# CHLOROPLAST DEVELOPMENT IN MAIZE LEAVES AT DIFFERENT LIGHT INTENSITIES

by

DR. ÁGNES FALUDI-DÁNIEL, ANNA H. NAGY and A. GYESKO

Department of Phylogenetics and Genetics of the Eötvös Loránd University, Budapest

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The area of the lamellar system which is the site of photosynthetic processes is determined by both the inner organization and the number of chloroplasts.

The inner organization of chloroplasts is governed by genetical and ecological factors (Eriksson et al. 1961). In higher plants the individual phases of conversion of proplastids into chloroplasts depend on several genes for the activity of which light of different energies and intensities is required. The conversion of protochlorophyll to chlorophyll and the formation of lamellar vesicles occur at relatively low light energies ($10^4$ erg/cm$^2$/200 sec, $\lambda = 650$ m$\mu$) (Virgin et al. 1963). A more intensive pigment synthesis and granum formation will start only upon illumination which is one order of magnitude higher. A functionally essential step in this process is the formation of a lipoprotein complex system (Boardman 1962) which is associated with active protein synthesis (De Deken-Grenson 1954). Processes maintaining the lamellar system of chloroplasts are also sensitive to changes of illumination. This is reflected in a volume decrease, in a lowering of pigment content (Godnev et al. 1960) and in a change of the electronmicroscopic picture (Wehrmeyer 1964) when chloroplasts are kept in the dark for prolonged periods of time.

The number of chloroplasts per cell is different with different species. By progressing towards higher organisms the number of plastids and its variation usually increase. In the angiosperms the number of plastids varies with different leaf tissues (Granick 1955). A correlation has been established between polyploidy and the number of plastids per cell (Butterfass 1964). Nitrogen supply of the plants (Tabentski 1953, Sargomsky 1956), spectral composition of light (Kahnovich 1960) and intesity of illumination (Lundegardh 1954, Kahnovich and Hodorenko 1964) are factors which influence the number of chloroplasts and/or the chlorophyll content of the cells.

The mechanism underlying the formation of a definite number of chloroplasts per cell is not fully understood. It is known that in seedling leaves the chloroplasts originate from proplastids. However, as to the size of the fraction of proplastids which will actually give rise to chloroplasts no detailed information is available. It is known that in leaves of higher plants grown at low light intersities fully developed plastids usually do divide (Schötz and Senser 1964) but nothing is known about the quantitative aspects of this process.

The present paper reports some quantitative studies on chloroplasts in various stages of differentiation. Different light intensities have been compared with respect to their effect on the pigment content of chloroplasts formed in maize leaves, on the number of chloroplasts per unit weight of leaves and on protein synthesis.

## Marerials and Methods

Grains from an inbred line (Mv 1143/53) of *Zea mays* L. convar. vulgaris were germinated in the dark at 26° C on a layer of cotton for 5 days and then exposed for 3 to 4 days to white light of intensities of 5, 25, 100 and 1000 lux, respectively (Tungsten lamps, 150 W).

The first and second leaves of the seedlings were cut to pieces about 0.5 cm long each. These were pooled to give samples of 5 to 20 g. Aliquots of 0.2 g were withdrawn for determinations of total pigment and total nitrogen contents, respectively. The pigments were extracted with a 1 : 1 mixture of acetone and petroleum ether (bp 40–60). Pigment content was measured spectrophotometrically at four different wavelengths (Faludi-Dániel et al. 1965).

Chloroplasts were isolated on glycerin-sucrose phosphate columns by gradient centrifugation (James and Das 1957). From fractions containing intact plastids known amounts were placed in a haemacytometer and microphotographs of an approximately 200 fold magnification were taken. Chloroplast counts have been expressed as the average values obtained from countings in 8 to 10 chambers of $0.2 \times 0.2 \times 0.1$ mm (the optimum dilution for counting was 20 to 20 chloroplasts per chamber).

Aliquots of the chloroplast suspensions were shaken with acetone-petroleum ether and the upper phase was subjected to spectrophotometric assay. The rest of the suspension was diluted two fold with dist. water and the protein precipitated with trichloroacetic acid in a final concentration of 3 per cent. The precipitate was washed with dist. water and after digestion with $H_2SO_4$ the nitrogen content was determined photometrically by the Nessler method (Bálint 1955).

