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CUTICLE FLUORESCENCE DURING EMBRYOGENESIS OF *ARABIDOPSIS THALIANA* (L.) HEYNH.

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Cuticle visualized by auramine O fluorescence on developing *Arabidopsis thaliana* embryos was investigated. Localization of the cuticle was studied on embryos of the zygotic wild Ler ecotype and nine lines of embryonic mutant: CS 2330, CS 3009, CS 3016, CS 3023, CS3025, CS 6330, CS 6340, CS 6343 and CS 6346. In *Arabidopsis* Ler ecotype embryogenesis, a fluorescing cuticle layer appears on the globular embryo and persists during successive stages of development. Such a layer does not occur on the suspensor. In a similar way, fluorescing cuticle envelops the entire globular and older embryo of embryonic mutants, although the embryos of different mutant lines reach different developmental stages.

Key words: *Arabidopsis*, embryo, embryonic mutants, cuticle, fluorescence.

INTRODUCTION

Several authors, particularly Yakovlev and Tshaban (1979) and Chamberlain et al. (1993) investigating embryogenesis in *Reseda lutea* and *Glicine max*, respectively, have described an electron-dense layer, which is considered to be a cuticle. The outer surface with cuticle on a heart-shaped *Helianthus annuus* embryo is described by Newcomb (1973) and in *Linum catharticum* by D'Alascio-Deschamps (1978). In all these plants, with the exception of *Linum*, cuticle is not observed on the suspensor surface.

A subtle cuticular layer fluorescing after auramine O treatment appears at the globular stage of embryo development. In *Stellaria* and *Alisma* it occurs on the apical part of the zygotic embryo proper, just after delimitation of the protoderm. Then the cuticular area extends above the entire surface of the globular embryo proper (Szczuka et al., 1995). The emergence of cuticle is quite different in orchid *Cymbidium sinense*. It starts from the lateral parts of the elongated, highly reduced, young embryo. In this orchid the cuticular layer surrounds the developing embryo proper. The branched and extended suspensor is not covered by cuticle (Yeung et al., 1996).

Fluorescing cuticle is described on zygotic embryos at various stages of development. Several monocots and dicots of angiosperm species and also the successive stages of developing somatic embryos of × *Triticosecale* have been investigated in this respect (for references: Szczuka et al., 1999).

This paper describes observations of cuticle fluorescence during *Arabidopsis thaliana* zygotic embryogenesis, and comparisons with embryos of embryonic mutants.

MATERIALS AND METHODS

The study used embryos of zygotic plants of the wild Landsberg *erecta* (Ler) ecotype and nine lines of embryonic mutant: CS 2330, CS 3009, CS 3016, CS

