CHLOROPHYLL AUTOFLUORESCENCE IN GLOBULAR AND HEART-SHAPED EMBRYOS OF SOME DICOTYLEDONS

B. FYK, J. BEDNARA, B. RODKIEWICZ

Department of Plant Anatomy and Cytology,
Institute of Biology, Maria Curie-Skłodowska University,
20-033 Lublin, Akademicka 19, Poland

ABSTRACT

The regions in early embryos of several species display chlorophyll autofluorescence in a certain order.

First, autofluorescence in *Pisum sativum* appears in the basal part of globular embryos; in *Lathyrus vernus* in the basal part of early heart embryos; in *Cardamine pratensis* at the sides of the hypocotyl or in *Phaseolus vulgaris* in the hypocotyl of elongating heart-shaped embryos.

Chlorophyll autofluorescence in an embryo proper of *Pisum* coincides with the development of a lamellar system in the plastids. The suspensorial plastids remain undifferentiated with one or two DNA positive nucleoids.

Cardamine, *Lathyrus*, *Phaseolus* and *Pisum* suspensors give no chlorophyll autofluorescence.

KEY WORDS: chlorophyll in embryogenesis, embryonic plastids, *Pisum*, *Phaseolus*, *Lathyrus*, *Cardamine*.

INTRODUCTION

Green embryos in developing seeds of angiosperms have been described for more than 100 years (ref. Pretova 1985). Among 1094 species investigated by Yakovlev and Zhukova (1973, 1980), 425 species have green immature embryos. The authors, therefore, distinguish two groups of angiosperms: chloroembryophyta and leukoembryophyta.

Greening often appears in heart-, and torpedo-shaped embryos (Mansfield and Briarty 1991). Globular embryos of *Linum* (Pretova 1985), *Capsella* (Rodkiewicz et al. 1994) give red chlorophyll autofluorescence.

The ultrastructural features of developing embryos of various species of angiosperms are very similar (Schulz and Jensen 1968, Zhukova 1981). Plastids in globular embryos contain simple lamellae, in heart-shaped embryos they begin to form rudimentary stacks which during the torpedo stage develop into grana or granalike systems.

It may be assumed that autofluorescence of globular embryos without developed chloroplasts is due to the presence of chlorophyllid, which is synthesised before the grana are set up (Rodkiewicz et al. 1994).

MATERIAL AND METHODS

Globular and heart-shaped embryos were dissected from living ovules of *Pisum sativum* L., *Lathyrus vernus* (L.) Bernh., *Phaseolus vulgaris* L. and *Cardamine pratensis* L.

For chlorophyll autofluorescence the dissected embryos were investigated in the fluorescent microscope with a 400 nm excitation light.

For the electron microscope, embryos of *Pisum* were fixed in 3.5% glutaraldehyde in 0.05 M cacodylate buffer at pH 7.0, embedded in Spurr's resin (Spurr, 1969). Ultrathin sections were stained with uranyl acetate and lead citrate (Reynolds, 1963). Sections were investigated with Tesla BS 500 and JEM 100B electron microscopes.

OBSERVATIONS AND DISCUSSION

In *Pisum sativum* embryogenesis, first chlorophyll autofluorescence appears in the basal region of the globular embryo (Fig. 1B) with a delimited protoderm (Fig. 1A). A more advanced globular embryo proper (Fig. 1C) becomes homogeneously autofluorescent (Fig. 1D). Autofluorescence persists during the heart stage (Figs 1E,F).

Non-autofluorescing early globular embryos proper contain undifferentiated plastids (Fig. 2A) often with starch grains. Lamellae are formed in the plastids of a late globular embryo proper (Fig. 2B) and rudimentary granal stacks in a heart-shaped embryo proper (Figs 2C, D).

The plastids in suspensor cells remain undifferentiated. They contain almost homogeneous stroma with one or two electron dense bodies (Figs 1G,I) as described by Marinos (1970). They are nucleoides with DNA fluorescing after DAPI treatment (Fig. 1H).

