CHARACTERISTICS OF CHLOROPLAST MUTANTS WITH ABNORMAL CAROTENOID SYNTHESIS*

by

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The chloroplast mutants are usually characterized by the leaf color. The leaf color, however, doesnot indicate the nature of the metabolic block and it is strongly influenced by environmental factors.

The great number of individually inherited chloroplast mutations suggest that the chloroplast characteristics are based on more than one metabolic process. One group of chloroplast mutants shows abnormal pigment synthesis, while in the other it is mainly the protein synthesis or respiratory system which deviates from the normal.

The work reported here was concentrated on maize mutants whith abnormal carotenoid synthesis, containing mainly ζ-carotene and lycopene respectively. These mutants are able to synthetize qualitatively normal chlorophyll. In dim light of several hundred lux they are "viridescens" though but in ordinary light of one thousand lux they show a high degree of photosensitivity. The leaves of these mutants bleach and in several hours appear to be perfect "albina"-s.

To explain this phenomenon, the question has been raised: in which way does the abnormal carotenoid synthesis affect the chlorophyll content.

It is known that the photosensitivity of the leaf pigments is very much dependent upon their bonds to proteins of the chloroplasts. Consequently the increased photosensitivity of the mutants might be related to decreased stability of the pigment-protein complex.

The stability of the pigment-protein complex in normal lycopene synthetizing corn seedlings and in ζ-carotene containing mutants was compared.

The seedlings were germinated at 25° C in darkness for three days, and then illuminated with 100 lux from a low pressure Xenon lamp. The leaf samples were treated with petrol ether of five-fold value at room temperature. The free (non protein bound) pigments went into solution. The remainder was

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extracted with acetone and the pigments were shaken into petrol ether. The extracts of free and protein-bound pigments were washed and then dried. The carotenoid and chlorophyll content was measured spectrophotometrically at characteristic wavelengths.

The carotenoid concentration and the percentage of free, non protein bound carotenoids is shown in Table 1.

Table 1.

The stability of carotenoid-protein complex in normal and carotenoid-mutant corn leaves

(6.5 day old seedlings, 100 lux)

Material	μM/g fr. w.	% of the labile fraction
Normal	0,23	5
Lycopenic	0,18	25
ζ-carotenic	0,13	42

It appears that the difference in the carotenoid content of normal and mutant leaves, grown at 100 lux, is not significant. The percentage of non-stable fraction in the normal leaves is very low, indicating that almost the whole carotenoid content is in protein-bound state. In the lycopenic mutant, which is unable to form ionon ring at the ends of the carotenoid molecule, the percentage of the free pigments is five-times higher. If the lack of the ionon rings is associated with partial saturation of the double bonds (as in the ζ-carotene containing mutant) the lability of the carotenoid-protein complex increases even to a higher degree.

The chlorophyll content and the percentage of non-protein bound chlorophylls is shown in Table 2.

Table 2.

The stability of chlorophyll-protein complex in normal and carotenoid-mutatnt corn leaves

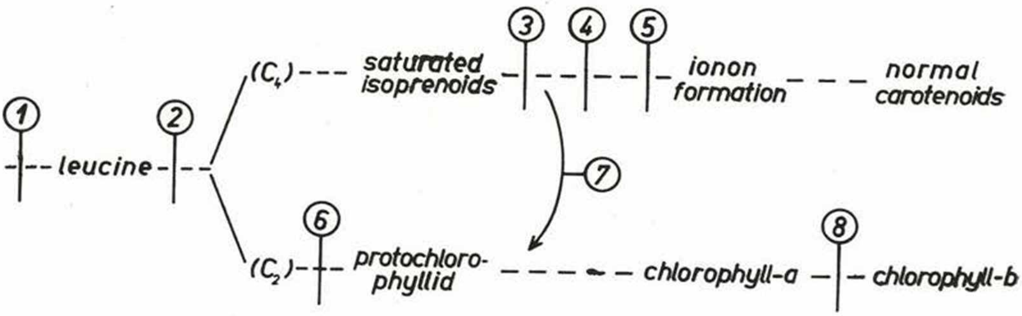
(6.5 days old seedlings, 100 lux)

Material	μM/g fr. w.	% of the labile fraction
Normal	0,37	2
Lycopenic	0,15	3
ζ-carotenic	0,03	4

From this Table it can be seen that the chlorophyll content of normal and carotenoid mutant leaves is considerably different. On the other hand the stability of the chlorophyll-protein complex is very much alike.