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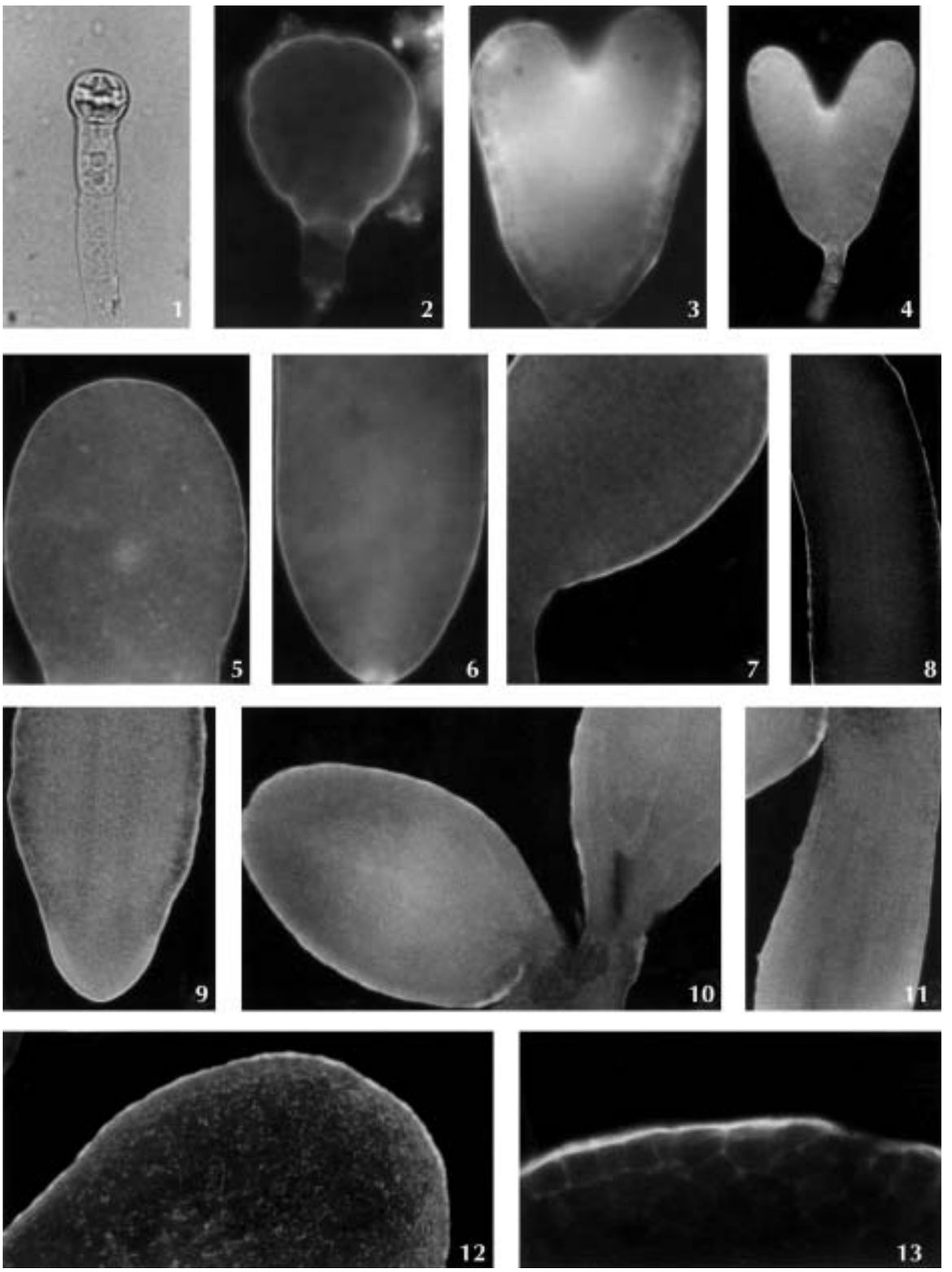
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3023, CS 3025, CS 6330, CS 6340, CS 6343 and CS 6346 of *Arabidopsis thaliana* (L .) Heynh. (Brassicaceae). Seeds of the Ler ecotype were obtained from the Department of Plant Anatomy and Cytology of Silesian University in Katowice (Poland), and embryonic mutant seeds from the Arabidopsis Biological Center of Ohio State University (U.S.A.). Both kinds of plants had been grown in greenhouse conditions. Fresh embryos at different stages of development were dissected from living ovules and placed in a drop of fluorochrome auramine O solution. The dye was made up at 0.01% in 0.05 M tris/HCl buffer, pH 7.2 (Heslop-Harrison, 1977).

RESULTS

The cuticle formed during embryogenesis can be easily shown, since cutin, its main component, fluoresces after auramine O staining. There is no fluorescence after auramine O treatment on the *Arabidopsis* proembryo surface (Fig. 1). At first the fluorescing cuticle is visible on the globular embryo (Fig. 2). At this stage of development the cuticular layer is found on the entire protoderm surface of the embryo proper. Only the cell adjoining the suspensor and suspensor cells show no fluorescence indicating the presence of cuticle. Cuticle is visible on early and late heart-shaped embryos (Figs. 3, 4). It envelops the embryo proper but not all suspensor cells. On the late heart-shaped embryo, fluorescence is visible not only on the embryo proper but also on the suspensor. The fluorescing cuticular layer remains on the torpedo embryo (Figs. 5, 6), and is particularly distinct on the late torpedo embryo (Figs. 7–9). Strong cuticle fluorescence persists on the developed embryo (Figs. 10–13).

