Chloroplast arrangement as a factor of photosynthesis in multilayered leaves

Z. LECHOWSKI

Institute of Molecular Biology, Jagiellonian University, Cracow

(Received: June 17, 1974)

Abstract

The dependence of photosynthesis on arrangement of the chloroplasts in *Ajuga reptans* and *Syringa vulgaris* differing in the ability of chloroplasts translocation was studied.

INTRODUCTION

The displacement of chloroplasts in the cells is frequently mentioned as one of the factors influencing the intensity of the process of photosynthesis (Senn, 1908; Voerke, 1934). Nevertheless studies on this relation are so far relatively scant.

The results of certain investigations found in the literature are only of qualitative character (Stahl, 1880; Schanderl and Kaempfert, 1933), and only those published by Zurzycki (1955, 1962) obtained on lower organisms present this relation in quantitative terms. Data are completely lacking as regards multilayered assimilation organs such as the leaves of land plants. The only work to date carried out on *Nicotiana* leaves by Babuskin reports quite opposite results from those obtained for other objects. This author established that photosynthesis declines at the very moment when the chloroplasts begin to translocate, both under light of high intensity and under weak illumination.

It results from other investigations that photosynthesis is dependent on the chloroplast arrangement only at low light intensities acting directly after the decline of strong light and that then photosynthesis gradually increases (Zurzycki, 1955). On the other hand, no correlation could be found between photosynthesis and the changes in the position of the chloroplasts occurring under the influence of high light intensities.

In earlier investigations on chloroplast translocation in the cells of multilayered leaves (Lechowski, 1970) it was found that the arrangement of chloroplasts in the cell can be revealed by measurement of the light transmitted by the leaf. It was ende-

avoured in the present study to establish, by measuring transmitted light and by simultaneous measurement of photosynthesis, the correlation between the arrangement of the chloroplasts and the intensity of the process of photosynthesis for objects of this type and to express numerically the differences in photosynthesis intensity.

MATERIAL AND METHODS

The experiments were performed on well developed leaves from natural sites with moderate insolation. Leaves of two plant species were used, differing in the reaction of their chloroplasts to light conditions. From among the group of plants exhibiting an ability of chloroplast translocation in the cell *Ajuga reptans* was chosen. In the leaves of this plant the chloroplasts move to a profile position and then absorption diminishes by about 6 per cent. As control the leaves of *Syringa vulgaris* were used, a species which shows practically no ability to chloroplast translocation and in which the changes in absorption amount to about 0.06 per cent (Lechowski, 1970). The leaves used for the experiments were first adapted in a moist chamber to conditions of low light intensity ($100 \text{ erg cm}^{-2} \text{ s}^{-1}$) for a period of 18–24 h in order to obtain a completely flat chloroplast arrangement.

The changes in chloroplast position and photosynthesis intensity were simultaneously investigated. For this purpose an assimilation chamber was used shown schematically in fig. 1.

Fig. 1. Scheme of assimilation chamber and illumination system:

I — iodine-quartz lamp, *wf* — water filter, *fa* — glass Antisol filter, *n* — net filter, *i* — microscope lamp, *ln* — lens, *m* — mirror, *gf* — glass filter, *l* — leaf, *o* — opal plate, *f* — photomultiplier, *w* — water jacket

The changes in chloroplast arrangement were followed by measuring the amount of light transmitted by the leaf. For this aim a photomultiplier type RCA-IP 21 was built into the lower part of the assimilation chamber. Over the light slit a neutral net filter was placed with a 12 per cent transmittance, which could be eliminated from the light course each time and replaced by an opal plate. Above the bottom of the assimilation chamber a nylon net was stretched on which the leaves were spread with petioles placed in small vessels filled with water. The leaf spread on the opal

plate was covered during light transmission measurements with a mobile glass filter of known light transmittance, placed within the chamber and removed after measurement beyond the surface area of the leaf.

As source of light for measurement of light transmittance a microscope lamp was used fed with stabilized current and placed in horizontal position outside the surface of the chamber. The light beam from the lamp was directed to the measured surface by a mirror which after measurement was shifted beyond the chamber surface. The photomultiplier current was measured with a Kipp and Zonnen type A-70 galvanometer. The principle on which measurement of light transmittance through the leaf was measured is described in another paper (Lechowski, 1970). The time of transmittance measurement did not exceed 3 seconds and followed immediately photosynthesis measurements. During measurement of transmittance the light used in the experiments was switched off. The source of light were 3 iodine-quartz Tungsum 1000 W, 220 V lamps. Infrared radiation was eliminated by placing in the light pathway a water filter (with flowing water) of 15 cm thickness and a 5-mm glass filter of Antysol type with characteristic close to the transmittance of the BG-17 filter. The light intensity was regulated by the application of natural net filters.

