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**Distribution of Plastid Quinones and Pigments along the Leaf
in Different Stages of Plastid Development of *Zea mays* L. Seedlings**

Rozmieszczenie chinonów plastydowych i barwników w liście o zróżnicowanej strukturze plastydów u siewek *Zea mays* L.

Размещение пластидовых хинонов и красителей в листе с дифференцированной структурой пластидов сеянцев *Zea mays* L.

INTRODUCTION

The "autonomy" of chloroplast is strictly connected with the presence of assimilatory pigments and specific structure of this organelle. Photosynthetic activity of chloroplast is correlated with chlorophyll biosynthesis and formation of thylakoids. Fine structure of the photochemically active thylakoids gives just now the ability of plant cells to photosynthesize.

Beside chlorophyll and carotenoids, active fat-soluble quinones of benzo- and naphthoquinone type with a prenyl side chain of various lengths are one of the main groups of compounds which occur in chloroplasts. This group comprises mainly plastoquinone-45 (involved in electron transport of chloroplasts), α -tocopherolquinone and their chromanols (plastoquinol-45 and α -tocopherol, respectively) and vitamin K₁ (2, 18, 22). Beside the predominant chloroplast quinone, PQA-45, chloroplasts of certain plants may contain a number of other quinones of the PQB and PQC series as well as β -, γ -tocopherolquinone and an uncharacterized vitamin K (5).

It appears from numerous studies, particularly those of Lichtenhaler (27), that lipoquinones are common components of green parts

of plants of various taxonomical groups and also of chlorophyll-free tissues of etiolated and aurea-variegated plants as well as of not fully functional plastids with coiled thylakoids (30); relative concentrations of these compounds are, however, quite different.

Phenotypic varieties of plastids such as leuco-, amylo-, etio- and chromoplasts are deprived of thylakoids but they also contain, though in smaller concentrations, plastid quinones particularly of the benzoquinone type (26). They are located in osmiophilic globules occurring at all stages in the growth and differentiation of plastids and they are an extra-thylakoidal reservoir for excess terpenoid lipids (3, 4, 15, 23, 25). The occurrence of quinones in this differentiation of plastids proves that their synthesis is also possible outside thylakoids. Thus there arises a question whether the promoting effect of light is confined only to form a fine structure of chloroplasts without which biosynthesis of these compounds would not be possible, or whether light directly effects the synthesis independently of that structure. The suggestion supporting the former assumption was put forward in reference to α -tocopherol (α -T) synthesis in streptomycin (SM) treated cell of *Euglena gracilis* (8). Light-stimulated synthesis of α -T during "greening up" of etiolated plants has been objected lately (12, 17, 33). The lack of distinct light effect on α -T synthesis after illumination of dark-grown plants, as Lichtenthaler thinks (28), could have been caused by photo-oxidation of α -T into α -tocopherolquinone (α -TQ).

The synthesis of main amounts of terpenoid quinones is, however, stimulated by light (16, 26). According to Lichtenthaler's hypothesis (28) the white light primarily promotes the synthesis of those plastid lipids that are present in etiolated plants in relatively low concentrations. In earlier studies it was found that concentration of plastidic quinones is governed by the degree of chloroplast development (34).

To prove these findings the author examined in this paper the dynamics and distribution of plastid quinones in leaves of maize seedlings characterized by various stages and differentiation of plastids. Gradual differentiation of plastid structure and chlorophyll formation along the leaf was obtained by treating the leaf with SM (31).

MATERIAL AND METHODS

For the experiments seeds of the *Zea mays* L. var. Mieszaniec from the Experimental Station at Ożańsk near Jarosław, collected in 1968, were used.

The seeds sterilized with mercuric chloride were soaked in darkness for 24 hrs and then they were put on wet lignin and placed in a thermostate at 23°C in the dark. After three days of germination they were exposed to light (4500 lx, 18 hrs light).