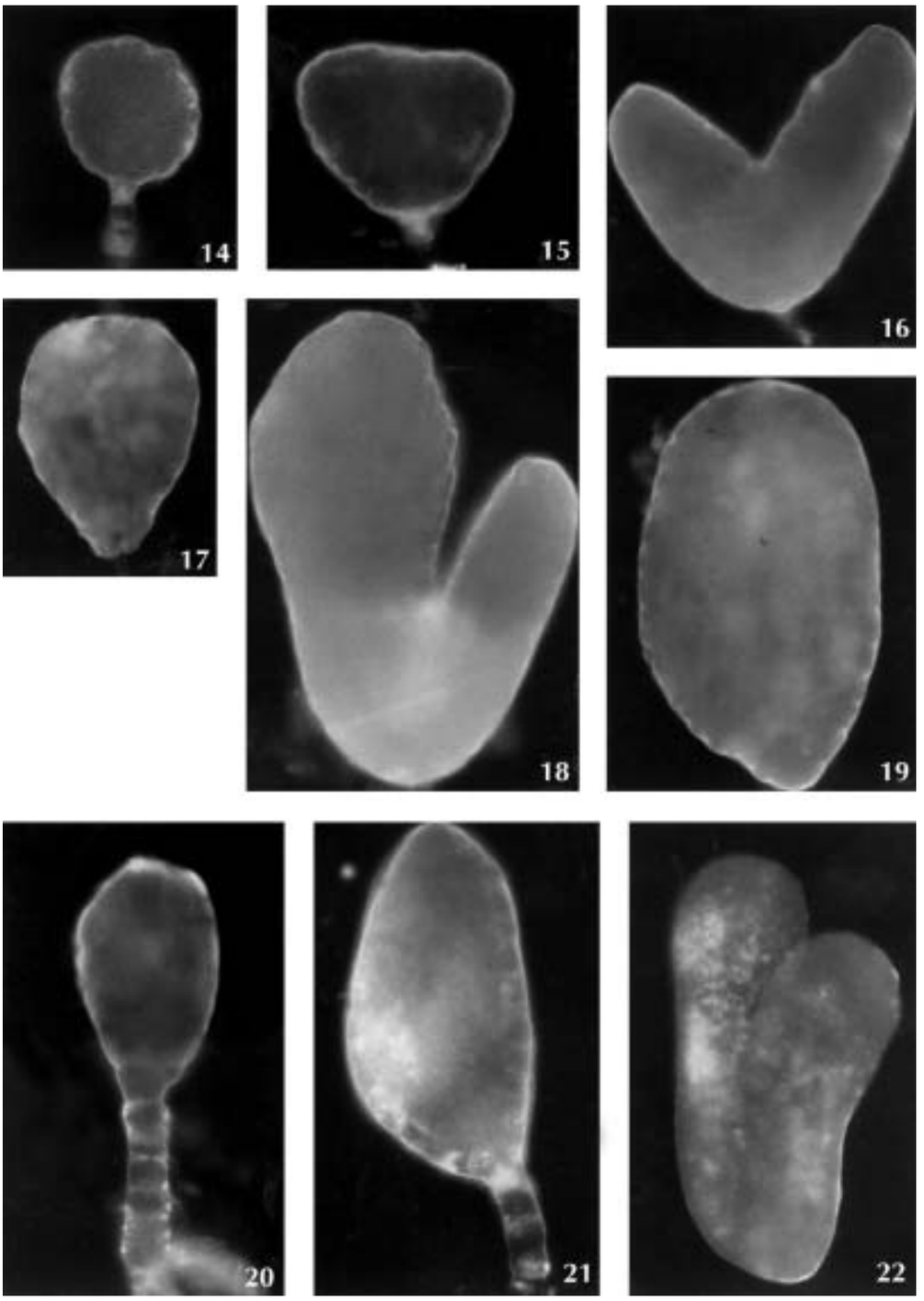
At the globular stage the embryo proper of the CS 3009 line embryonic mutant is covered by fluorescing cuticle (Fig. 14). There is no fluorescing cuticle on the suspensor cell surface of such a globular embryo. In a similar way, cuticle envelops the embryos at the transition from the globular to heart-shaped stage (Fig. 15) and advanced heart-shaped stage (Fig. 16). Fluorescence is visible on irregularly built embryos: the mace-shaped embryo of CS 6343 (Fig. 17), the embryo with different-length cotyledons of CS 3016 (Fig. 18) and the oval embryo of CS 6346 (Fig. 19) lines. Fluorescing cuticle is distributed in a different way in CS 3023 and CS 3025 line embryos. Cuticle surrounds the mace-shaped embryo proper of the CS 3023 line, and additionally covers the external walls of suspensor cells (Fig. 20). In the same way, cuticle occurs on the CS 3025 line embryo (Fig. 21). The surface of the CS 2330 line embryo, the most advanced in development, is covered by cuticle of irregular thickness, which is not uniformly distributed (Fig. 22).

DISCUSSION

In *Arabidopsis thaliana* (L.) Heynh. wild Ler ecotype embryogenesis, a fluorescing cuticle layer appears on the globular embryo and persists during the successive stages of development. The proembryo does not show any cuticle. In the same way, fluorescing cuticle envelops the entire globular and older embryos of over twenty monocots and dicots of angiosperm species, as it does at successive development stages of somatic embryos of × *Triticosecale* (for references: Szczuka et al., 1999). Similarly, globular and older atypically shaped embryonic mutant embryos of *Arabidopsis* are covered by fluorescing cuticle, although the embryogenesis of different mutant lines ends at different stages of development.