The number of chloroplasts per unit leaf weight was calculated from the pigment content of the leaves divided by that of the chloroplasts. In the Tables averages of four experiments and their mean errors are given.

## Results

Table 1 shows the amount of chlorophyll per unit leaf weight and per chloroplast, respectively, in leaves kept at different light intensities.

*Table 1.*

**Effect of different light intensities on the chlorophyll content of maize leaves and chloroplasts and on the number of plastids per unit weight**

| Light intensity lux | $10^{-9}$ M / g fresh weight (leaves) | $10^{-16}$ M / chloroplasts | $10^9$ chloroplasts / g fresh weight (leaves) |
|---|---|---|---|
| 5 | 281 ± 150* | 1.0 ± 0.5 | 2.8 |
| 25 | 780 ± 73 | 3.5 ± 0.3 | 2.2 |
| 100 | 989 ± 154 | 6.3 ± 0.8 | 1.6 |
| 1000 | 1200 ± 144 | 7.2 ± 1.4 | 1.7 |

* $P_{\bar{x}} \geq 10$, for the test, $P \ll 5$

It may be seen that with increasing light intensities chlorophyll content increases at a decreasing rate, as expected. The increase in chlorophyll content occurs at a higher rate in intact plastids obtained by density gradient centrifugation. The number of chloroplasts as calculated on the basis of chlorophyll content decreases with increasing light intensities.

Table 2 shows the carotenoid content of chloroplasts and of leaves grown at different light intensities and the number of chloroplasts per unit leaf weight as calculated on the basis of caroteinoid content.

*Table 2.*

**Effect of different light intensities on the carotenoid content of maize leaves and chloroplasts and on the number of plastids per unit weight**

| Light intensity lux | $10^{-9}$ M / g fresh weight (leaves) | $10^{-16}$ M / chloroplasts | $10^9$ chloroplasts / g fresh weight (leaves) |
|---|---|---|---|
| 5 | 96 ± 35 | 0.3 ± 0.1 | 3.2 |
| 25 | 191 ± 25 | 0.6 ± 0.1 | 3.2 |
| 100 | 216 ± 32 | 1.4 ± 0.2 | 1.5 |
| 1000 | 270 ± 40 | 1.6 ± 0.2 | 1.7 |

As it is seen, at higher light intensities the carotenoid content shows an increase similar to chlorophyll content but to a lesser extent. At low light intensities the number of chloroplasts based on carotenoid contents is higher than that based on chlorophyll contents. In the case of more intensive illumination the number of chloroplasts is the same whether calculated on the basis of chlorophyll or carotenoid contents.

The formation of chloroplasts is associated with active protein synthesis. The state of differentiation of plastids is reflected in their protein content or

rather in the fraction of total cellular protein contained in the plastids. The protein content of chloroplasts and of leaves grown at different light intensities and the fraction of proteins in the plastids are shown in Table 3.

*Table 3.*

**Effect of different light intensities on the protein content of maize leaves and chloroplasts**

| Light intensity lux | mg protein / g fresh weight | $10^{-9}$ mg / chloroplasts | Per cent protein in the chloroplasts* |
|---|---|---|---|
| 5 | 19.3±1.8 | 1.4±0.3 | 20 |
| 25 | 23.1±1.7 | 2.3±0.2 | 22 |
| 100 | 23.8±1.1 | 4.1±0.7 | 27 |
| 1000 | 26.6±1.6 | 4.9±0.8 | 31 |

* $\frac{\text{Protein /chloroplast} \times \text{number of plastids/g fresh weight (leaves)}}{\text{protein/g fresh weight (leaves)}} \times 100$

It is clear from Table 3 that at high light intensities the protein content of both leaf tissues and individual chloroplasts is higher than at lower illumination. This increase upon illumination is about 40 per cent with the leaves and about 250 per cent with the chloroplasts, under identical conditions. Consequently leaves kept at high light intensities have fewer plastids, the amount of protein contained in the plastids is nevertheless higher than in the control.

