

SEED COAT MICROSCULPTURING IN *GINKGO BILOBA* L. CULTIVARS

MAŁGORZATA KLIMKO, STANISŁAWA KORSZUN, MARCIN KOLASIŃSKI

M. Klimko, Department of Botany, Poznań University of Life Sciences, ul. Wojska Polskiego 71 C, 60-625 Poznań, Poland, e-mail: klim@up.poznan.pl
S. Korszun, M. Kolasiński, Department of Dendrology, Pomology and Nursery, Poznań University of Life Sciences, Dąbrowskiego 159, 60-594 Poznań, Poland, e-mail: kolamarc@up.poznan.pl

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ABSTRACT. This article presents the results of a study on seed coat micromorphology and epicuticular structure of *Ginkgo biloba* cultivars. The micromorphology of the seed coat of 10 cultivars and one clone of female *G. biloba* were analysed. The taxa differed in the size and shape. Homogeneous epicuticular wax deposited in the form of wax tubules on the sarcotesta and sclerotesta surfaces. There were great differences in wax between the outer and inner layer of the sarcotesta. In the endotesta wax was diversified in the form of tubules, platelets or it was mixed. The article includes descriptions and illustrations of several micromorphological features which have not been published previously. The results of the SEM and LM investigation confirmed the taxonomic significance of the micromorphological traits in the *Ginkgo* genus. In the seeds many characters are present in of all the taxa and may be typical of *Ginkgo*.

KEY WORDS: *Ginkgo biloba*, seed coat sculpture, SEM, LM

INTRODUCTION

Ginkgo biloba L. is a unique living representative of the order Ginkgoales, and is usually described as a 'living fossil' (LIU et al. 2006). *G. biloba* is a now rare species in the wild but has been widely and long cultivated as an ornamental (KORSZUN & KLIMKO 2014). The ginkgo is a dioecious tree. In Poland few ginkgo cultivars are grown. They are to be used as ornamental trees in green spaces. Usually these are male clones. The literature does not provide much information about the micromorphology of vegetative and generative organs of *G. biloba* cultivars (KORSZUN & KLIMKO 2014, TOMASZEWSKI & ZIELIŃSKI 2014, KLIMKO et al. 2015). A mature *Ginkgo* seed is relatively large and consists of an embryo embedded in the tissue of the female gametophyte surrounded by a thick seed coat. The sporangial wall is multi-layered. This seed-coat consists of a soft, orange and brown, glaucous fleshly outer layer (sarcotesta), a hard stony middle layer (sclerotesta) and a thin membranous inner layer (endotesta). Each ovule consists of a nucellus and a single integument. The endotesta protects the endosperm (WANG et al. 2007b). The endosperm is the main edible part of the seed, which contains nutri-

ents, starch and proteins (WANG et al. 2007a, b, JIN et al. 2012). The aims of this study was to describe and document microsculpturing of the seed coat in *G. biloba* cultivars.

MATERIALS AND METHODS

In November 2011 the authors collected mature *G. biloba* seeds after they had naturally fallen from 10 cultivated ornamental trees: '10-98', 'DFS', 'Epiphyl-la', 'Gui-Zhi', 'Hunan', 'Korinek', 'Mei-Her', 'Mieszko I', 'Ohazuki', 'Variegata' and a female form. They were selected from around 60 varieties in the collection of the Department of Dendrology, Pomology and Nursery, Poznań University of Life Sciences, Poland. For about 15 years the trees had been grown in the ground, in similar habitats. The cultivars under study were grafted with scions collected from trees producing seeds. A scanning electron microscope (SEM) and light microscope (LM) were used to obtain comprehensive information about the seed-coat sculpture. Micrographs were taken with a SEM type EVO 40 (Carl Zeiss, Jena, Germany) microscope at an accelerating voltage of 15kV, at the Confocal and the Electron Microscopy Laboratory, Faculty of Biology,

Adam Mickiewicz University, Poznań, Poland. Prior to the observation the prepared material was sputtered with gold (for 15 s) by means of an SCB 050 ion sputter (Balzers AG, Liechtenstein). Five seeds of individual taxa were examined for the following traits: inner and outer epidermis of the sarcotesta, outer epidermis of the sclerotesta, and endosperm surface, wax and cuticle sculpture. The study was documented with five microphotographs for each seed, with magnified photographs ($\times 500$, $\times 1000$ and $\times 20000$) taken during the observation. The terminology of epicuticular waxes follows RIEDERER (1989) and BARTHLOTT et al. (1998).

