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Morphology and Development of Hard Seed Coat in *Melilotus indicus* (L.) All

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ABSTRACT

Mature seeds of *Melilotus indicus* (L.)All. are considered as one of the hardest leguminous seeds that develop inside single- seeded small pods. The young ovary is covered with small, multi-celled uni-seriate glandular trichomes that persist long up to the dicotyledonous embryo stage, at a time when seed coat is hard enough. During ontogeny the outer integument modifies gradually into a thick and hard seed coat composed of outermost, palisade like, macro-sclereid layer, a hypodermal osteo-sclereid layer followed by two to three parenchymatous layers. Like other legumes, the macro-sclereid layer represents the modified epidermis of the outer integument while the cells of its hypodermal layer change to osteo-sclereids imparting additional hardness to the seed coat. The tight packaging and cuticularization of macro-sclereids make the seeds of *Melilotus indicus* hard and impermeable to water. The inner integument does not participate in seed coat formation instead helps provide nutrition to the developing embryo before its lyses. The mature dry seed is dorsal-ventrally flat with a complex hilar region.

Keywords: *Melilotus indicus*, Seed coat, Integuments, ontogeny

Melilotus of the tribe Trifolieae of family Fabaceae is a green manure crop with 19 species that are also used as fodder and folk medicines. Three species are found in India and one of them, *Melilotus indicus* (L.) All., commonly called as Indian sweet clover is a small, herbaceous wild fodder legume that appears as a common winter weed in the month of November in Delhi. Its reproductive phase starts in February and continues till March end. It branches profusely and attains a height of 50-60 cm at maturity (Fig. 1). Leaves are trifoliate with spiral phyllotaxy. Flowers develop in axillary racemes and each inflorescence bears 20-30 small, yellow, papilionaceous flowers. Almost all flowers develop into tiny one-seeded pods. Mature seeds are brown enveloped by a rough hard seed coat.

The testa in leguminous taxa is well known for its refractory nature and macro- sclereid layer that contributes to hard seededness. A waxy cuticle and a subcuticular pectin layer veneer this layer. The macro- sclereid cell walls are composed of a cellulose pentosan matrix and do not show lignifications (Reeve, 1946; Weber *et al.* 1979). Subjacent to the macro-sclereid layer is osteo-sclereid layer whose structure and development at both light and Electron microscopic levels is reported only in few legumes (Harris, 1983, 1984; Seth & Vijayaraghavan, 1990). The developmental changes at light and scanning electron microscope when correlated with *in situ* localization of major metabolites like insoluble polysaccharides and total proteins will help to understand the developmental biology of the seed coat in *Melilotus indicus* L. (All).

MATERIALS AND METHODS

Young buds of *Melilotus indicus* (L.) All. at progressive developmental stages were collected from the botanical garden of University of Delhi, Delhi. Conventional methods for the fixation, embedding and staining of material were followed as mentioned by Gautam (2006).

Observations

The small and spindle-shaped ovary of *M. indicus* encloses two ovules on marginal placenta. The surface of young green ovary is covered all over by small 2-3 celled glandular hair. Like other legumes, ovules are campylotropous and bitegmic. Both integuments of the young ovule up to preglobular embryo stage are two-layered thick (Fig.1A). During ontogeny of seed coat, the layers of outer integument divide by both anticlinal and periclinal divisions (Fig.1 B). The cells of the outermost layer (epidermis) of the outer integument elongate radially and cuticularize (Fig.1 C), deposit secondary wall material in their upper region as caps and ultimately develop into hard, tightly- packed, PAS positive macro-sclereids (Fig.1 D,2 A-C). The nucleus moves to the middle of the cell (Fig. 1D), thereafter, cell contents disappear gradually.

The inner layer of the outer integument divides periclinally into two PAS rich layers (Fig.1B). The cells of the hypodermal layer also become thick-walled and modify to hourglass- shaped osteo-sclereids, imparting more hardness to the seed coat (Fig. 2A-C) while the inner integument layers degenerate gradually. Interestingly the inner integument does not contribute to the seed coat development in *M. indicus*, its cells show enlargement in size at early stages and later disintegrate (Fig. 1 B).