After shooting of the leaf through the coleoptile a part of plants were sprayed with 0.5% aqueous solution of SM (11,31) every one hour 12 times during the day. After some time of growth the second leaf of SM-treated seedlings partly decolorized (the apex remained green, the middle part of leaf became light-green and its base lacked completely chlorophyll). Green and etiolated seedlings growing under identical conditions but not treated with SM were the control.

For examinations the second leaf of 14-day-old seedlings was taken and to estimate the concentration gradient of plastid quinones and pigments along the leaf of *Zea mays* seedlings it was divided into five equal segments.

Chromatography of the lipid fraction on alumina was carried out according to Griffiths and his co-workers (16). The amounts of quinones and β -carotene present in leaf tissues sections were determined spectroscopically by Threlfall and Goodwin (32).

Quantitative determination of chlorophyll (a + b) was carried out after Arnon (1).

The fraction containing tocopherols was determined colorimetrically according to the reaction of Emmerie and Engel with FeCl_3 and α , α' -dipyridyl.

Moreover, photosynthetic activity of the analysed segments of the leaf was examined by Zurzycki's microrespirometric method (36).

The values given in the figures represent mean values of five separate determinations.

RESULTS AND DISCUSSION

Plastid quinones and pigments in *Zea mays* seedlings were examined several times (7, 10, 14, 16, 33). It was found that in early stages of their growth, beside chlorophyll and β -carotene, there also occur plastoquinones (PQ), phyloquinone, α -tocopherolquinone, α -T, γ -T and ubiquinone.

In the present studies the author examined the distribution of plastid quinones and pigments along the second leaf of etiolated and green maize seedlings. The green seedlings were exposed to light, the intensity of which was normal for lamellar system development (20). The distribution of the above components in green and etiolated leaves was compared with that in SM-treated leaves. As it appears from Signol's studies (31), what was confirmed in these studies, the basal part of the leaf treated with SM has plastids, the structure of which is close to that of plastids of etiolated seedlings. The apical part, however, contains plastids with fine thylakoidal structure. The leaf with such differentiated structure of plastids is thus a good object of investigations on the relationship between light dependent synthesis of plastid quinones and different stages of plastid development.

Taking into consideration the relationship which exists between chlorophyll formation and structural changes in plastids (21, 35), the control of the structure of the segments with electron microscope was

abandoned and only measurements of chlorophyll accumulation reflecting chloroplast structure were carried out. Besides, photosynthesis was measured being an additional criterion of changes caused by SM.

In this paper a close relationship between photosynthetic activity and the amount of chlorophyll in green leaves was found. In plants treated with SM the photosynthetic activity decreased parallelly to the

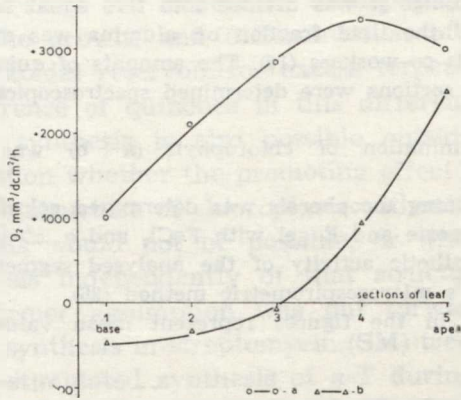


Fig. 1. Photosynthetic oxygen evolution along the leaf of green and SM-treated maize seedlings; a—green leaf; b—SM-treated leaf

decrease of chlorophyll accumulation (Fig. 1). However, the decrease of photosynthetic activity is greater than it might appear from slight decrease of chlorophyll content. Thus it seems that, irrespective of the inhibition of chloroplast development, disturbances in the activity of definite enzyme systems upon which photosynthetic activity of mesophyll cell depends are presumably the reason of low photosynthetic activity in SM-treated leaves. This interpretation can be supported by the fact found by Margulies (29) that the change of the activity of enzymes involved in photosynthesis need not go together with chlorophyll synthesis.

Chlorophyll distribution along the leaf occurs analogically as the distribution of α -T by Booth and Hobson-Frohock (9). The basal part of the green leaf contains more than four times less of chlorophyll than the apical one (Fig. 2). This is in agreement with the known principle referring to α -T that fast growing parts of the leaf contain less of the compound examined than those growing slower.