RESULTS AND DISCUSSION

The size and shape of the seeds varies in the years of collection (KORSZUN & KOLASIŃSKI, unpub. data). The length of the *Ginkgo* arillate seeds ranged from 17.3 mm ('Gui-Zhi') to 32.3 mm ('Hunan'), and width from 18.01 mm ('Gui-Zhi') to 42.6 mm ('10-98'). The length to width ratio ranged from 0.64 to 1.55 ('10-98'). The length and width of the *Ginkgo* without arils seeds ranged from 16.6 mm ('Ohazuki') to 29.5 mm ('DSF') and 12.4 mm ('Mieszko I') to 18.4 mm ('Hunan'), respectively. The length to width ratio ranged from 1.01 ('Ohazuki') to 1.62 ('DSF').

Sarcotesta sculpture. Under the SEM the sarcotesta surface (Fig. 1A) is covered by thick wax and the epidermal cells were not visible. There are epicuticular wax deposits in the form of wax rodlets projecting outwards. Each wax rodlet is erect and tubular in shape (Fig. 1A) (RIEDERER 1989, BARTHLOTT et al. 1998). This type of wax was found in the seed coat (in the sarcotesta layer) from Beijing, China (MAJUMDER et al. 2013). As results from the study by MAJUMDER et al. (2013), the EDX analysis revealed that wax tubules in the sarcotesta were composed of two different elements – carbon and oxygen, thus indicating the hydrocarbon nature of the wax rodlets. Under the LM all the taxa are characterised by a reticulate sarcotesta pattern (Fig. 1B). The epidermis cells are polygonal. Their length is nearly equal to their width (Fig. 1B). They have largely straight anticlinal walls. The anticlinal walls that form a boundary between the cells protruded above the outer tangential surfaces of the cells. The periclinal walls are slightly concave and smooth. There are a few differences observed in the sarcotesta surface. MAJUMDER et al. (2013) report that this surface was undulated, with small elevations of various shapes and different sizes. The anticlinal walls were distinct and the periclinal walls of the epidermal cell were convex, thus giving it a dome-shaped appearance. Differences in the sarcotesta structure result from the degree of the sarcotesta maturity. This study showed that there are rounded resin canals (secretory cavities) on the outer and inner surface of the sarcotesta (Figs 1B,

C). The inner layer of the sarcotesta differed from the outer layer in the absence of wax. This surface is characterised by diversified cuticular layer, which is smooth, slightly striate and granulate (Fig. 1C). The micromorphological sculpture of the sarcotesta is the same in all cultivars.

Sclerotesta surface. Beneath the sarcotesta is the hard sclerotesta. The surface of the sclerotesta is covered by the same wax tubules as the sarcotesta surface (Fig. 1E, arrow). The sclerotesta is white or creamy and have irregular small pores (Fig. 1D). The epidermis cells are polygonal and elongate, with acute to rectangular ends. The form of the anticlinal walls is largely straight and the periclinal cell walls are convex (Fig. 1F). The cuticle on the sclerotesta surface is smooth and it is the same in all taxa.

Endotesta. The endotesta of *Ginkgo* seeds is very thin. In SEM individual cells are invisible, because the surface is covered by a thick epicuticular layer. The outer surface could be smooth and slightly folded without wax (Fig. 2A), or with wax platelets (Fig. 2B), rodlets or a mixture of both (Fig. 2C). The reticulate endotesta of the outer and inner surfaces is visible in LM (Figs 2D, E). The cells are elongated, axially parallel with the long axis of the endosperm. The anticlinal walls are straight or slightly undulate, whereas the periclinal walls are slightly convex. The epidermal cell walls between the outer and inner surface differed in their thickness (Figs 2D, E). This feature is the same in all taxa.

Endosperm. All the taxa are characterised by a reticulate endosperm pattern on the upper surface. The anticlinal walls are thin and slightly undulate (Fig. 2F).

Our results suggest that although some cultivated plants are morphologically well characterised by size and shape of leaves, they cannot be identified on the basis of the micromorphology of the seed coat, because all taxa are very similar and there were no absolute micromorphologic diagnostic characters between the taxa under study. The micromorphology of some *Ginkgo* cultivars provided some important new data, e.g. wax rodlets on sarcotesta and sclerotesta, resin canals in sarcotesta, the inner layer of the sarcotesta differed from the outer layer in the absence of wax and form anticlinal and periclinal cell walls. In general, seeds, as a generative organs that are only slightly influenced by environmental conditions, are important diagnostic feature. In analyzing features we have shown similarities in seed sculpture between different cultivars not reported earlier. The epicuticular features may help to identify and classify dispersed cuticular fragments in fossil seeds (FAHN 1979) and therefore they can be used as an additional tool to identify this unique gymnosperm. The results of the SEM and LM study confirm the taxonomic significance of micromorphological traits in the *Ginkgo* genus.

