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THE EFFECT OF GAMMA RAYS ON THE ULTRASTRUCTURE OF DEVELOPING PLASTIDS UNDER ANOXIC CONDITIONS

(With 3 figures)

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It has been shown that the differentiation of plastids during the exposure of etiolated plants to light is considerably changed if they are previously irradiated with gamma rays (Wrischer and Devidé 1964, 1966). The first phase of plastid differentiation (i. e. the disappearance of the crystal-lattice-like structure of the prolamellar body) cannot be affected by irradiation, even if doses of 500 kr are applied. The second phase of plastid differentiation, i. e. the formation of grana and consequently the greening of leaves are however, considerably delayed and reduced in irradiated plants. This delay, or even inhibition is dose dependent, i. e. the higher the dose used, the stronger the effect.

Among the factors which could possibly modify the effects of gamma rays on the plastid development the anoxic treatment has been studied. As known, anoxic conditions during irradiation considerably reduce the radiation damage (Bacq and Alexander 1961).

Materials and Methods

For the experiments 8 days old etiolated bean seedlings (*Phaseolus vulgaris* cv. top crop and for some experiments cv. Butterfisole) grown in total darkness were used. The shoot apices with their cotyledons and primary leaves were cut off 5—7 cm from the top at an illumination of a non-effecting dark green light and placed in glass bottles containing some tap water. By means of two glass tubes, each having a stop cock, nitrogen was drawn through the bottles at least 1 hour. After turning off both stop cocks the plants were irradiated in darkness with gamma rays (Co^{60}-source, 350 Curie, 15 r. sec^{-1}; doses 10, 25 and 50 kr). Then the material was immediately transferred to the air and exposed to con-

tinuous artificial white light (fluorescent tubes) at an illuminating intensity of 4.000 Lux. At certain intervals small pieces of leaf lamina were cut out and fixed in a 5 p. c. $KMnO_4$-solution. The material was embedded in araldite and ultrathin sections observed in the electron microscope. (For further technical details see Wrischer and Devidé 1966).

Results

The experiments showed that the plastid development was sometimes less delayed or inhibited in the treated material than in the irradiated control only at the beginning of the experiments. In comparison with the non-irradiated control the leaves of plants, which had been irradiated with 10 kr in nitrogen atmosphere, began sometimes to become green nearly simultaneously, i. e. they showed a green colour in such cases already after an exposure to light of 2—3 hours; however, the plants irradiated with higher doses (25 and 50 kr) still showed some retardation in the process of greening.

The analysis of ultrathin sections also showed that at the beginning of the experiment the differentiation of plastids of plants irradiated in nitrogen was sometimes less delayed in comparison with that of the irradiated control (Fig. 2, 3). The formation of the first grana in plastids always occurred simultaniously with the greening of such leaves. Similarly to the non-irradiated control (Fig. 1) the plastids of the plants, which were irradiated in nitrogen, often contained a certain number of grana already after 6 hours illumination (Fig. 2). This reduction of the delay in differentiation of irradiated material seemed to be a remarkable one, if one considers that in the cv. top crop irradiated with 50 kr in the air the first grana appeared after about 12 to 15 hours of continuous illumination. However, this retardation partially depends upon the stage of the development, the plant variety and, to some extend, also on the nutritive conditions of the leaf (Wrischer and Devidé 1966).

Although the differentiation of the plastids in the leaves of plants irradiated in nitrogen atmosphere seemed to be frequently less delayed at the beginning of the experiment than in those irradiated in air, after one day such plants were less developed than the non-irradiated control in the process of greeening as well as in the number of grana per each plastid. This secondary delay is also dose dependent, i. e. the higher the dose used the more striking the delay. At 10 kr it is hardly perceivable, because as in the differentiation of plastids after 24 hours the leaves of plants irradiated in air practically reach the non-irradiated control (Wrischer and Devidé 1966). At 25 and especially at 50 kr the secondary delay is however very striking. The experiments were repeated several times and their results were always consistent.

Regardless whether the radiation damage appeared to be modified by the anoxic treatment at the beginning of the plastid development or not, at the end of the experiment there were never any perceivable differences between the irradiated control and the plants irradiated with the same amount of gamma radiation in nitrogen atmosphere. Prelimi-

nary measurements of the chlorophyll content in the leaves also showed that after 24 hours there is no difference between plants irradiated in nitrogen and in air. A definite and final effect of the anoxic treatment during gamma radiation of etiolated bean seedlings on the differentiation of plastids in light could thus not be proved.

Discussion

If the irradiation occurs in the absence of oxygen in biological systems (cells and tissues) the effects of ionizing radiation — irrespective of the death of organisms, reduction of growth, or cytological changes (chromosome breakage, biochemical changes) — are reduced, but not thorougly eliminated (Bacq and Alexander 1961). Under anoxic conditions the possibility of the formation of dangerous radicals is considered to be lowered. The reduction factor usually equals 2 to 3. This effect has recently been observed also in the differentiation of autothropic plant cells, the reduction factor beeing 2,5 (Howard 1965).

In the course of differentiation of plastids in etiolated plants under the action of light important biochemical processes occur, such as the sustained synthesis of chlorophyll (Butler 1961, Virgin 1964) and the protein synthesis (Rhodes and Yemm 1963). Ionizing radiation probably affects some biochemical reactions which are connected with these processes. It is therefore difficult to explain why at the beginning of the experiment anoxia had only some apparent and inconsistent effect on the development of plastids in irradiated etiolated plants and why it is not possible to state finally a true protective effect.

