth, glossy
	Group VIII	Small	Yellowish brown	Round	Wrinkled

(*continued*)

Table 1. (*continued*)

Accession	Category	Size	Colour	Shape	Surface
IC95227	Group I	Large	Brown	Spheroid	Smooth, glossy
	Group II	Medium	Brown	Spheroid	Smooth, glossy
	Group III	Large	Blakish brown	Round	Smooth, glossy
	Group IV	Medium	Blakish brown	Round	Smooth, glossy
	Group V	Medium	Yellow	Spheroid	Wrinkled
	Group VI	Medium	Blakish brown	Spheroid	Wrinkled, glossy
	Group VII	Medium	Brown	Round	Smooth, glossy
	Group VIII	Medium	Blakish brown	Spheroid	Wrinkled, glossy
	Group IX	Small	Blakish brown	Round	Smooth, glossy
IC112417	Group I	Large	Brown	Spheroid	Smooth, glossy
	Group II	Large	Blakish brown	Round	Smooth, glossy
	Group III	Large	Brown	Spheroid	Smooth, glossy
	Group IV	Medium	Brown	Spheroid	Smooth, glossy
	Group V	Medium	Brown	Round	Smooth, glossy
	Group VI	Medium	Yellow	Spheroid	Wrinkled
	Group VII	Medium	Yellow	Round	Smooth, glossy
	Group VIII	Large	Blakish brown	Spheroid	Wrinkled
	Group IX	Medium	Blakish brown	Spheroid	Wrinkled
	Group X	Small	Yellow	Spheroid	Wrinkled
EC38955A	Group I	Large	Dark brown	Spheroid	Smooth, glossy
	Group II	Medium	Dark brown	Spheroid	Smooth, glossy
	Group III	Medium	Yellowish brown	Spheroid	Smooth, glossy
	Group IV	Medium	Yellowish brown	Round	Smooth, glossy
	Group V	Medium	Dark brown	Spheroid	Wrinkled, glossy
	Group VI	Medium	Dark brown	Round	Smooth, glossy

seed polymorphism was found in accession EC38154. Light microscopic features supplemented with these under SEM proved to be important to achieve more accurate seed identification (Gandhi et al. 2011). The SEM study revealed seed coat diversity among different accessions. SEM showed no differences in surface characteristics for the three successive generations of each accession. Thus, the surface characteristics of each accession can be used as taxonomic marker. In this case, the size of seed may not be useful for delimiting the accessions. Although seed size is subject to ecological and physiological variation, we found no differences between the three successive generations of each accession.

According to Skvortsov and Rusanovitch (1974) seed coat characteristics are genetically determined and are the main source of intra- or inter-specific variation. In contrast, Heydecker (1973) demonstrated that day length affects seed coat structure, while Sharma et al. (1977) concluded that edaphic factors are responsible for the differences found.

The hilar region is taxonomically characteristic for *P. tetragonolobus*, as Papilionaceous seeds have a very specialised organisation. The following characteristics had distinctive taxonomic value. Halos varied among accessions. Seeds of accessions EC38154, EC38825 and IC95227 had a prominent localised flap-like growth of the hilar rim. IC112417 and EC38955A also showed the same characteristics. In accession IC95226, deposition of corky material in the hilar rim was uneven and the flap was not prominent. In IC95224 and EC38821B, halos were thick and uneven, flap present, but not prominent. In EC38954 and EC27886, deposition of corky material was uneven, and the flap was not prominent.

The testa ornamentation pattern was very much specific to each accession and could be used as a taxonomic tool to identify the accession. Accession EC38154 had a papillose type of ornamention. This pattern was not observed in other accessions of the species. Accession IC95226 could be identified by a granulose papillose type of ornamentation. Accessions IC95224 and EC38825 had granulose-type ornamentation, while IC95224 showed striations and small pits along with a granulose type. In accession EC38821B, seed showed tuberculate-aculeate type of surface pattern. Accession EC38954 had large pits on the texture. Finely interwoven and reticulate foveolate type of surface features were found in EC27886 and IC95227 respectively. Granulose texture with ridges was observed in accession IC112417, and accession EC38955A showed an incomplete reticulate of testa ornamentation.