There is no autofluorescence in *Cardamine*, *Lathyrus* and *Phaseolus* globular embryos (Fig. 3). Initially, autofluorescence appears in the basal region of the early heart-shaped embryo (Figs 3B, D), but later the whole embryo proper becomes autofluorescent (Fig. 3C).

Fig. 1. *Pisum sativum*; A. A proembryo with delimited protoderm and multinuclear suspensor. B. Autofluorescence of chlorophyll in a basal part of the embryo shown in Fig. A. C. An older globular embryo. D. Even chlorophyll autofluorescence of the embryo shown in the previous picture. E. Heart-shaped embryo F. Autofluorescence in embryo shown in Fig. E. G. The fragment of suspensorial cell of a heart-shaped embryo. H. DNA fluorescence after DAPI in nuclei and nucleoids of the plastids shown in Fig G. I. The electron dense bodies in plastids shown in Fig G. A, B x 230; C-F x 130; G, H x 750; I x 4000.

Fig. 2. *Pisum sativum*; A. Protoplastids in an early globular embryo. B. Plastid with lamellae in a late globular embryo. C, D. Plastids with rudimentary grana in a heart-shaped embryos. $\times 22000$.

Fig. 3. *Lathyrus vernus*; A. A globular embryo with a suspensor there is no autofluorescence at this stage. B. An early heart-shaped embryo with the autofluorescing basal region. C. Autofluorescence of a somewhat older embryo.

A-C x 140. *Phaseolus vulgaris*; An embryo in transition to the torpedo stage. D. Hypocotyl autofluorescence. E. Autofluorescence stronger in a hypocotyl than in cotyledones. F. Uneven autofluorescence in a growing early torpedo embryo.

G. *Cardamine pratensis*; Autofluorescence along the sides of a heart-shaped embryo. D, E, G x 120; F x 140.

Fig. 4. *Pisum sativum*; A. Yellowish pod with yellow-greenish seeds 28 days after anthesis. The flower 3 days after anthesis was wrapped in black paper and covered with a sheet reflecting light.

B. Yellowish pod with degenerating seeds. The flower was only wrapped in black paper.

C. Heart-shaped embryo 12 days after anthesis.

D. Autofluorescence of the embryo show in the previous picture.

A, B x 1; C, D x 200.

Fig. 5. Chlorophyll autofluorescence in embryos of: A. *Capsella* and *Pisum*. First fluorescence occurs in globular embryos.

B. *Lathyrus* and *Cardamine*. First fluorescence in heart-shaped embryos.

In *Cardamine* first autofluorescing regions are located along the sides of the slightly elongated heart embryo (Fig. 3G) and subsequently in cotyledons.

In *Lathyrus* and *Phaseolus*, autofluorescence first appears in the hypocotyl of the elongated heart embryo (Figs 3B, D) and later in cotyledons. Some elongated heart embryos emit uneven fluorescence, which is distinctly weaker at the tips of the cotyledons and in the basal part of the embryo axis (Fig. 3F).

These investigations show that chlorophyllid or chlorophyll autofluorescence begins at different stages of early embryogenesis: globular (in *Pisum*), heart (in *Lathyrus* and *Phaseolus*) or at a stage intermediate between heart and torpedo (in *Cardamine*), as shown in Fig. 5.

At least in some species, chlorophyll autofluorescence first occurs in a certain region of the embryo. This region is situated in the basal part of globular or heart embryo proper (*Lathyrus*, *Phaseolus* and *Pisum*). But in *Cardamine* it is situated along the sides of a heart-shaped embryo and in *Phaseolus* in the hypocotyl of an somewhat older embryo, apparently early torpedo.

There are conflicting views on the role of chlorophyll in developing embryos (ref. Pretova 1985).

According to Ryczkowski and Szewczyk (1975, 1983) green embryos of *Lunaria* and *Haemanthus* are able to photosynthesise.

