Chlorophyll content and culture of flax embryos *in vitro*

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Abstract

During *in vitro* cultivation of green flax embryos excised 14 days after fertilization on White's medium the chlorophylls *a* and *b* are gradually destroyed and the lamellar system of plastids is disintegrated. It was possible, to prolong somewhat the presence of chlorophyll and to enhance embryo growth by culture in the dark and by adding kinetin. Light has a stimulating effect on the germination of embryos. Streptomycin inhibits chlorophyll synthesis not earlier than at germination. Young, green embryos are unable to survive and develop on media without sucrose. It is supposed that photosynthesis does not occur in flax embryos *in vitro*, and that green embryos are not more autotrophic *in vitro* than those of leucoembryophytes.

The embryos of some angiosperms contain green pigment either throughout their whole embryogenesis or during some part of it. Therefore, the term "*Chloroembryophyta*" has been introduced for plants with green embryos, and "*Leucoembryophyta*" for those with embryos without green pigment (Yakovlev, Zhukova, 1973).

Light access is difficult to most embryos through the pericarp and the seed envelope, making the role of chlorophyll in photosynthesis *in situ* improbable. Szewczyk (1974) and Ryczkowski and Szewczyk (1975), however, found that green embryos of some species are able to photosynthesize in light, immediately after excision. We were interested first of all in the possibility of a higher autotrophy of green embryos, which could influence their culture *in vitro*. In this way, simpler nutrient media could be used, as some necessary substances would be synthesized by the embryos themselves.

In our experiments, flax embryos were used, as representatives of *Chloroembryophyta*, the embryos of which remain green almost up to maturity. We investigated the quantitative and qualitative pigment content, ultrastructure of plastids in 14-day old embryos, and after 10-day culture *in vitro*. Stimulators and inhibitors of the chlorophyll synthesis and the influence of light were investigated.

The qualitative and quantitative pigment composition of 14-day old embryos was determined by the method of chromatography (Hašpellová-Horvátovicová, 1963). The embryonal green pigment was determined as chlorophyll *a* and *b*. The absorption maximum of chlorophyll *a* is shifted towards the longer wavelengths (670 nm), as compared with the absorption maximum of chlorophyll *a* from normal leaves of flax. In 14-day old embryos the relation of chlorophyll *a* to chlorophyll *b* is low, only 2:1 or even 1:1 (Pretová, 1977a). Such a relation is characteristic for chlorophylls from poorly illuminated tissues (ref. in Thornber, 1975). In embryos of *Haemanthus katherinae* Bak. the chlorophyll *a* and *b* relation varied during embryogenesis (Ryczkowski, Szewczyk, 1972).

The plastids of cytoledons of 14-day old embryos belong, according to their structure, to the category of chloroplasts. They are of oval shape, having one or two starch grains. The inner lamellae are arranged into grana. The chloroplast structure of 14-day flax embryos, in general is the same as that described by Deschamps (1970) for the heart-like embryo of flax. It does not differ much from the typical chloroplast structure in the mesophyll of the flax leaf. The chloroplasts of the embryos are, however, smaller and have a lower number of grana (Ciamporová, Pretová, 1980). This is in good correlation with the lower chlorophyll content in the 14-day flax embryos (Pretová, 1977a).

Flax embryos (14-day old) are cultured on White's medium with 5% sucrose, at 25°C and under illumination of 2 000 lux for 16 hours daily. The embryos lose green pigment after 7-10 days of culture (Pretová, 1978). After 10 days *in vitro* conditions there occurs: size reduction of the plastids, disintegration of granal and intergranal lamellae, an increase in the number of plastoglobuli. Many lipid bodies resemble those in the typical structure of dormant embryos (Ciamporová, Pretová, 1981). In the dark, the destruction of chlorophyll is retarded by a few days. During the first two weeks of embryo culture, light is not necessary. Culture in the dark during this period leads to stimulation of embryo germination (Erdelská, Pretová, unpublished data).

Addition of kinetin, the stimulating effect of which on chlorophyll synthesis is well known (Fletcher, McCullagh, 1971; Kulaeva, Kliachko, 1965), prevents total destruction of chlorophyll, shortening the dormancy period and stimulating germination with intensive greening of the cotyledons of cultured embryos (Pretová, 1977a). The character of the developmental processes in embryos cultured under the influence of kinetin, was not so unequivocal as to be considered as being connected with preservation of the embryonal chlorophyll.

Addition to the culture medium of streptomycin which inhibits chlorophyll synthesis and total proteosynthesis, does not influence the

content of embryonal chlorophyll, but causes development of chlorotic seedlings growth. The degree of chlorosis depends on the concentration of streptomycin (Pretová, 1980).

It has been proved that flax embryos *in vitro* are unable to photosynthesise even a part of the necessary products. We cultured flax embryos on media without sucrose or with mannitol. Sucrose cannot even partially be replaced by mannitol (Pretová, 1974). Embryos cultured without sucrose soon become totally bleached and die very quickly.

Basing on these processes observed in flax embryos cultured *in vitro*, we suppose that there is not photosynthesis in green flax embryos, except probably immediately after excision. The green embryos are, therefore, not more autotrophic than those of leucoembryophytes.

REFERENCES

- Ciamporová M., Pretová A., 1981. The New Phytologist 87: 473-479.
Deschamps R., 1970. 7th Cong. Int. de Micr. Electron., Grenoble pp. 451-452.
Fletcher R. A., McCullagh D., 1971. Planta 101: 88-90.
Haspelová-Horvatovicová A., 1963. Biologia (Bratislava) 5: 334-345.
Kulaeva O. N., Kliachko N. L., 1965. Dokl. Akad. Nauk USSR 164: 458-461.
Pretová A., 1974. Biologia Plantarum (Praha) 16: 14-20.
Pretová A., 1977a. Photosynthetica 11: 217-219.
Pretová A., 1977b. In: Dni rast. fyziol. I. (Bratislava) pp. 260-263.
Pretová A., 1978. Biologia (Bratislava) 33: 29-34.
Pretová A., 1980. Biologia (Bratislava) 35: 413-416.
Ryczkowski M., Szewczyk E., 1972. Bull. Acad. Polon. Sci. Ser. Sci. Biol. 20: 49-53.
Ryczkowski M., Szewczyk E., 1975. Zeitschr. für Pflanzenphys. 75: 175-180.
Szewczyk E., 1974. Bull. Acad. Polon. Sci. Ser. Sci. Biol. 22: 53-56.
Thornber J. Ph., 1975. Ann. Rev. Plant Physiol. 26: 127-158.
Yakovlev M. S., Zhukova G. Ya., 1973. Pokrytosemyanniye rasteniya s zelenim i bestsvetnym zarodyshem. Nauka, Leningrad p. 115.