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Chlorophyll and cutin in early embryogenesis in *Capsella*, *Arabidopsis*, and *Stellaria* investigated by fluorescence microscopy

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Abstract Advanced globular embryos of *Capsella* and early heart-shaped embryos of *Arabidopsis* begin to show chlorophyll fluorescence. It is not present in the suspensor, epiphysis, radicle and embryo of *Stellaria*. Cutin fluorescence appears on the protoderm of all advanced globular embryos. Fluorescence disappears during the early torpedo stage. It is not present on suspensors.

Key words *Arabidopsis* · *Capsella* · *Stellaria* Embryogenesis · Chlorophyll · Cutin

Introduction

Maturing embryos of some angiosperm species form chlorophyll and become green, while in some other species, they remain nongreen. Therefore, two categories of plants were distinguished: chloroembryophyta and leukoembryophyta (Yakovlev and Zhukowa 1980). Greening appears in heart-shaped and torpedo *Arabidopsis* embryos (Mansfield and Briarty 1991) and in globular embryos of *Linum* (Pretova 1985).

Using a fluorescence microscope we have shown chlorophyllid, chlorophyll, and cutin fluorescence in globular, heart-shaped, and torpedo embryos.

Materials and methods

Globular, heart-shaped and early torpedo embryos dissected from living ovules of *Capsella bursa-pastoris* L. and *Arabidopsis thaliana* (L.) Heynh. (Brassicaceae) and *Stellaria media* Vill. (Caryophyllaceae) were investigated with a fluorescence microscope with a 400-nm excitation light. In UV light, chlorophyll and chlorophyllid give red autofluorescence. Cutin fluorescence was demonstrated after application of fluorochrome auramine O according to the procedure used by Heslop-Harrison (1977).

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Results

In *Capsella*, young 4–16 cell embryos do not show chlorophyll red fluorescence, but it appears in larger globular embryos. Primarily, fluorescence is stronger in the peripheral layer (Figs. 1, 2); later, it is equally strong in the entire embryo proper (Fig. 3). Cotyledon primordia of the early heart-shaped embryo show weak fluorescence, while the remaining embryo cells give strong fluorescence. Cotyledons of older heart-shaped and torpedo embryos become wholly fluorescent. The suspensor, hypophysis and primordial radicle remain non-fluorescent.

In *Arabidopsis*, chlorophyll fluorescence begins to appear in early heart-shaped embryos (Fig. 4) and follows the pattern observed in *Capsella* (Figs. 5 and 6).

Stellaria embryos give no chlorophyll fluorescence. *Capsella*, *Arabidopsis*, and *Stellaria* embryos show a similar pattern of cutin fluorescence. It appears on the protoderm of large, globular embryos and remains during the heart-shaped stage. It disappears in the early torpedo stage (Figs. 7–12). There is no cutin fluorescence on the suspensor.

Discussion

Ultrastructural features of plastids are very similar in the developing embryos of *Arabidopsis* (Mansfield and Briarty 1991), *Capsella* (Schulz and Jensen 1968) and some other plants, e.g., *Helianthus* (Newcomb 1973), *Linum* (Deschamps 1968), and *Quercus* (Singh and Mogensen 1975). Plastids in globular embryos contain single lamellae; in a heart-shaped embryo, they begin to form rudimentary granal stacks which during the torpedo stage develop into grana. It may be assumed that red autofluorescence of globular and heart-shaped embryos is due to the presence of chlorophyllid which is synthesized before the grana are set up.