The assimilation chamber was conditioned to a temperature of 27°C ($\pm 0.5^\circ\text{C}$) by way of circulation of thermostated air and by applying water flow in a special water jacket surrounding the chamber.

Gas exchange was measured by recording changes in CO_2 concentration with an IF gas analyzer of Infralyt III type (Junkalor, Dessau, West Germany). A closed measuring system was used. CO_2 concentration was measured within the range of 300–400 ppm.

Measurements of photosynthesis and transmittance were taken at 5-min intervals under strong light and 2-min intervals under low intensity light over a 10 min period of exposure, and later at 5 min intervals.

Photosynthesis and respiration intensity were expressed as milligrams of CO_2 consumed or released by a 1 dm² surface area of the leaf blade over a period of 1 h ($\text{mg CO}_2 \text{ dm}^{-2} \text{ h}^{-1}$). Each result given is a mean from a series of 8 experiments.

RESULTS

Before starting the investigation proper on the influence of the position of the chloroplasts on the process of photosynthesis, the dependence of this process on light intensity was studied for both the specie investigated. The values of the rate of photosynthesis marked on the light intensity curves (fig. 2) were obtained after 1 h of exposure to light of known intensity. The value of light intensity at which compensation point and the point of saturation appear are listed in table 1.

Further experiments were performed with the use of two different light intensities. The species studied show certain differences as regards light requirements, therefore for each of them appropriate light intensity were established. As light high intensity (I_1) for both species could be considered $191\,000 \text{ erg cm}^{-2} \text{ s}^{-1}$. This intensity exceeded

Fig. 2. Light curve of photosynthesis for: I — *Ajuga reptans* II — for *Syringa vulgaris*

by far the point of photosynthesis saturation, but at the same time it was so adjusted that under exposure to it a maximal increase of light transmitted by the leaf occurred. On the other hand weak illumination (I_2) of different intensity was applied to each species.

Table 1

Object	Compensation point erg cm ⁻² s ⁻¹	Saturation point erg cm ⁻² s ⁻¹
<i>Ajuga reptans</i>	1200	32 800
<i>Syringa vulgaris</i>	2100	72 500

The value of I_2 was so adjusted as to completely arrest the increase in transmittance obtained under strong light, and it lay above the compensation point. Such light intensity depressed photosynthesis in both species by about 70 per cent of the values reached under light of I_1 intensity. The corresponding intensities were read on the curves in fig. 2 they were:

for *Ajuga reptans* $4260 \text{ erg cm}^{-2} \text{ s}^{-1}$

for *Syringa vulgaris* $11700 \text{ erg cm}^{-2} \text{ s}^{-1}$.

The results obtained in photosynthesis measurement and the leaf transmittance values at the tested light intensities are shown in fig. 3.

Strong light (I_1) provokes in both species an increase of photosynthesis up to 25 min, after this period the rate becomes constant to the end of exposure lasting 90 min (curve I).

Simultaneous measurement of light transmittance indicates that, for the *Ajuga reptans* leaf, at the same time the maximal increase of transmittance equal to 4.98 per cent (curve II) is reached. The increase in transmittance is associated with the translocation of chloroplasts from flat to profile arrangement. The transmittance value reached in the first 30 min changes no more under further exposure to light.

In the leaf of *Syringa vulgaris* transmittance increases by barely 0.06 per cent and this value is reached as early as after 5–10 min. As demonstrated earlier (Le-

Fig. 3. Course of photosynthesis and light transmittance of leaf in dependence on light conditions I_1 and I_2 and respiration in time: photosynthesis (curve I), transmittance (curve II), respiration in darkness (curve III)

a — *Ajuga reptans*, b — *Syringa vulgaris*

chowski, 1970) this species has no ability to chloroplast translocation, and the change in transmittance is connected with conformation changes occurring in the chloroplasts. The course of the photosynthesis curve for the period investigated is for both species the same, in spite of their different ability of chloroplast translocation. The gradual rise of the photosynthesis curve in the initial period of the experi-

ment is not associated with chloroplast translocation to a profile arrangement but with the adaptation of the measuring system to the experimental conditions and with the period of induction of photosynthesis.

After 90 min of exposure to light of I_1 intensity the conditions were changed to an intensity of I_2 . The action of weak light causes a drastic decrease of photosynthesis. At the first minute of measurement very intensive photorespiration could still be recorded, its value exceeding many times the values of respiration in darkness (curve III).

The picture of the photosynthesis curve in fig. 3a is obscured in the first minute of exposure to low intensity light by the superposition of the effects of photosynthetic induction over the photorespiration process. The present experiments were not conducted with the aim of characterizing these two processes. In order to obtain the probable course of the photosynthesis curve in the initial period of exposure to weak light, the curve was extrapolated in relation to the remaining points lying on it. Further application of low intensity light may have a different effect in both the species examined. In *Ajuga reptans* the rate of photosynthesis consistently rises up to the 25th min and then remains at a constant level.

