Occurrence of wax rodlets in the seed coat of *Ginkgo biloba* L.

Plant surface provides the largest biological interface on earth. Variability of these surface structures, evolved over millions of years, has led to a great diversity of highly adapted functional features for specific environmental conditions. In plants the outer covering consists of a hydroxy fatty acid polymer called cutin, integrated with intra- and epicuticular waxes. The cuticle is water-resistant and functions as a physical barrier that prevents penetration by virus, bacteria, spores or growing filaments of fungi and helps the plant to resist drought[1]. It also provides structural and chemical modifications for surface wetting in plants, with the help of surface sculpturing of the cells, such as variable folding of the cuticle or by epicuticular wax depositions[2]. The wax projections are thought to be mainly of crystalline nature[3].

Wax production in different plant parts such as leaves, stems and fruit walls is a common phenomenon. Occurrence of wax in angiosperms is common, but rare in gymnosperms, particularly on the seed coat[1].

Wax in the form of variously shaped rodlets occurs in angiosperms, as in the leaves of *Aristolochia gigantea* Mart. and Zucc., *Centranthus ruber* (L.) DC., *Fritillaria pallidiflora* Schrenk, *Gypsophila acutifolia* Fisch., *Laurus nobilis* L., *Leucojum aestivum* L., *Nicotiana glauca* Graham, *Paeonia mlokosewitschii* Lom. and *Paeonia officinalis* L.[3], in the young seeds of *Cercidium floridum*[4] and *Phyllanthus amarus* Schum & Thonn[5].

In gymnosperms, the presence of wax in the seed coat of *Picea abies* (L.) Karst[6], seed coat and seed wing of *Chamaecyparis nootkatensis*[7] and leaves of *Ginkgo biloba* L.[1] has been reported earlier. *G. biloba* L., commonly known as a 'living fossil', is the sole surviving species of the once dominant gymnosperm flora and probably one of the coal-forming plants in North China[8]. During the Early Tertiary period, it reduced to a circumpolar distribution in the northern hemisphere[9,10]; only a few have been reported from the southern hemisphere[11].

Most of the research is focused on the study of epidermal features of the stem and leaf of *G. biloba* L. and its biochemical and therapeutic attributes, but no such attempt has been made to study the ultrastructural characteristics of the seed coat. The present study records the ultrastructural features of the seed coat of *G. biloba* L. with special emphasis on the occurrence of wax rodlets.

Fresh mature seeds of *G. biloba* L. were collected from Beijing, China in July 2011, for the present investigation. Study of the seed coat was made by stereo microscope (Stemi SV 11) and photographs of entire seeds were taken under digital camera (Nikon P-90).

For ultrastructural features, seed-coat fragments were cleaned, air-dried, mounted on aluminium stubs with glue,

