CUTIN FLUORESCENCE IN EARLY EMBRYOS OF *PINUS* AND *TSUGA*EWA SZCZUKA¹ and IRENA GIEŁWANOWSKA²¹Plant Anatomy and Cytology Department,
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ABSTRACT

Embryos of *Pinus nigra* Arnold and *Tsuga canadensis* Carr. (*Pinaceae*) at different stages of development were dissected from fresh, unfixed seeds and examined in a fluorescence microscope with 400 nm excitation light.

The embryos of the investigated species showed cutin fluorescence after auramine O staining. At first the fluorescing cutin layer was formed on the apical part of the embryo with a well developed secondary suspensor, then it extended over the lateral surface of the embryo; the suspensor remained nonfluorescent. The fluorescing cutin layer occurred on the apical and side surface of the embryo, undergoing differentiation into the shoot axis and root initials.

It is assumed that polarization and nutrition of the embryo may be influenced by presence of the cuticle.

KEY WORDS: embryonic cutin, gymnosperm embryogenesis.

Developing embryos of angiosperms formed a thin cutin layer which gave fluorescence after application of auramine O. This fluorescence appeared on the embryo proper at the globular stage and persisted over the heart stage, but there was no fluorescing layer on the suspensor.

During the following stages of embryogenesis the fluorescing areas of the embryo changed according to a certain pattern (Rodkiewicz et al. 1994; Szczuka and Rodkiewicz, in press).

We also noticed cutin fluorescence in early embryogenesis in two species of gymnosperms.

Embryos of *Pinus nigra* Arnold and *Tsuga canadensis* Carr. (*Pinaceae*) were dissected from fresh, unfixed seeds at early development stages. For cutin fluorescence, these unfixed embryos were directly placed in auramine O solution in 0.05 M Tris/HCl buffer at pH 7.2 (Heslop-Harrison, 1977). Preparations were investigated in the fluorescence microscopy with 400 nm excitation light.

There was no fluorescing cutin on embryonal cells with one embryonal tube (Fig. 1). The first weak fluorescing cutin layer was formed on the apical part of the embryo with a well developed secondary suspensor. The fluorescing layer extended later on the embryonal cells but not on the suspensor formed by several embryonal tubes (Figs 2 and 3). Later, the fluorescing layer extended over the lateral surface of the embryo, forming a large secondary suspensor (Figs 4, 5, 6, 7 and 8). The cutin layer was present on the apical and side surface of the embryo; presumably the embryo without cotyledonary

primordia is undergoing differentiation into shoot axis and root initials. The area of the cutin layer assumingly corresponds to the shoot axis. The basal part of the embryo with root initials, the root cap and, a large number of cells differentiating into the embryonal tubes – displayed no cutin fluorescence (Fig. 9).

In the investigated species of gymnosperms the first cutin fluorescence appeared on the apical part of the early embryo with a secondary suspensor. Then the fluorescing layer of the cutin extended over the entire embryo proper, but the suspensor remained nonfluorescent in all investigated embryos of gymnosperms. A similar appearance and extending fluorescing cutin were observed in some species of angiosperms (Szczuka et al. 1995; Szczuka and Rodkiewicz, 1996). It may be assumed that this phenomenon is common to a great number of angio- and gymnosperms. In both groups of these plants the fluorescing cutin envelops the embryo proper, but is almost absent around the suspensor. The presence of the cuticle over the embryo and its absence on the suspensor supports the view that in a number of angiosperms and gymnosperms the suspensor plays an active role as the major route for the transport of materials into the early embryo (ref. in Szczuka and Rodkiewicz, 1996). We may assume that the fluorescing layer of the cutin partially isolates the embryo and limits the exchange of solutes. The stream of substances entering the embryo of gymnosperms from the suspensor may enhance polarization of the embryo and be involved in the formation of the root-shoot axis.

Fig. 1. *Tsuga* embryo, Nomarski microscope. Embryonic cells and an embryonal tube (secondary suspensor) – no cutin fluorescence at this stage, x 150. Figs 2 and 3. The early embryo of *Pinus*, x 150; 2. Light microscope. 3. Fluorescence microscope. Embryo with secondary suspensor; apical surface gives cutin fluorescence.

Figs 4-6. *Tsuga* embryogenesis, x 160 4. Nomarski microscope. Early embryo with secondary suspensor. 5. Similar embryo as in Fig. 4. in fluorescence microscope. 5 and 6. The embryos invested with fluorescing cutin layer. Secondary suspensor without cutin fluorescence.

Figs 7-9. *Pinus* embryogenesis. 7. Embryo with secondary suspensor. The embryo, except the secondary suspensor, enveloped in fluorescing cutin layer, x 160; 8. Older embryo than in Fig. 7. with fluorescing cutin layer, x 75; 9. The embryo differentiated in the shoot axis and root initials – the plane of root initials shown by arrowheads. The base part is formed by divisions of the root cap and embryonal tube primordia, x 80.

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FLUORESCENCJA KUTYNY W MŁODYCH ZARODKACH *PINUS* I *TSUGA*

STRESZCZENIE

Zarodki *Pinus nigra* Arnold i *Tsuga canadensis* Carr. (*Pinaceae*) po zabarwieniu auraminą O wykazują fluorescencję kutyny. Fluoryzująca kutyna pojawia się na wierzchołku zarodka z wieszadelkiem wtórnym, a następnie rozszerza się na boczną powierzchnię zarodka; nie ma kutyny na wieszadelku. Warstwa kutyny fluoryzuje w górnej części zarodka różnicującego się na na oś pędową i zaczątek korzenia.

Przypuszcza się, że kutykula na powierzchni zarodka wpływa na polaryzację i odżywianie zarodka.

SŁOWA KLUCZOWE: embrionalna kutyna, embriogeneza nagozalążkowych.