These results obtained by fluorescence microscopy are confirmed by electron microscopy (Szczuka et al., 1995; Szczuka and Rodkiewicz, 1996; Rodkiewicz and Szczuka, 1997; for references to earlier research see the introduction to this paper). According to Yakovlev and Tshaban (1979), in *Reseda lutea* the cuticle persists on heart-shaped embryos, but gradually disappears with the disintegration of endosperm cells. In contrast, in *Glicine max* the cuticle covers the embryo proper at the later stages of embryogenesis also (Chamberlain et al., 1993). Timmers (1993) used electron microscopy to show a very thin cuticle on a torpedo-shaped zygotic embryo of *Daucus carota*.

Figs. 1–13. Embryogenesis of *Arabidopsis*. **Fig. 1.** Proembryo. Bright-field microscopy. × 450. **Figs. 2–13.** Cuticle fluorescence after auramine O treatment. **Fig. 2.** Globular embryo with fluorescing cuticle. × 450. **Fig. 3.** Heart-shaped embryo. × 450. **Fig. 4.** Late heart-shaped embryo. × 220. **Figs. 5, 6.** Early torpedo embryo with cotyledon (**5**) and hypocotyl and radicle (**6**). × 300. **Figs. 7–9.** Late torpedo embryo; part of cotyledon (**7**), × 200, hypocotyl (**8**), × 70, and hypocotyl and radicle (**9**), × 150. **Figs. 10, 11.** Developed embryo with fluorescing cuticle on cotyledons (**10**) and hypocotyl (**11**). × 150. **Figs. 12, 13.** U-shaped embryo, older than in Figs. 10 and 11. Fragments of cotyledon with fluorescing cuticle. Fig. 12. × 70. Fig. 13. × 400.



Figs. 14–22. Embryos of *Arabidopsis* embryonic mutants with fluorescing cuticle after auramine O. **Fig. 14.** CS 3009 line. × 200. **Fig. 15.** CS 6330 line. × 270. **Fig. 16.** V-shaped embryo of CS 6340 line. × 270. **Fig. 17.** CS 6343 line. × 200. **Fig. 18.** CS 3016 line. × 180. **Fig. 19.** CS 6346 line. × 180. **Fig. 20.** CS 3023 line. × 300. **Fig. 21.** CS 3025 line. × 300. **Fig. 22.** Torpedo embryo with fragments of thicker fluorescing cuticle on the surface; CS 2330 line. × 130.

Our observations on the presence of fluorescing cuticle on the developing *Arabidopsis* embryo are generally in line with the results obtained by Yeung et al. (1996). These authors reported the presence of fluorescing cuticle in the successive stages of *Cymbidium sinense* embryogenesis. In this orchid species, cuticle is present on the surface of the elongated, cylindrical, highly reduced embryo proper.

Cuticle is seen over the surface of the embryo proper, but in most investigated species it is entirely or almost entirely absent over the suspensor. In the *Arabidopsis* wild Ler ecotype, cuticle was observed only on the suspensor cell walls in the heart-shaped embryo, in a small part of the suspensor adjacent to the embryo proper. The upper part of the suspensor has been clearly shown to be covered by cuticle in *Linum catharticum* (D'Alascio-Deschamps, 1978), *Linum usitatissmum* (Szczuka et al., 1995) and *Brassica napus* (Szczuka, 1995). The presence of fluorescing cuticle surrounding the upper part of the suspensor seems to be more common.

Among the embryonic mutant lines described in this paper, fluorescing cuticle was found on the suspensor cell walls in only two. However, as mentioned above, the embryos of the embryonic mutant lines reach different developmental stages typical of a given line. In some embryonic mutant lines such as CS 6343 the embryo develops to reach the globular stage. In the suspensor, cuticle has been observed (in fluorescence or electron microscopy) in zygotic embryogenesis only during the transition from globular to heart-shaped, and heart-shaped stages (Szczuka, 1995; Szczuka et al., 1995).

It is common knowledge that nutrients are transferred to the embryo proper via the suspensor. The cuticle present in the upper part of the suspensor can make it more rigid, which can affect the stream of substances being exchanged between the embryo and endosperm. The rings strengthening the cellular joints in *Brassica napus* (Szczuka, 1995) can play the same role as the cuticle of the cells of the upper suspensor. In the embryos of the embryonic mutant lines the suspensor cuticle fulfills a similar function.

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