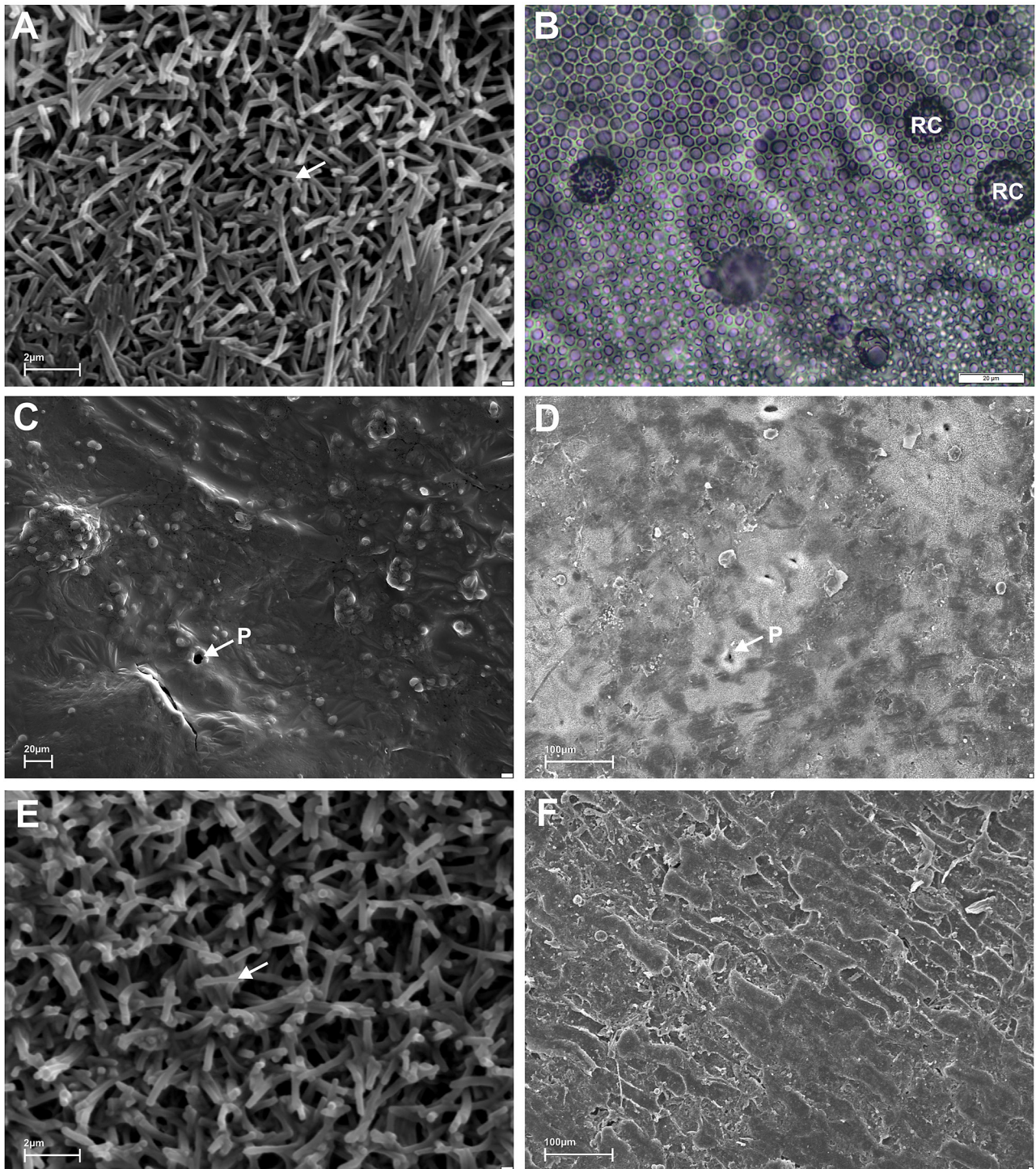


Fig. 1 A–F. Micromorphology of seed coat of *Ginkgo biloba*: A – ornamentation of wax on sarcotesta ‘GUI Zui’ (SEM), B – sarcotesta outer epidermis of ‘10–98’ (LM), C – sarcotesta inner layer ‘Epiphylla’ (SEM), D – sclerotesta outer layer ‘Mei-Her’ (SEM), E – ornamentation of wax on sclerotesta ‘Korinek’ (SEM), F – sclerotesta outer epidermis, clone of female (SEM). Abbreviations: arrow-wax tubule, P – pores, RC – resin canals