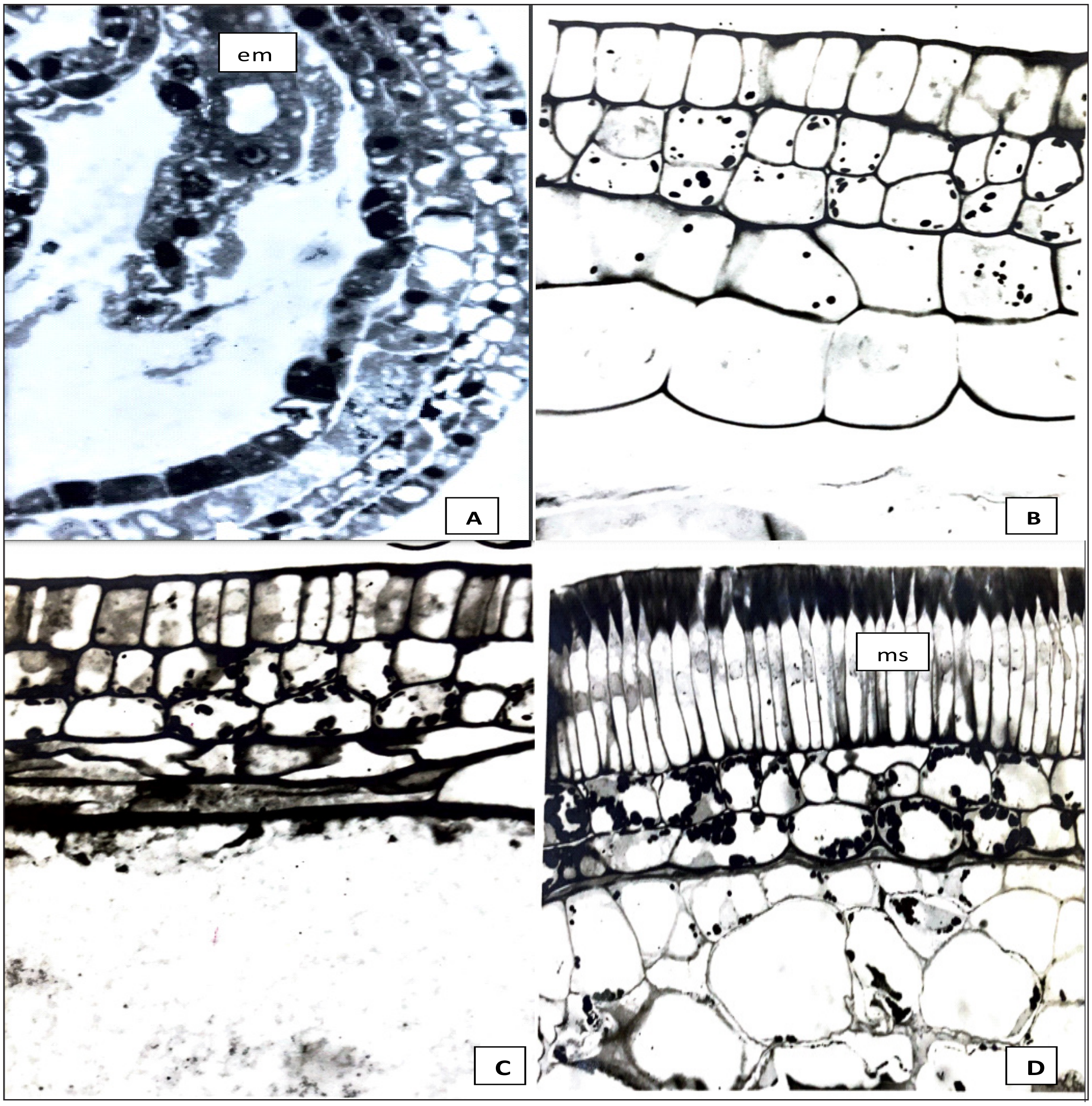

Fig. 1: (A-D) Seed coat differentiation. **(A)** Two-layered inner and outer integuments at pre-globular embryo stage. **(B, C)** Radially elongated cells of outermost layer, PAS rich inner layers in the outer integument and degeneration of inner integument. **(D)** Radial enlargement and deposition of secondary wall material in the apical region of developing macro-sclereid layer (em- embryo; ms- macro-sclereid layer)