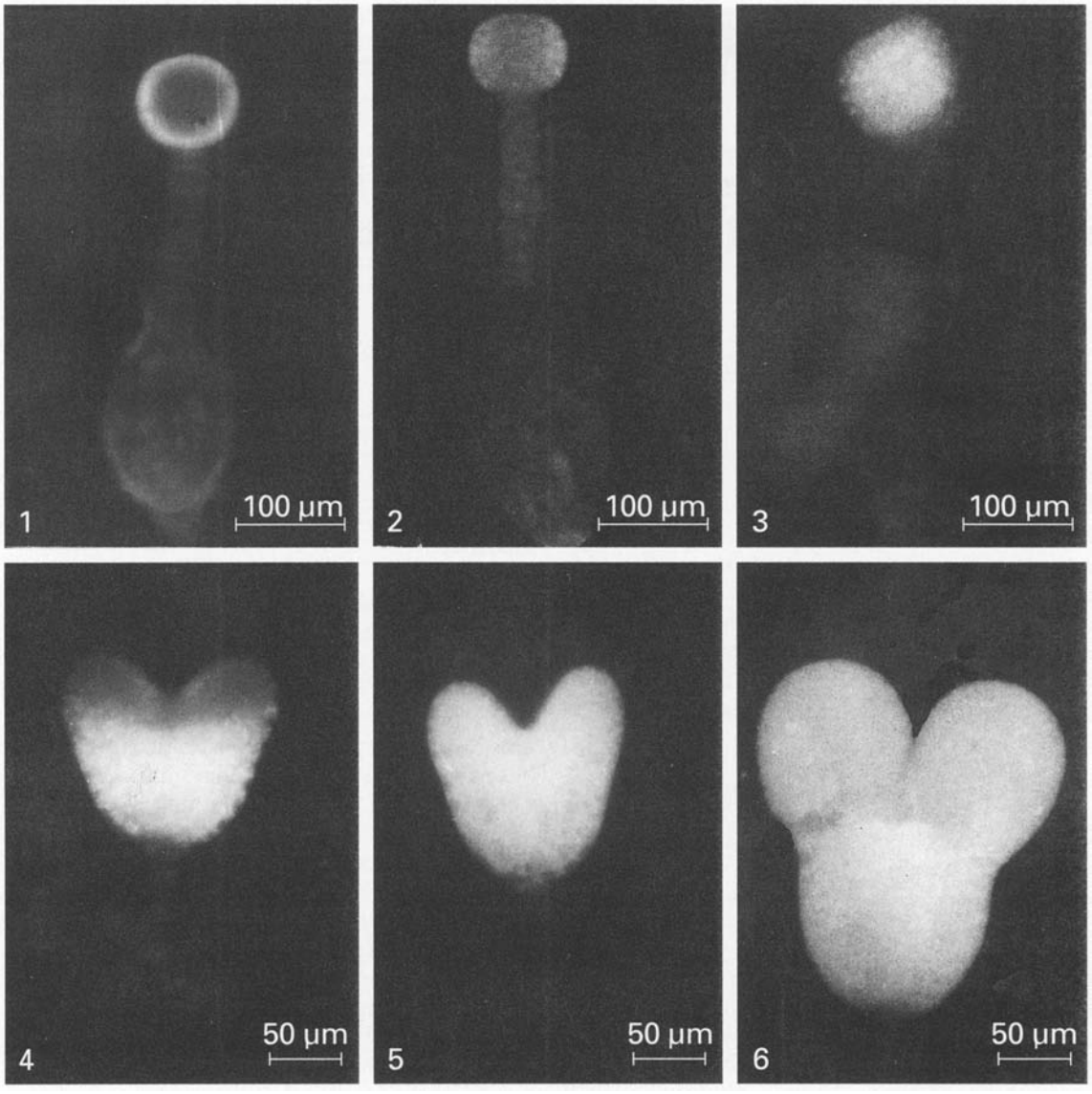

Fig. 1–6 Autofluorescence of chlorophyllid and chlorophyll. **1** Globular embryo of *Capsella* with strongly fluorescent peripheral layer. **2** Globular embryo of *Capsella*. **3** Older embryo of *Capsella*. **4** Early heart-shaped embryo of *Arabidopsis* with weak fluorescence in primordial cotyledons. **5** Heart-shaped embryo of *Arabidopsis*. **6** Torpedo embryo of *Arabidopsis*

Cutin is laid down on the protoderm of globular embryos with six to eight cellular suspensors and disappears from torpedo embryos when the suspensors begin to degenerate. A layer of cutin isolates the embryo from the endosperm, therefore most or all nutrients must be transferred through the suspensor whose cell walls are without cutin. The stream of substances entering a globular embryo from one direction may enhance polarization of the embryo and formation of the root-shoot axis.