Summary

After gamma irradiation of etiolated bean seedlings in nitrogen atmosphere the delay or inhibition of the development of the grana in the plastids and of the greening process of leaves is inconsistently reduced only at the beginning of the light exposure. After 24 hours the effect, so far present, completely disappears and therefore a definite and final protective effect could never be proved.

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SADRŽAJ

DJELOVANJE GAMA-ZRAKA U UVJETIMA ANOKSIJE NA ULTRASTRUKTURU PLASTIDA U RAZVITKU

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(S 3 slike)

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Ranija su istraživanja (Wrischer i Devidé 1964, 1966) pokazala da ozračivanje etioliranih biljaka graha gama-zrakama (doze od 10 do 500 kr) upravno proporcionalno dozi usporava ili čak koči razvitak grana i sintezu klorofila koji se zbivaju pod utjecajem svjetlosti. Od faktora koji modificiraju djelovanje zračenja istraženo je u prvom redu djelovanje anoksije.

Etiolirane biljke graha (*Phaseolus vulgaris* cv. top crop i cv. Butterfisole), stare 8 dana, ozračene su u tami gama-zrakama (izvor Co^{60}, doze 10, 25 i 50 kr) paralelno u dušiku i u zraku te zatim izložene zajedno s neozračenim biljkama bijeloj svjetlosti fluorescentnih svjetiljki uz intenzitet osvjetljenja od 4 000 luksa.

Eksperimenti su pokazali da je utjecajem anoksije doduše katkada smanjeno usporavanje diferencijacije plastida u listovima ozračenih biljaka, no samo u svojoj početnoj fazi. U takvim se slučajevima u biljnom materijalu ozračenom u dušiku pojavljuju u plastidima grana već nakon 6-satnog osvjetljenja (sl. 2.) kao i u neozračenoj kontroli (sl. 1), dok u plastidima biljaka ozračenih u zraku u to vrijeme grana još nema (sl. 3). Ovo smanjenje usporavanja diferencijacije se, međutim, u toku kasnijeg razvitka gubi, tako da u ultrastrukturi plastida biljaka ozračenih u dušiku i onih ozračenih u zraku već nakon 1 dan, a pogotovo nakon 2 dana praktički nema više nikakvih razlika. Taj je efekt osobito uočljiv nakon ozračivanja biljaka dozama od 25 do 50 kr.

Ako anoksični tretman nije odmah na početku pokazao neki efekt, diferencijacija plastida odvijala se posve jednako kao kod materijala ozračenog u zraku istom dozom.

Prema tome, nije bilo ni u kojem slučaju moguće dokazati bilo kakvo određeno i konačno zaštitno djelovanje anoksije u pogledu diferencijacije plastida ozračenih etioliranih biljaka izloženih svjetlosti.


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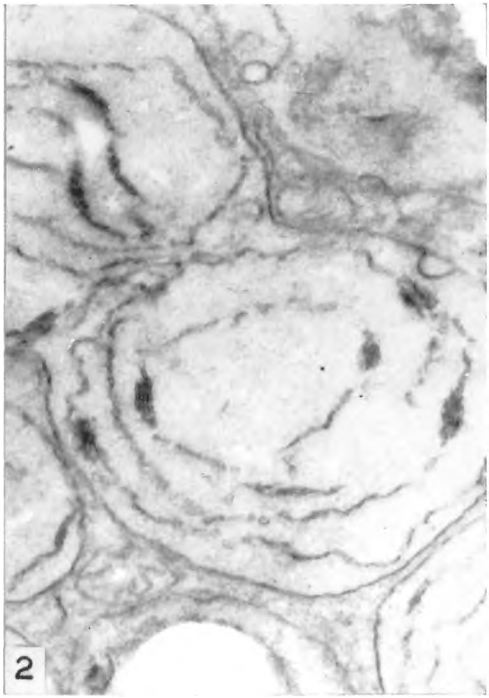
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EXPLANATION OF FIGURES

Fig. 1—3. *Phaseolus vulgaris*, etiolated seedlings. Plastids in cells of primary leaves. $KMnO_4$-fixation, araldite, 30.000 : 1.

Fig. 1. Non-irradiated control, 6 hours illumination. The grana are already well developed.

Fig. 2. Irradiated with 50 kr gamma rays in nitrogen atmosphere, 6 hours illumination. Due to apparent protective effect some grana are already developed.

Fig. 3. Irradiated with 50 kr gamma rays in air, 6 hours illumination. No grana are present in the plastid.

TUMAČ SLIKA

Sl. 1—3. *Phaseolus vulgaris*, etiolirane mlade biljke. Plastidi u stanicama primarnih listova. $KMnO_4$-fiksacija, araldit, 30.000 : 1.

Sl. 1. Neozračena kontrola, 6 sati osvjetljenja. Grana su već jasno razvijena.

Sl. 2. Ozračeno s 50 kr gama-zraka u dušiku, 6 sati osvjetljenja. Kao posljedica prividnog zaštitnog djelovanja pojedina grana su već razvijena.

Sl. 3. Ozračeno s 50 kr u zraku, 6 sati osvjetljenja. Nikakva grana nisu prisutna u plastidu.