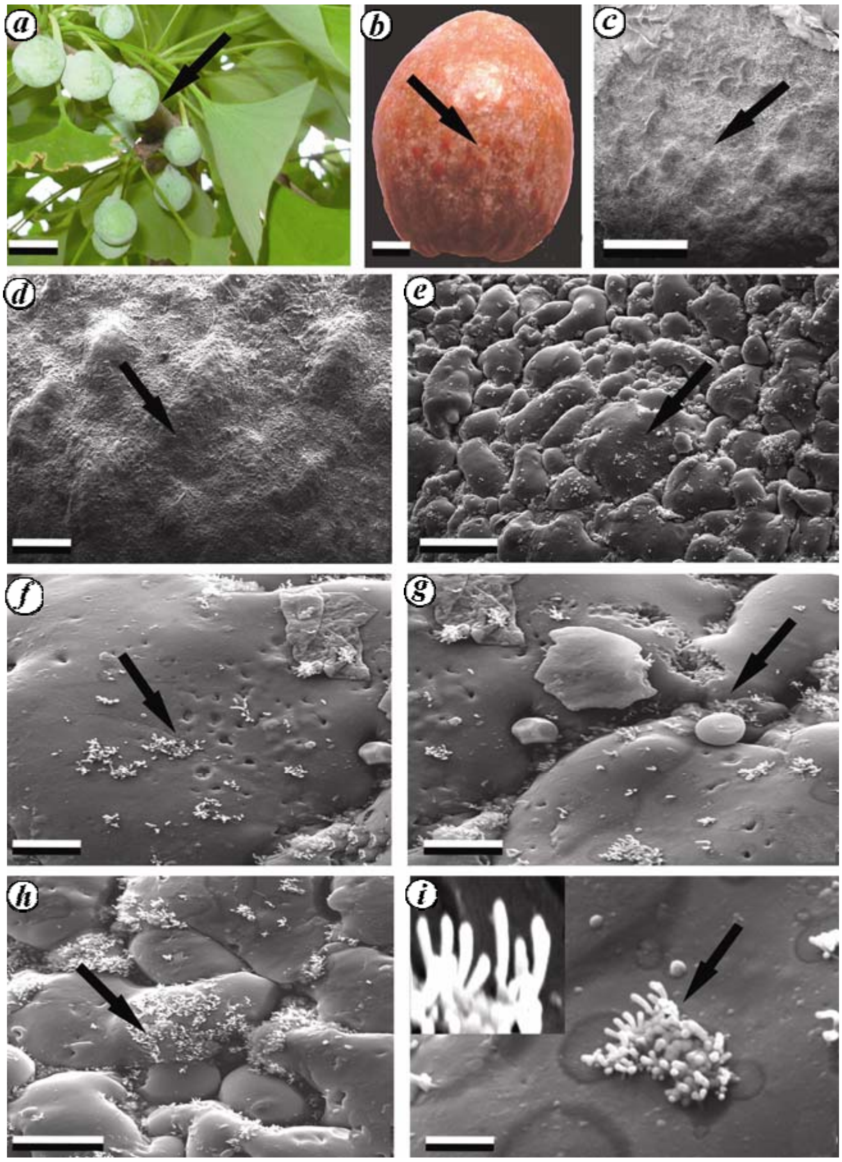

Figure 1. ***a***, A twig of *Ginkgo biloba* L. containing mature green seeds. Scale bar = 15 mm. ***b***, A single ripened seed of *G. biloba* L., showing brown colour and glossy texture of the seed coat. Scale bar = 4 mm. ***c***, A portion of the seed coat under SEM showing elevations on the seed coat surface. Scale bar = 3 mm. ***d***, Elevations magnified, reflecting the papillose nature under SEM. Scale bar = 600 μm. ***e***, Portion of seed coat magnified under SEM showing polyhedral to irregular-shaped epidermal cells of the seed coat. Scale bar = 60 μm. ***f***, Epidermal cells with small pores, distributed irregularly, indicated by arrow. Scale bar = 10 μm. ***g***, Epidermal cell showing sub-surface wax deposition at the ruptured portion (arrow marked). Scale bar = 10 μm. ***h***, Diffused clusters and solitary wax rodlets (arrow mark) on the seed coat surface of *G. biloba* L. under SEM. Scale bar = 20 μm. ***i***, Cluster of wax rodlets on the seed coat surface. (Inset) Magnified view showing tubular shape and erect nature of the rodlets under SEM. Scale bar = 5 μm.

coated with palladium in a sputter coater and scanned under SEM. Photographs were taken using a scanning electron microscope (Zeiss EVO 40) at an accelerating voltage of 15 kV at SEM Laboratory of the Geological Survey of India (GSI), Kolkata.

Energy dispersive X-ray (EDX; Oxford Instruments INCA, UK) analysis was done to confirm the chemical nature of the wax rodlets on the seed coat. The elemental composition of the wax rodlets was determined from the X-ray spectra excited by high-energy electron bombardment of the specimen. In the EDX graph the vertical axis displays the number of X-ray counts and the horizontal axis displays energy in keV.