## Discussion

Upon illumination pigment content increases to about the same extent in both leaves and plastids as long as the intensity of light is kept at a low level. At 100 and 1000 lux, however, whereas the pigment content shows a 3 to 4 fold increase per unit leaf weight, it increases by a factor of 5 to 7 per plastid.

The different rate of pigment accumulation in leaves and plastids may be connected with the fact that for the numerical increase of plastids low light energies suffice. On the other hand, the formation of stable structures in the plastids is not triggered under these conditions which is indicated by the lower pigment concentration. At higher light intensities differentiation is accompanied by a reduction in the number of plastids. It can be supposed that during the differentiation of plastids some sort of a regulatory mechanism will set an upper limit to the amount of protein which can be withdrawn form the total protein pool for synthetic processes in the chloroplasts. As a result of the operation of such a regulatory process the differentiation of some plastids may stop completely. This may in turn lead to their destruction. This hypothesis is supported by Arnason and Walker (1949) who have reported the occurrence of three zones in variegated barley leaves with respect to the size of chloroplasts. In the green mesophyll they found plastids of 5 to 7 $\mu$ in size, in the transition zone in addition to few such plastids a larger

number of bodies of 1 to 3.5 $\mu$ and in the white zone many small bodies which were shown to be mutated plastids uncapable of further differentiation.

In *Euglena*, too, there is a difference in the number of proplastids and fully developed chloroplasts, respectively. 30 proplastids were observed to give rise to only about 10 to 12 fully developed plastids (Schiff and Epstein 1966).

Another possibility is that at lower light intensities fragile plastids of lower mechanical stability are formed which are broken down during isolation increasing thereby the apparent plastid counts.

The number of plastids per unit leaf weight is not quite the same depending on whether it is based on chlorophyll or carotenoid contents. At hight light intensities the two values are in agreement. With low light intensities the much higher plastid counts based on carotenoid contents are due to the fact that the mechanically less stable plastids with less chlorophyll and more carotenoids were eliminated during density gradient centrifugation. A similar phenomenon was observed when pigment contents of normal and mutated corn leaves and plastids were compared (Osipova et al. 1967). Neish's (1939) results point to a similar phenomenon. He reported different dry weights for the plastids depending on wheter chlorophyll and carotenoid or xanthophyll contents served as a basis for the calculations.

Studies on carotenogenesis in etiolated and illuminated leaves of maize have led Goodwin (1958) to conclude that it is mainly $\beta$-carotene, forming about 30 per cent of total carotenoid content, which is most hightly bound to the structure of chloroplasts. Upon illumination this particular $\beta$-carotene will accumulate.

During the formation of plastids most of the increase in total protein content is confined to the plastids. This increase occurs to such an extent that while the number of plastids decreases, the fraction of total protein content in the plastids increases by 10 per cent. According to De Deken-Grenson's (1954) data upon illumination of etiolated leaves the protein content of plastids increases by 210 per cent whereas that of microsomes only by 10 per cent.

Fully developed plastids contain about 30 per cent of the total cell protein (Menke 1938).

Taking an average molecular weight of 25,000 for the structural proteins of chloroplasts (Criddle 1966) the number of chlorophyll molecules per one protein molecule will be around 0.6 with illuminations of 25, 10 and 1000 lux. At an illumination of 5 lux, however, the ratio of chlorophyll to protein molecules will drop to 0,3. This suggests that even in plastids not completely differentiated (25 lux) the chlorophyll-protein complex will be formed. This is in line with the data indicating that at 25 lux the ratio of chlorophyll in a free state to that bound in the complex is small as shown by high equilibrium constants (Faludi-Dániel et al. 1965).

## Summary

Differences in the pigment and protein contents of leaves and chloroplasts of maize seedlings grown at different light intensities have been studied.

It has been shown that with increasing light intensities the amount of pigments localized in the plastids increases to a higher extent than the total pigment content of leaves.

The number of plastids calculated on the basis of chlorophyll and carotenoid contents decreases with increasing light intensities.

The fraction of total protein represented by the plastids increases at higher light intensities.

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