The mature seed coat is well differentiated into four layers, the outer macro-sciereid layer, light line, osteo-sclereid layer and the innermost parenchymatous layer (Fig. 2B, C). The cuticularization of macro-sclereids and cuticle layer deposition give rise a characteristic rugose-tuberculate sculpturing to the seed coat surface.

The mature seeds are brown, small, dorsal-ventrally flat with a complex, elliptic hilar region (Fig.3 A) and prominent strophiole. The maro-sclereid cells of the seed coat appear tetragonal in surface view9 Fig. 3 B). Scanning Electron micro-graph shows the presence of mounds on the surface of mature seeds imparting further roughness (Fig. 3 C).

DISCUSSION

The development of suberin rich palisade layer of the seed coat and deposition of phenol cause hardness and impermeability of legume seeds (Baskin *et al*. 2000; Dueberrn de Sousa & Marcos-Filho, 2001; Zohary *et al*. 2012; Abbo *et al*. 2014). According to Bradford and Nonogaki (2009), testa or the seed coat derives from the inner, outer or both integuments in plants. During the development of seed coat in *M. indicus* (present study), the major contribution is made by the outer integument while inner integument disappears at an early stage as reported in many other members of Fabaceae (Esau, 1965; Miller *et al*. 2010; Smykal *et al*. 2014). Weber *et al.* (2005) reported that during early legume seed growth the maternal seed coat controls the nutrient supply to the embryo and therefore, the embryo development is preceded by endosperm and seed coat development. The hard seed coat is water impermeable and provides physical dormancy to seeds. Such physical dormancy is reported in many