Mature seeds are green with a whitish wax deposition (Figure 1 *a*), turning brown when ripe, giving the seed coat surface a glossy, uneven texture (Figure 1 *b*).

Under SEM, the seed coat surface appears undulated with small elevations (Figure 1 *c* and *d*). At higher magnification the elevations show variously shaped epidermal cells of different sizes ranging from 20 to 65 μm in length and 10 to 35 μm in breadth, giving the whole surface a papillose appearance. Epidermal cell boundaries are distinct and compactly arranged, more or less polyhedral to mostly irregular in shape (Figure 1 *e*). The periclinal wall level of the epidermal cell is convex, thus giving a dome-shaped appearance, accompanied with scattered, diffused small particles, which are present singly or in clusters (Figure 1 *f*–*i*), throughout the entire seed coat surface. Few epidermal cells show small pores on the periclinal wall (arrow mark in Figure 1 *f*). In more magnified view the scattered, diffused small particles of the seed coat are the epicuticular wax depositions in the form of wax rodlets projecting outwards. Each wax rodlet is erect, tubular in shape, narrowing towards the base, measuring 1.5–2.5 μm in length, mostly aggregated in clusters; few are also present in solitary form (Figure 1 *h* and *i*). Sub-surface wax is also present below the seed coat surface visible at the ruptured portion of the epidermal cell (Figure 1 *g*).

Graphical presentation and quantitative data of the elements present in the wax rodlets from EDX analysis are presented in Figure 2 and Table 1 respectively. The figure shows the peaks, manifesting the presence of carbon and oxygen as major elements, indicating the hydrocarbon nature of the wax rodlets.

The wax extrusions on the seed coat, present in the form of rodlets, referred to as 'epicuticular wax', exhibit a prominent waxy bloom. This may be due to the reflection and scattering of light on the surface by the waxy deposits whose dimensions are close to or only slightly above the wavelength of light[1]. This layer is microcrystalline in structure and forms the outer architecture of the cuticular membrane.

Table 1. Quantitative data of the elements present in the wax rodlets of the seed coat surface of *G. biloba* L. from EDX analysis

Element	Weight (%)	Atomic (%)
C	58.47	65.22
O	41.53	34.78
Total	100.00	100.00

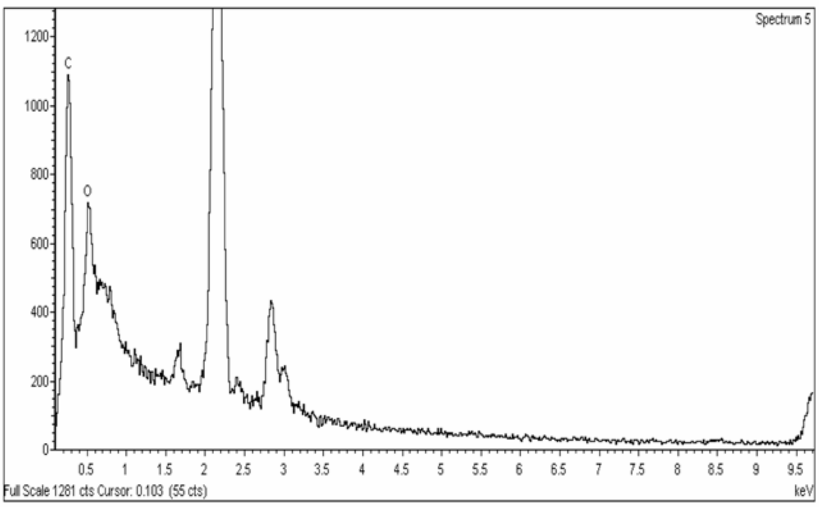

Figure 2. Graphical representation of qualitative data from EDX analysis showing occurrence of different elements present in the seed coat wax rodlets of *G. biloba* L.