Transmittance measurements show its decrease from 4.98 to 0.55 per cent after 25 min. Further exposure to low intensity light decreases transmittance to the value recorded before illumination with light of high intensity. This decrease is due to the translocation of chloroplasts to a flat arrangement.

The curves in fig. 3a reveal a distinct correlation between the increase of the photosynthesis rate and the decrease of light transmittance by the leaf. These processes occur within the same time. Once the chloroplasts assume their position in the flat arrangement, they exert no more influence on photosynthesis.

The mean photosynthesis value obtained in dependence on the chloroplast arrangement and the percent of photosynthesis reduction at the time when the chloroplasts are in profile position are shown in table 2.

Table 2

Respiration mg CO ₂ dm ⁻² h ⁻¹	True photosynthesis mg CO ₂ dm ⁻² h ⁻¹		Reduction of photosynthesis %
	profile arrangement	flat arrangement	
0.2693	0.5768	1.1693	50.7

The value of true photosynthesis was calculated without taking into account photorespiration. The per cent of photosynthesis reduction when the chloroplasts are in profile arrangement was calculated with the value of photosynthesis in flat arrangement assumed as 100 per cent.

It results from the data of the photosynthesis curve in fig. 2a that the mean value of photosynthesis, at the time when the chloroplasts are in profile arrangement, obtained at light intensity I_2 (4260 erg cm⁻²s⁻¹) is already reached at an intensity of 2520 erg cm⁻²s⁻¹ when the chloroplasts lie completely flat. The difference between

light intensities giving the same photosynthesis values at various positions of the chloroplasts is 41.4 per cent. The decrease in light absorption for the flat chloroplast arrangement is for the leaves of *Ajuga reptans* 6 per cent. Conversion of this 6 per cent decrease of absorption at light intensity I_2 to light intensity units gives a value of $255 \text{ erg cm}^{-2}\text{s}^{-1}$. If we introduce this correction at light intensity I_2 and then read the photosynthesis value on the light curve in fig. 2a, we obtain true photosynthesis equal to $1.0693 \text{ mg CO}_2 \text{ dm}^{-2}\text{h}^{-1}$. Thus light absorption diminished by 6 per cent should reduce photosynthesis by about 8.6 per cent. Reduction of photosynthesis is about 50.7 per cent that is about 5.9 times more than do changes in the values of light absorption. Of course the conversion given here is only of orientational value.

In the case of *Syringa vulgaris* (curves in fig. 3b) the new constant photosynthesis value under low light intensity is reached as early as after 2 min, at the same time transmittance reaches the initial value.

After 40 min of exposure to light of I_2 intensity, light of I_1 intensity was switched on again. The course of the photosynthesis curve is opposite to that in the initial period of the experiment. The action of strong light in this period causes maximal photosynthesis values to be reached in the leaves of both species. On the other hand, transmittance in *Ajuga reptans* increases up to 40 min of exposure and thereafter remains at a constant level.

From the course of the photosynthesis and transmittance curves for the leaves, within this range of light intensities, the conclusion may be drawn that there is no correlation between photosynthesis and the changes in the arrangement of the chloroplasts to profile position.

Attempts were also made to establish whether some other factor, beside chloroplast arrangement, could be responsible for the differences in the rate of the process of photosynthesis observed, for instance the degree of opening of the stomata. The application of extremally different light intensities could cause the closure of the stomata which during further action of low intensity light might open gradually. Such gradual opening could cause a better gas exchange with the ambient atmosphere. In such a case the process of photosynthesis would be limited by the concentration of CO_2 present in the intercellular spaces.

Therefore the degree of opening of the stomata was measured by the method of collodion imprints. No differences were, however, revealed in the degree of opening of the stomata in both the objects examined, under the influence of change in the light conditions, notwithstanding the order of succession of the light intensities applied; $I_1 \rightarrow I_2$ or $I_2 \rightarrow I_1$. Since the stomata in both the species under study behave in the same way, that is they remain the whole time opened to the same extent, the influence of this factor may as it seems be ruled out.

DISCUSSION

The results of photosynthesis measurements obtained for objects with a multi-layered structure such as leaves of land plants show that species differing in their ability of chloroplast displacement in the cell exhibit also differences in the course of

the photosynthesis process. Such a relationship was observed only in the case of weak illumination. Data obtained in these experiments are similar to the results obtained for lower plants (Zurzycki, 1955).