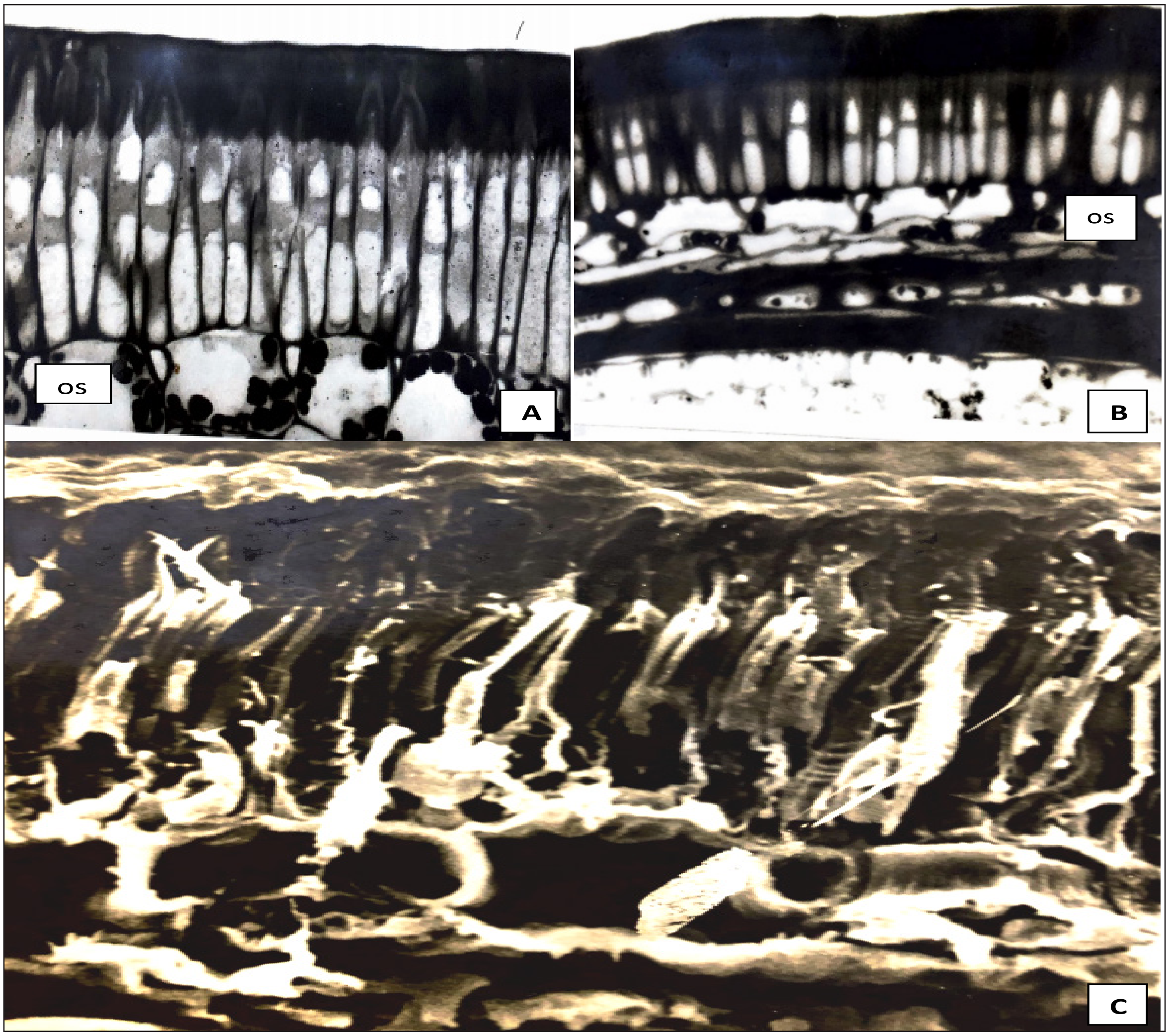

Fig. 2: (A-C) Seed coat differentiation. **(A)** Deposition of secondary wall material in the upper 1/3rd region of macro-sclereids as caps. **(B)** Light line appears below the cap layer and well defined hourglass osteo-sclereids. **(C)** SEM of Seed coat in sectional view