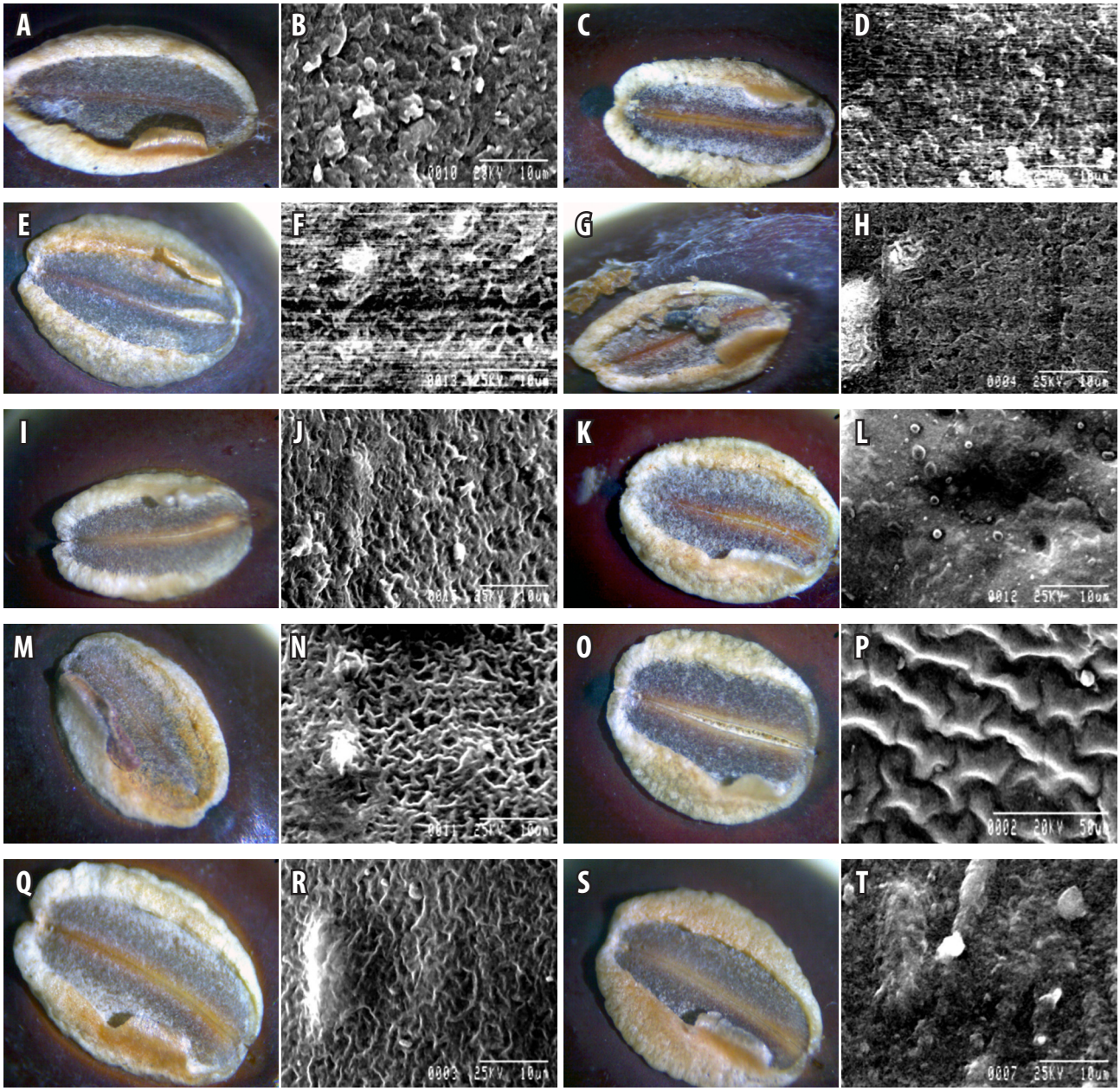

Fig. 1. Testa ornamentation pattern under stereo microscope and hilum features by scanning electron microscopy of *Psophocarpus tetragonolobus* seeds of different accessions. A, B, EC38154; C,D, IC95226; E, F, IC95224; G, H, EC38825; I, J, EC38821B; K, L, EC38954; M, N, EC27886; O, P, IC95227; Q, R, IC112417; S, T, EC38955A.

Thus, *P. tetragonolobus* showed wide variety of seed characters. Seed character alone can not be used for distinguishing the cultivars (Chernoff et al 1992). However, when used together with general morphological characteristics, it might be helpful in distinguishing different cultivars.

References

National Research Council. 1981. The winged bean. A high-protein crop for the tropics. 2nd Ed. National Academy Press, Washington DC.

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.