The axially differentiated embryo loses its cutin layer and may absorb nutrients directly from the surrounding endosperm.

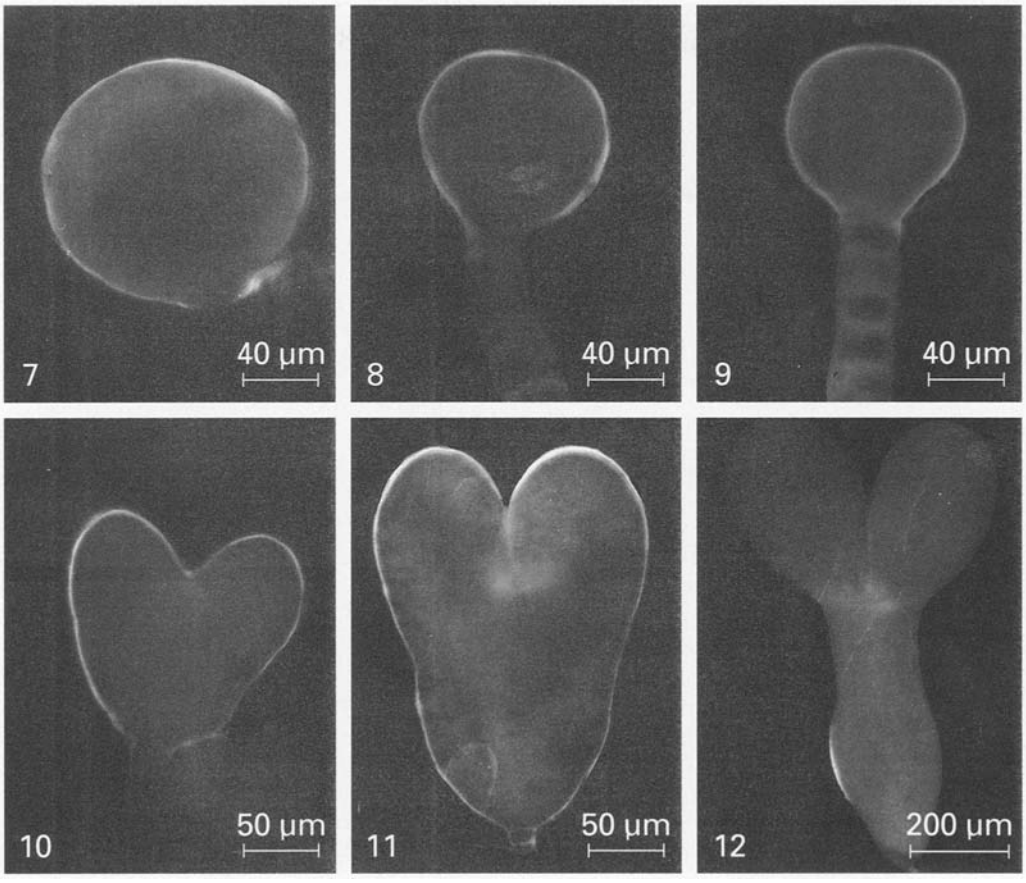

Fig. 7–12 Cutin fluorescence after auramine O. **7** Globular embryo of *Stellaria*. **8** *Arabidopsis*. **9** *Capsella*. **10** Heart-shaped embryo of *Stellaria*. **11** *Capsella*. **12** Torpedo embryo of *Arabidopsis*

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