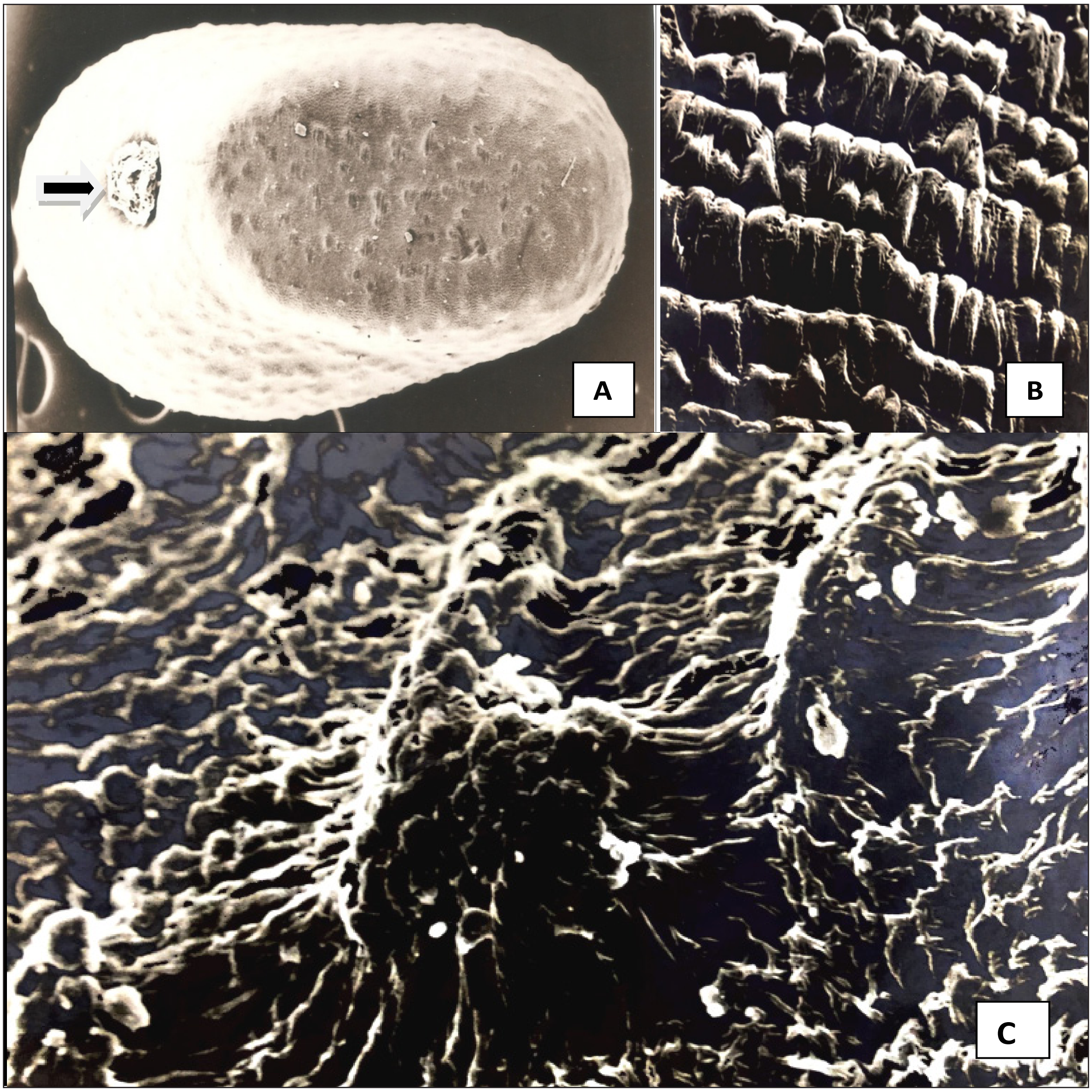

Fig. 3: (A-C) Scanning Electron micro graphs. **(A)** Mature seed with hilum (arrow), **(B)** Macro-sclereids in surface view, **(C)** Seed coat with mounds

families including Papilionaceae (Baskin & Baskin, 1998, 2000; Paulson *et al*. 2013). In *Melilotus alba* the presence of callose in the light line area and palisade cells and also the seed coat hardness is attributed to the entire wall of the palisade layer, except their inner tangential side (Riggio Bevilacqua *et al*. 1989). A direct and positive correlation is seen between the seed hardness and physical dormancy in legumes responsible for the distribution of germination over a long period to enhance the survival and existence of taxa under harsh circumstances (Lush & Evans, 1980; Bewley *et al*. 2013). Bibbey (1948) reported the survival of dormant seeds in *Melilotus* for as many as 17 years. Hard seed coat not only regulates the germination process but also protects it against fungal and microbial pathogens (Dalling *et al*. 2011).

The presence of mould on the mature seed provides it the roughness and is used for taxonomic purposes. Ceter *et al*. (2012) recognized seven morphological types on the basis of shape, colour and surface ornamentation pattern of seed coat in 37 species of *Trigonella.*

Melilotus indicus is used as a traditional medicine, its leaves and seeds find a special mention. This weed legume is able to survive well in salt rich soil (Al Serif, 2009), therefore, is a potential forage plant for the restoration of waste and degraded land. It can be an excellent choice for crop rotation and mixed cropping practices.

REFERENCES

Abbo, S., Pinhasi van-Oss, R., Gopher, A., Saranga, Y., Ofner, I. and Peleg, Z. 2014. Plant domestication versus crop evolution: a conceptual framework for cereals and grain legumes. *Trends Plant Sci.,* **19**: 351–360.

Al Sherif, E.A. 2009. *Melilotus indicus* (L.) All., a salt-tolerant wild leguminous herb with high potential for use as a forage crop in salt-affected soils. *Flora,* **204**: 737–746.

Baskin, J.M. and Baskin, C.C. 1998. Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination. Academic Press, San Diego.

Baskin, C.C., Baskin, J.M. and Li, X. 2000. Taxonomy, anatomy and evolution of physical dormancy in seeds. *Plant Species Biol.*, **15**: 139–152.