In the naked seeded plants, the seed coat is the outermost covering of the seed and so protection is important for retention of viability in case of germination. In *Ginkgo* the seed surface has pores, arranged in a diffused manner (Figure 1 *f*). Entry of water into the seed is greatly influenced by this nature of the seed coat. Heavy deposits of wax and lipid on the surface may repel moisture because of their hydrophobic nature. The wax deposits also plug the pores and intercellular spaces on the seed coat[2].

As wax rodlets cover almost the entire seed surface it may prevent escape of water and resist drying of seeds. It thus plays an essential role in reducing the variation of moisture content during the stratification of seeds, thereby helping in seed germination[12].

The self-cleaning of plant surfaces by water repellence is a novel mechanism against particle accumulation. Superhydrophobicity is biologically significant as a mechanism to protect against plant pathogens such as fungi and bacteria, because germination and penetration of many micro-organisms such as fungi within the plant tissues and reproduction of bacteria are limited by water access[13]. Superhydrophobicity prevents the formation of water films on the surface, which also reduces the gaseous exchange[2].

Results of the present study reflect mostly physiological and mechanical adaptive parameters underlying the seed coat pattern. The morphological architecture of seed coat epidermis may give additional advantages to *G. biloba* for long-time survival. Moreover, the cuticle which is resistant and indestructible, retains the shape and structure of the epidermal cells. Such cuticular features may help in identification and classification of dispersed fragments of cuticle of extinct plants[14] and therefore can be used as an additional tool in identifying this unique gymnosperm.

1. Martin, J. T. and Juniper, B. E., *The Cuticles of Plants*, Edward Arnold, Edinburgh, 1970.
2. Koch, K. and Barthlott, W., *Philos. Trans. R. Soc.*, 2009, **367**, 1487–1509.

3. Meusel, I., Neinhuis, C., Markstädter, C. and Barthlott, W., *Can. J. Bot.*, 1999, **77**, 706–720.
4. Murray, F., Scott, B., Bystrom, G. and Bowler, E., *Am. J. Bot.*, 1962. **49**, 821–833.
5. Machado, C. A., de Oliveira, P. L. and Mentz, L. A., *Braz. J. Pharmacog.*, 2006, **16**, 31–41.
6. Tillman-Sutella, E. and Kauppi, A., *Trees*, 1995, **9**, 269–278.
7. Tillman-Sutella, E. and Kauppi, A., *Can. J. Bot.*, 1998, **76**, 1458–1466.
8. Shenghui, D., Xiaoju, Y. and Zhiyan, Z., *Chin. Sci. Bull.*, 2004, **49**, 1774–1776.
9. Stewart, W. N. and Rothwell, G. W., *Paleobotany and the Evolution of Plants* (eds Stewart, W. N. and Rothwel, G. W.), Cambridge University Press, London, 1993, pp. 385–412.
10. Zhou, Z. Y., *Acta Bot. Yunnanica*, 2003, **25**, 377–396.
11. Hill, R. S. and Carpenter, R. J., *Aust. J. Bot.*, 1999, **47**, 717–724.
12. Pawuk, W. H., *Tree Planter's Notes*, 1993, **44**, 21–24.
13. Stosch, A. K., Solga, A., Steiner, U., Oerke, C., Barthlott, W. and Cerman, Z., *Appl. Bot. Food Qual.*, 2007, **81**, 49–55.
14. Fahn, A., *Secretory Tissues in Plants*, Academic Press, London, 1979, pp. 151–221.

ACKNOWLEDGEMENT. We thank Swapan Chowdhury, Assistant Geologist, GSI, Kolkata, for co-operation and assistance in EDX analysis.