It is known that the carotenoids play an important role in stabilizing chlorophyll against photodestruction. The absorption maxima of the pigment-protein complexes are shifted towards longer wavelengths than those characteristic for the free pigments. This is particularly true for the carotenoids, the absorption maxima of which in protein-bound form might be shifted theoretically even to 1100 mμ. Therefore it depends on the quality and quantity of bound carotenoids to what extent these pigments are able to fulfill their protective and energy accumulating role. As shown by the data obtained, the carotenoids of the mutants are less able to form a stable complex. This might offer an explanation of the increased photosensitivity of the lycopenic and ζ-carotenoid leaves.

To fit these data into a phenogenetical system of chloroplast mutants on the basis of the data published so far, a hypothetical scheme was advanced. This scheme concerning the localization of the genetic blocks in the pathway of pigment synthesis is shown in Fig. 1. (3).



Block 1 refers to the paper of Eriksson et co-workers in which it was shown that a leucine supply is needed for chloroplast development. In the mutant xantha-23 of barley, the chloroplast structure and chlorophyll content was partially normalized when the seedlings were supplied with leucine.

The role of the leucine in this process is supported also by the paper of Fujii and Ono, who describe wheat mutants with increased leucine content. In this case the metabolization of the leucine might be retarded, as shown in Block 2.

The leucine is known as an important component of proteins, determining their primary and secondary structure. Moreover as we found earlier, leucine is incorporated into the pigments themselves. It was proved to be an effective precursor of carotenoids and part of it appears in the chlorophyll molecule.

Blocks 3. to 5. show chloroplast mutants described in maize with abnormal carotenoid synthesis, containing partially saturated phytoene, phytofluene, ζ-carotene or lycopene. As referred to before, these genetical blocks affect indirectly the chlorophyll content, loosening the chloroplast structure at molecular level. This increases the photosensitivity of the photosynthetizing apparatus.

In Block 6. a barley mutant containing protoporphyrin IX but no chlorophyll is shown.

Block 7. represents maize mutants with decreased phytolization of the chlorophyll. Block 8. refers to mutants which contain chlorophyll-a but no chlorophyll-b.

This hypothetical localization of the genetical blocks in chloroplast mutants with deviating pigment synthesis may be necessarily somewhat onesided and perhaps a fairly rough one, and further studies are needed for its improvement.

РЕЗЮМЕ

В значительной части хлоропластовых мутантов высших растений хлорофильный синтез является нормальным, и генетический блок помешен в другом месте. В процессе изучения синтеза каротиноида в листьях кукурузы-альбиноса и ячмени нами найдены характерные аномалии.

Нами установлено, что лейцин представляет собой важный прекурсор синтеза каротиноида в ходе хлоропластовой дифференциации. Хотя и один из альбиносных типов синтезирует каротиноиды, но последние являются отчести насыщенными (фитоен, фитофлюен, ζ-каротин). И в случае другого типа смыкание иононного кольца не осуществляется. (Ликопин).

Как количественная, так и качественная разница в содержании каротиноида влечет за собой повышенную стабильность комплексов пигментов и протеинов хлоропластов. Отсутствие иононного кольца уменьщает стабильность на 30 проц. Если отсутствие связано с аномалией насыщенности, то стабильность снижается на 48 проц.

На основе наших исследований нами удалось установить гипотетическую схему на месте генетических блоков.

RETERENCES

1. Eriksson, G. – Kahn, A. – Walles, B. – Wettstein, D. v. 1961: Zur makromolekularen Physiologie der Chloroplasten III. Ber. deutsch. Bot. Ges. **74.** 221 – 232.
2. Fujii, T., Ono, Y. 1960: Amino acid content of Einkorn wheat chlorophyll mutants. Proc. Japan. Acad. Sci. **36.** 609 – 617.
3. Faludi-Dániel, Á. 1963: Amino acids in the biosynthesis of leaf pigments in higher plants I. Ann. Univ. Sci. Bp. **6.** 83 – 88.