Baskin, J.M. and Baskin, C.C. 2000. Evolutionary considerations of claims for physical dormancy-break by microbial action and abrasion by soil particles. *Seed Sci. Res.,* **10**: 409–413.

Bewley, J.D., Bradford, K., Hilhorst, H. and Nonogaki, H. 2013. Seeds: Physiology of Development, Germination and Dormancy, 3rd Edn. New York: Springer-Verlag.

Bibbey, R.O. 1948. Physiological studies of weed seed germination. *Plant Physiol.,* **23**(4): 467-484.

Bradford, K. and Nonogaki, H. 2009. Seed development, dormancy and germination. *Ann. Pl. Rev.,* **27**. Oxford: Blackwell.

Ceter, T., Pinar, N.M., Akan, H., Ekici, M. and Aytac, Z. 2012. Comparative seed morphology of *Trigonella* L. species (Leguminosae) in Turkey. *Afr. J. Ag. Res.,* **7**(3): 509-522.

Dalling, J.W., Davis, A.S., Schutte, B.J. and Arnold, A.E. 2011. *Seed survival in soil: interacting effects of predation, dormancy and the soil microbial community,* **99**(1): 89-95.

Dueberrn de Sousa, F.H.D. and Marcos-Filho, J. 2001. The seed coat as a modulator of seed-environment relationship in Fabaceae. *Braz. J. Bot.,* **24**: 365–375.

Esau, K. 1965. Plant Anatomy, 2nd Edn. New York, John Wiley.

Gautam, N. 2006. Studies on ovarian trichomes in *Melilotus indica* All. *Phytomorphology,* **56**(1&2): 107-112.

Harris, W.M. 1983. On the development of macrosclereids in seed coats of *Pisum sativum* L. *Am. J. Bot.,* **70**: 1528–1535.

Harris, W.M. 1984. On the development of osteosclereids in seed coats of *Pisum sativum* L. *New Phytol.,* **98**: 135–141.

Lush, W.M. and Evans, L.T. 1980. The seed coats of cowpeas and other grain legumes: structure in relation to function. *Field Crops Research,* **3**: 267-286.

Miller, S.S., Jin, Z., Schnell, J.A., Romero, M.C., Brown, D.C. W. and Johnson, D.A. 2010. Hourglass cell development in the soybean seed coat. *Ann. Bot.,* **106**: 235–242.

Paulsen, T.R., Colville, L., Kranner, I., Daws, M.I., Högstedt, G., Vandvik, V. *et al*. 2013. Physical dormancy in seeds: a game of hide and seek? *New Phytol.,* **198**: 496–503.

Reeve, R.M. 1946. Ontogeny of the Sclereids in the Integument of *Pisum sativum* L. *Am. J. Bot.,* **33**(10): 806-816.

Riggio Bevilacqua, L., Roti-Mihelozzi, G. and Modenesi, P. 1989. The watertight dormancy of *Melilotus alba* seeds: further observations on the palisade cell wall. *Can. J. Bot.,* **67**: 3453–3456.

Seth, N. and Vijayaraghavan, M.R. 1990. Differentiation of the seed coat in *Sesbania speciosa. Proc. lnd. Acad. Sci.* (Plant Sci.), **100**(5): 301-309.

Smykal, P., Vernoud, V., Blair, M.W., Soukup, A. and Thompson, R.D. 2014. The role of testa during development and in the establishment of dormancy of the legume seed. *Front. Plant Sci*.

Weber, H., Borisjuk, L. and Wobus, U. 2005. Molecular physiology of legume seed development. Ann. Rev. *Plant Biol.,* **56**: 253–279.

Weber, H., Borisjuk, L., Heim, U., Sauer, N. and Wobus, U. 1997. A role for sugar transporters during seed development: molecular characterization of a hexose and a sucrose carrier in faba bean seeds. *Plant Cell,* **9**: 895–908.

Zohary, D., Hopf, M. and Weiss, E. 2012. Domestication of Plants in the Old World, 4th Edn. Oxford, Oxford University Press.