SM inhibits chlorophyll synthesis in leaves, and under conditions of our experiment the inhibition increased from apex to base of the leaf. The basal part of the leaf (the youngest) does not contain any chloro-

phyll at all. It should be noticed that in the apical part of the leaf with well developed thylakoids a partial inhibition of chlorophyll can be observed. Photosynthetic activity of this part of the leaf, as it was mentioned above, is more limited. Thus Kirk's opinion (19) seems to be right because blanching SM activity is not caused exclusively by inhibition of chloroplast formation but it is also the result of the inhibition of chlorophyll biosynthesis.

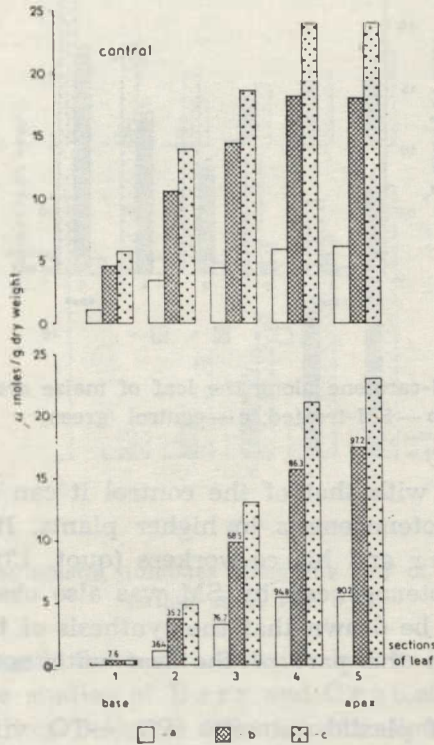


Fig. 2. Chlorophyll distribution along the leaf of green and SM-treated maize seedlings; a—chlorophyll a, b—chlorophyll b, c—total (a + b). The number above the columns presents the percentage of compounds content in comparison to control sections (100%)

The second plastid pigment, β -carotene, in etiolated leaves occurs in minor amounts and its distribution along the leaf is slightly changed (Fig. 3). Leaves of green plants possess higher level of β -carotene than do etiolated ones. Higher level of β -carotene in green plants in relation to etiolated is a commonly known fact (among others 24,28). In the younger basal part of the leaf the amount of β -carotene is ten times higher than in the corresponding part of etiolated leaf. Towards the

apex β -carotene accumulation increases gradually so as to exceed its amount 22 times in the apical part as compared with that of etiolated plants. On examining the distribution of β -carotene in SM-treated leaves it can be found distinctly that this compound is quantitatively bound to thylakoids. When comparing the amount of β -carotene in

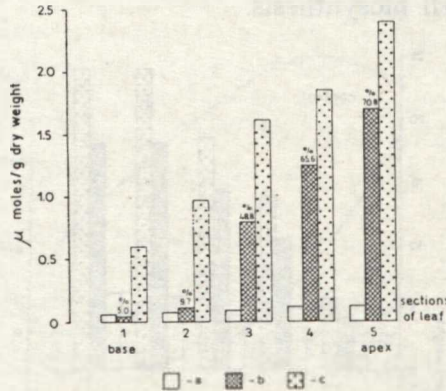


Fig. 3. Distribution of β -carotene along the leaf of maize seedlings; a — etiolated, b — SM-treated, c — control (green)

the apex of the leaf with that of the control it can be seen distinctly that SM inhibits carotenogenesis in higher plants. It confirms earlier studies of Schopfer and his co-workers (quot. 13). In lower plants the inhibition of carotenogenesis by SM was also observed (13, 19).

A conclusion may be drawn that the synthesis of both plastidic pigments is inhibited in the part of the leaf with not fully developed structure of thylakoids.

The distribution of plastid quinones (PQ, α -TQ, vit. K_1) in etiolated and green leaves takes a similar course (Fig. 4). In green leaves, however, the level of quinones is considerably higher. The increase of these compounds from base to apex in green plants in relation to etiolated ones is for α -TQ about 10-fold, PQ 4,7-10-fold, vit. K_1 3-7,6-fold, respectively.