Photosynthesis in developing green embryos of *Pisum* may be inhibited or prevented simply by wrapping the flowers in black paper. In spite of this pods with seeds apparently grow normally (Fig. 4A) except for yellowish colour. These preliminary observations suggest that photosynthesis in *Pisum* embryo is not indispensable for development but heart-shaped embryos (Fig. 4C) dissected from the seeds give abnormal fluorescence (Fig. 4D) (control – Fig. 1F).

LITERATURE CITED

- MANSFIELD S.G., BRIARTY L.G., 1991. Early embryogenesis in *Arabidopsis thaliana*. II. The developing embryo. *Can. J. Bot.* 69: 461-476.
- MARINOS N.G., 1970. Embryogenesis of the pea (*Pisum sativum*). II. An unusual type of plastids in the suspensor cells. *Protoplasma* 71: 227-233.
- PRETOVA A. 1985. The assimilation pigments, fats and the ultrastructure of plastids during the embryogenesis in flax *in situ* and *in vitro*. *Acta Bot. Slov. Ser. B.* 4: 1-73.
- REYNOLDS E.S. 1963. The use of lead citrate of high pH as an electron-opaque stain in electron microscopy. *J. Cell Biol.* 17: 208-212.
- RODKIEWICZ B., FYK B., SZCZUKA E. 1994. Chlorophyll and cutin in early embryogenesis in *Capsella*, *Arabidopsis* and *Stellaria* investigated by fluorescence microscopy. *Sex. Plant Reprod.* 7: 287-289.
- RYCZKOWSKI M., SZEWCZYK E. 1975. Further research on the photosynthesis in the green developing embryos (Dicotyledonous plants). *Zeitschr. Pflanzenphys.* 75: 175-180.
- RYCZKOWSKI M., SZEWCZYK E. 1983. Chlorophyll and their function in the developing embryo. In: *Fertilization and embryogenesis in ovalated plants*. Erdelska (ed.), Slovak. Acad. Sci. Bratislava: 303-306.
- SCHULZ P., JENSEN W.A. 1968. *Capsella* embryogenesis: the early embryo. *J. Ultrastruct. Res.* 22: 376-392.
- SPURR A.R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. *J. Ultrastruct. res.* 26: 31-43.
- YAKOVLEV M.S., ZHUKOVA G.YA. 1973. Angiosperms with green and colourless embryos (chloro- and leukoembryophyta). *Nauka, Leningrad* pp 116. (in Russian).
- YAKOVLEV M.S., ZHUKOVA G.YA. 1980. Chlorophyll in embryos of angiosperm seeds. A review. *Bot. Not.* 133: 263-281.
- ZHUKOVA G.YA. 1981. Embryogenesis of angiosperms: ultrastructural transformation of plastids. *Acta Soc. Bot. Pol.* 50: 303-306.

AUTOFLUORESCENCJA CHLOROFILU W GLOBULARNYCH I SERCOWATYCH ZARODKACH NIEKTÓRYCH ROŚLIN DWULIŚCIENNYCH

STRESZCZENIE

W młodych zarodkach licznych gatunków roślin autofluorescencja chlorofilu pojawia się w określonym porządku.

U *Pisum sativum* autofluorescencja po raz pierwszy pojawia się w bazalnej części globularnych zarodków, u *Lathyrus vernus* w bazalnej części wczesnosercowatych zarodków, u *Cardamine pratensis* w bocznych częściach hypocotyłu a u *Phaseolus vulgaris* w hypocotyłu wydłużających się sercowatych zarodków.

Autofluorescencja chlorofilu w zarodku właściwym *Pisum* jest związana z rozwojem lamelarnego systemu w plastydach. Suspensorialne plastydy, pozostają niezróżnicowane z jednym lub dwoma nukleoidami DNA pozytywnymi. Suspensory *Cardamine*, *Lathyrus*, *Phaseolus* i *Pisum* nie wykazują autofluorescencji chlorofilu.

SŁOWA KLUCZOWE: chlorofil w embriogenezie, embrionalne plastydy, *Pisum*, *Phaseolus*, *Lathyrus*, *Cardamine*.