Chandel P.R., Arora R.K., Pant O.K.G. 1978a. Winged bean in India, its present status and prospects. The winged bean proceedings, 1st International Symposium on developing the potential of winged bean, Manila, Phillippines, pp. 393–395.

Chandel P.R., Arora R.K., Pant O.K.G. 1978b. Genetic resources of winged bean (*Psophocarpus tetragonolobus* L. DC) in India. National Symposium of Plant and Animal Genetic Resources. New-Delhi (Dec 1973). Proc. Ind. J. Genet.

Chandel P.R., Arora R.K., Pant O.K.G. 1979. Winged bean a legume for better nutrition. *Ind. Hort.* 24: 15–17.

Chernoff M.U., Kislev M.E. 1992. Seed characters and testa texture in species of Vicieae: their taxonomic significance. *Israel J. Bot.* 41: 167–186.

El-Sahhar K.F., Emara, K.S., Ali W.A. 2013. Comparative systematic studies of *Astragalus* L. in flora of Arab Republic of Egypt and Syrian Arab Republic: Seed features and germination. *Res. J. Agricult. Biol. Sci.* 9: 79–88.

Erol O., Uzen E., Kucuker O. 2006. Preliminary SEM observations on the seed testa structure of *Gladiolus* L. species from Turkey. *Int. J. Bot.* 2: 125–127.

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

Farrington R., Meyers Z., Ickert-Bond S.M. 2008. Comparative study of seed coat micromorphology and seed anatomy in Alaskan *Oxytropis* (Loco-weed, Fabaceae) using SEM, and their taxonomic significance. Poster Presentation. Botany 2008. Vancouver, Canada.

Fawzi N.M., Fawzy A.M., Mohamed A.A.H.A. 2010. Seed morphological studies on some species of *Silene* L. (Caryophyllaceae). *Int. J. Bot.* 6: 287–292.

Gandhi D., Albert S., Pandya N. 2011. Morphological and micromorphological characterization of some legume seeds from Gujarat, India. *Environ. Exp. Biol.* 9: 105–113.

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.

Gunn C.R. 1972. Seed characteristics. In: Hasen A.A. (ed.). *Alfalfa Science and Technology*. American Society of Agronomy, Madison, Wisconsin, pp. 677–687.

Gunn C.R. 1981. Seeds of Leguminosae. In: Pothill R.M., Raven P.H. (eds.). *Advances in Legume Systematics*. Part 2. Royal Botanic Garden, Kew, UK, pp. 913–925.

Gunn C.R. 1981. Seed topography in the Fabaceae. *Seed Sci. Technol.* 9: 737–757.

Gunn C.R. 1991. Fruits and seeds of genera in the subfamily Caesalpinioideae (Fabaceae). 1st Ed., U. S. Department of Agriculture, Agriculture Research Service, Washinton, D. C., pp. 408.

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

Idu M., Omoruyi O.M. 2002. Seed coat structure and anatomy of some Nigerian pulses. *J. Plant Biol.* 45: 189–195.

Jafari A., Fathi Z., Bemani M. 2009. Using morphology and micromorphology of characters for identification of Silene L. species in North-East of Iran. *Res. J. Environ. Sci.* 3: 667–676.

Kaplan A., Hasanoglu A., Ince I.A. 2007. Morphological, anatomical and palynogical propertiesof some Turkish *Veronica* L. species (Scrophulariaceae). *Int. J. Bot.* 3: 23–32.

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

Lersten N.R., Gunn C.R., Brubaker C.L. 1992. Comparative morphology of the lens on legume (Fabaceae) seeds, with emphasis on species in subfamilies Caesalpinioideae, and Mimsoideae. 1st Ed., U.S. Department of Agriculture, Washington, DC., pp. 44.

Meireles J.E., Tozzi A.M.G.A. 2008. Seed and embryo morphology of Poecilanthe (Fabaceae, Papilionoideae, Brongniartieae. *Bot. J. Linn. Soc.* 158: 249–256.

Paulino JV, Pessine E, Teixeira SP 2010. Estudos morphoanatomicos da semente e da plantula de species de anileiras (*Indigofera* L., Leguminosae). *Acta Bot. Bras.* 24: 1–7.

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.

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

Teixeira de Queiroz R., de Azevedo Tozzi A.M.G., Lewis G.P. 2013. Seed morphology: an addition to the taxonomy of *Tephrosia* (Leguminosae, Papilionoideae, Millettieae) from South America. *Plant Syst. Evol.* 299: 459–470.

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

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