In leaves with differentiated structure of plastids under the influence of SM, a characteristic variation in accumulation of the above compounds is observed. The basal part of the leaf contains as few quinones as the etiolated leaf; going in the apical direction the amount of plastid quinones increases so as to reach in the apical part the value close to that found in the apex of green leaf. Among the quinones PQ is characterized by the highest concentration. Particularly in the apical part of the leaf with high chlorophyll level and full photosynthetic com-

petence, the highest level is shown by PQ. This seems to be obvious since PQ is a member of the electron transport chain of chloroplast and biological oxidation-reduction catalyst in the photochemically active thylakoids.

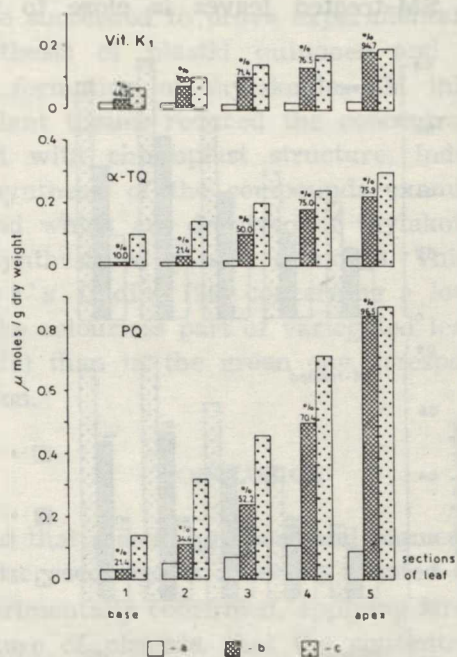


Fig. 4. Distribution of plastid quinones along the leaf of maize seedlings; explanation as in Fig. 3

On the other hand, the apex of maize leaf is its oldest part, but it is known from the studies of Barr and Crane (5) that there exists a positive correlation between PQ concentrations and the stage of maturity of the plant.

In the analysed parts of leaves of all combinations of the experiment the presence of α -T and γ -T was found. This result confirms earlier studies of Green (14) and Griffiths and his co-workers (16) concerning the occurrence of these two homologues in early phases of maize growth. Their distribution along green, etiolated and SM-treated leaves coincides with the curve presented earlier by Booth and Hobson-Frohock (9). In etiolated leaves the level of tocopherols is lower than in green ones (Fig. 5), i.e. in the basal part it is about 59%, in the apical part 96% as compared with the amount present in green leaf, respectively. Concentrations of tocopherols were maximal near the apices. This was also found by the above authors for such plants as *Dactylis glomerata*, *Eryngium pandandifolium*, *Plantago lan-*

ceolata, *Alium porum* and *Iris germanica*. Moreover, they found that in *Narcissus* leaves α -T gradient was slight along young leaves which were growing throughout, whereas in older leaves the gradient rose sharply towards the slower growing apex. The level of tocopherols in the basal part of SM-treated leaves is close to that in leaves of

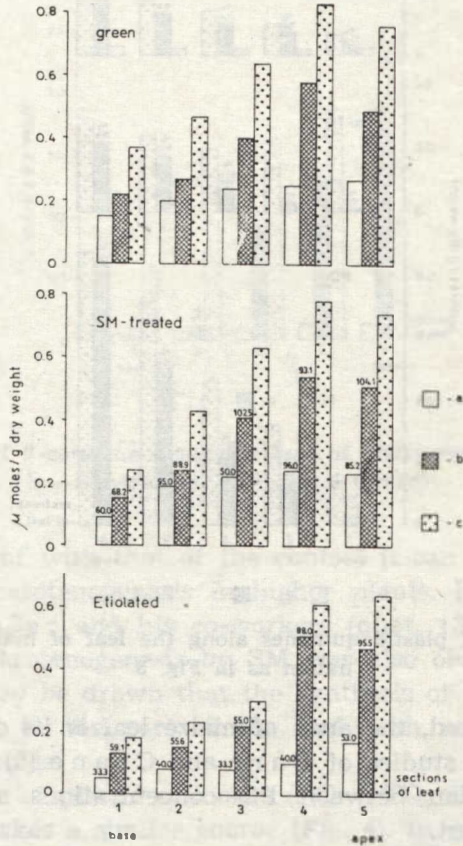


Fig. 5. Distribution of tocopherols along the leaf of maize seedlings; a — γ -tocopherol, b — α -tocopherol, c — total