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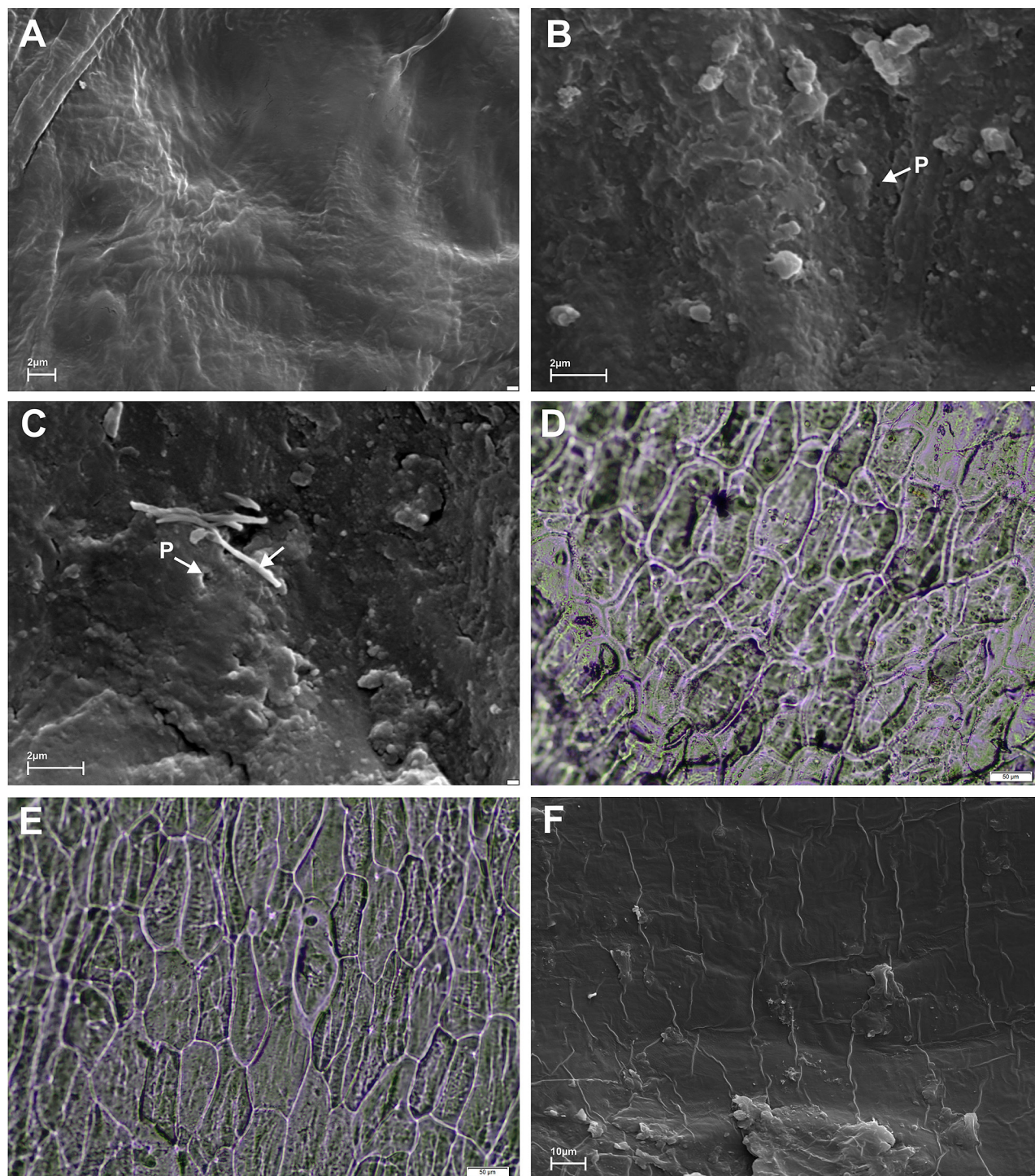


Fig. 2 A–F. Micromorphology of seed coat of *Ginkgo biloba*: A–C – endotesta outer epicuticular layer ‘GUI-Zui’ (SEM), D – endotesta outer epidermis ‘Mieszko I’ (LM), E – endotesta inner layer ‘Variegata’ (LM), F – endosperm outer epidermis clone of female, (SEM). Abbreviation: arrow – wax tubule, P – pores.

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