Received 15 July 2012; revised accepted 23 November 2012

SOMA MAJUMDER[1]
ASHALATA D'ROZARIO[2]
SUBIR BERA[1,*]

[1]*Centre of Advanced Studies, Department of Botany, University of Calcutta, Kolkata 700 019, India*
[2]*Department of Botany, Narasinha Dutt College, Howrah 711 101, India*
**For correspondence.*
e-mail: berasubir@yahoo.co.in

Identification of regional-scale cuspate–lobate folds in Singhbhum region, India using satellite remote sensing

In the Precambrian Singhbhum craton of Eastern India, the area north of the Singhbhum shear zone and south of Chota Nagpur Plateau is known as the North Singhbhum Mobile Belt (NSMB). Many researchers have made valuable contributions towards the understanding of stratigraphy, structural and tectonic evolution, and economic geology of this region. The objective of the present study is to report regional-scale cuspate–lobate folds[1] in the Proterozoic NSMB based on the study of satellite imagery.

The NSMB comprises Proterozoic Singhbhum Group and the Dalma Lavas[2]. The Singhbhum Group of rocks occurs between Singhbhum shear zone and Dalma syncline (Figure 1). The area extends over a strike length of more than 150 km with a broadly east-west arcuate trend that is convex northward.

The Singhbhum Group is composed of a thick sequence of mainly amphibolite facies meta-sedimentary rocks of Chaibasa

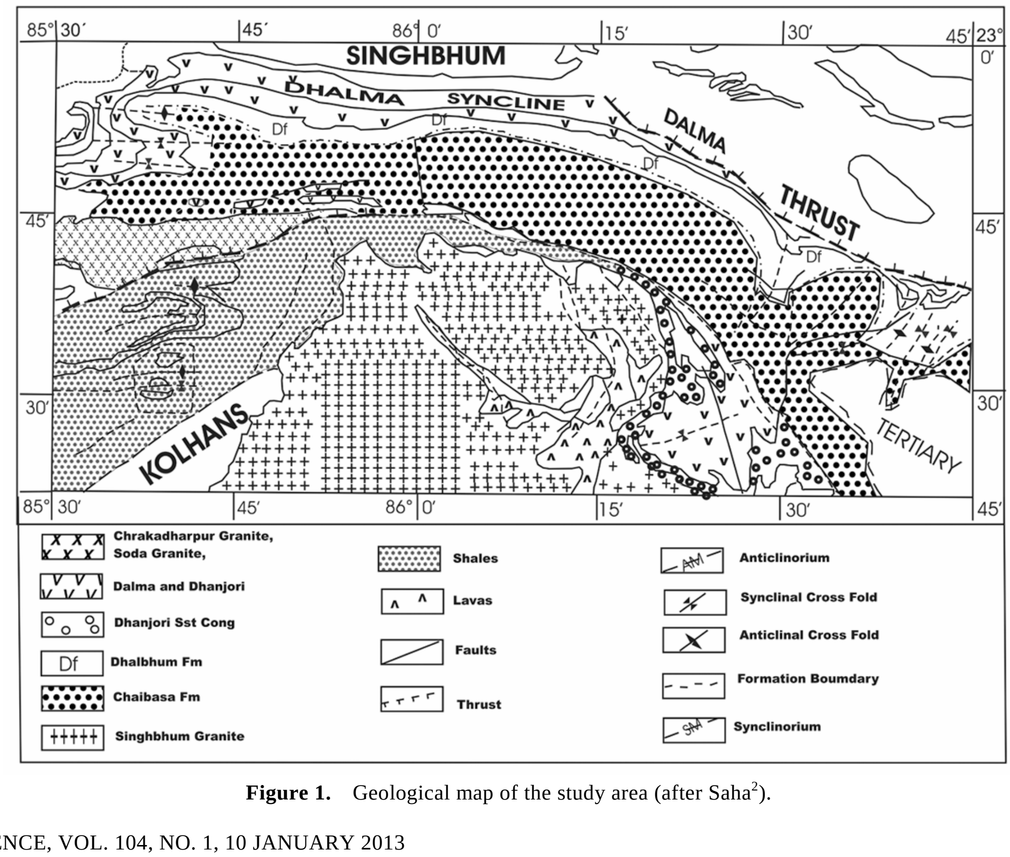

Figure 1. Geological map of the study area (after Saha[2]).