etiolated plants, whereas in the apical part it is close to that in leaves of green plants. Our earlier opinion (8) that most of α -T present in cells of *Euglena gracilis* is synthesized in chloroplasts with developed thylakoidal structure seems to be here still actual, although the relationship between the amount of tocopherols and chloroplasts development is not so distinct because of high tocopherol level in etiolated plants. Small differences in the content of tocopherols in etiolated and green maize seedlings were already observed by other authors (7, 16).

Taking all results obtained into consideration we can supplement the conclusion of Booth and Hobson-Frohock (9) that not only α -T, but also plastid quinones and pigments content of maize leaves are inversely related to growth rate.

The author also succeeded to prove experimentally the opinion that light-induced synthesis of plastid quinones and pigments proceeds parallelly to the formation of thylakoids. SM inhibiting chloroplast development in plant tissues reduced the concentration of these compounds, associated with chloroplast structure, independently of light. Lack or limited synthesis of the compounds examined in illuminated parts of leaves and which are deprived of thylakoids defines the role of light in the synthesis of plastid quinones. This corresponds with Lichtenthaler's finding (26) concerning a lower content of plastid quinones in the colourless part of variegated leaves (*Acer negundo*, *Pelargonium zonale*) than in the green one, irrespective of the conditions of illumination.

CONCLUSION

1. It was found that plastid quinones and pigments content of leaves of 14-day-old maize seedlings is inversely related to growth rate.
2. It was experimentally confirmed, applying streptomycin to differentiate the structure of plastids, that the concentration and synthesis of the above components is related to the degree of chloroplast development.
3. Light does not stimulate the synthesis of plastid quinones in thylakoid-free plastids of maize leaves.

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STRESZCZENIE

Badano rolę światła podczas syntezy chinonów i barwników plastydowych w liściach siewek kukurydzy, zawierających plastydy w różnym stopniu rozwoju. Różnicowanie rozwoju plastydów osiągnęto działając na młode siewki streptomycyną. Porównywano rozmieszczenie i koncentrację chlorofilu, β -karotenu, plastochinonu, α -tokoferolochinonu i α -, γ -tokoferoli wzdłuż drugiego liścia 14-dniowych siewek zielonych, etiolowanych i poddanych działaniu streptomycyny.

Stwierdzono, że:

1) synteza chinonów i barwników plastydowych wzdłuż liścia kukurydzy jest odwrotnie proporcjonalna do szybkości wzrostu liścia;

2) synteza badanych w liściu związków zależy od stopnia rozwoju plastydów;

3) światło nie stymuluje syntezy chinonów plastydowych w odcinkach liścia kukurydzy, w których plastydy pozbawione są tylakoidów.

РЕЗЮМЕ

Исследовали значение света в период синтеза пластидовых хинонов и красителей в листьях сеянцев кукурузы, содержащих пластиды развитые в разной степени. Дифференциацию развития пластидов получали действием на молодые сеянцы стрептомицина. Сравнивали размещение и концентрации хлорофилла, β -каротина, пластохинона, α -токоферолохинона и α -, γ -токоферола вдоль другого листа 14-дневных зеленых сеянцев, этиолированных сеянцев и сеянцев, подвергнутых действию стрептомицина.

Констатировали, что:

1) синтез пластидовых хинонов и красителей вдоль листа кукурузы обратно пропорционален скорости роста листа;

2) синтез исследованных в листе соединений зависит от степени развития пластидов;

3) свет не стимулирует синтеза пластидовых хинонов в участках листа кукурузы, в которых пластиды были лишены тилакоидов.