The change in chloroplast arrangement in the cells of the assimilation organs is not without influence on the amount of light energy absorbed (Stahl, 1880; Detlefson, 1887; Zurzycki, 1953, 1961; Lechowski 1970). Exposure to strong light causes the chloroplasts to assume a profile position. This arrangement is, however, reached at light intensities exceeding many times the point of saturation (Zurzycki, 1955), and the amount of light energy obtained by the chloroplasts is excessive. Thus within this range of intensity the arrangement of chloroplasts has no influence on the process of photosynthesis.

Translocation of the chloroplasts to the profile arrangement reduces light absorption by about 12 per cent in plants in which the structure of the assimilation organs is simple (Zurzycki, 1961) and by 5—6 per cent in multilayered leaves (Seybold, 1956; Lechowski, 1970). Low intensity light is the factor limiting the process of photosynthesis. The amount of light energy absorbed is additionally diminished by the profile arrangement of the chloroplasts. In spite of certain differences in the percentual decrease of light absorption in the case of this position, photosynthesis reduction reaches in both kinds of objects about 50 per cent. Orientational calculations show that the decrease in the rate of photosynthesis exceeds about 5.9 times the reduction of light absorption. The value obtained for photosynthesis reduction may be lower if we take into account in true photosynthesis calculations the values of photorespiration intensity. The question arises what is the cause of these results. One probable factor may be the influence of an additional decrease in light intensity which was not taken into account when calculating its value.

Absorption may also be influenced, beside transmittance, by light reflexion, changes in which were not measured in the present experiments. The degree of light reflexion is not a constant value but may change in dependence on the conditions. Reflected light consists of light reflected from the outer surface and partly of light returning from the inner surface of the leaf (Pokrowski, 1925). The results of investigations on the changes in reflexion of the leaves of *Lemna trisulca* (Zurzycki, 1961) indicate that the profile position of chloroplasts enhances light reflexion. This factor was taken into account in the presently given changes in absorption. The degree of dispersion of incident light is an additional factor increasing light reflexion (Seybold, 1933; Zurzycki, 1961). Thus, light dispersion would exert an additional effect on the decrease in absorption, the degree of which was not determined, because in experiments to date both leaf irradiation and transmittance measurements were performed in light concentrated by an optic system, thus falling parallelly on the leaf surface. In the present investigations a certain discrepancy occurred. Transmitted light was measured with the use of light concentrated by the optic system of a microscope lamp, whereas the light applied in experiments on photosynthesis was strongly dispersed (no concentrating system and additional dispersion owing to the application of various kinds of filters).

Therefore the true decrease of light absorption in the case of profile position of the chloroplasts may be higher. On the other hand, the contribution of other factors cannot be excluded. The results obtained may have also been due to the different ability of photosynthesis when the chloroplasts are arranged flat or in profile position, or else the effect of changes in absorption might be enhanced by effects of photosynthesis induction (Zurzycki, 1961). The first hypothesis would seem to be confirmed by the fact of the lamellar structure of the chloroplasts and the partial orientation of photosynthetic dye molecules in these structures.

A protraction of the effects of photosynthetic induction to a dozen minutes or so was not observed in the case of plants lacking the ability of chloroplast displacement in cells such as *Fontinalis* (Zurzycki, 1955) and *Syringa* used in the present experiments. Since the effects of induction are very sensitive to changes in the environment and the physiological state of the cell, their action may exert a certain enhancing influence, particularly in the first minutes of change in illumination.

SUMMARY AND CONCLUSIONS

Results are reported of investigations on the relation between the process of photosynthesis and the position of chloroplasts for two plant species differing in their ability of chloroplast translocation. Changes in the chloroplast arrangement were established by measuring transmitted light. Photosynthesis was measured in a closed system by means of a gas IR analyzer type Infralyt III. A special assimilation chamber was used in which light transmission through the leaf could be measured simultaneously with photosynthesis.

The dependence of photosynthesis on the arrangement of the chloroplasts was found only at low light intensities following immediately the action of strong light, when the chloroplasts are still arranged in profile. Translocation to a flat arrangement enhances photosynthesis for a period of 25 min.

The mean value of photosynthesis reduction in the case of profile arrangement of chloroplasts is for *Ajuga reptans* about 50.7 per cent. Absorption in this arrangement is depressed by about 6 per cent. The effect exerted on photosynthesis is several times greater than the occurring diminution in light absorption.

Such a relation was not noted in *Syringa vulgaris* which does not exhibit any ability to chloroplast translocation.

Acknowledgements

The author wishes to thank professor J. Zurzycki for advice and his kind guidance in the course of performing the experiments and in preparing this paper.

Thanks are also due to doctor M. Czarnowski for acquainting me with the method of measurements on a gas IF analyzer.

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Author's address

Dr. Zbigniew Lechowski

Institute of Molecular Biology,

Jagiellonian University

ul. Grodzka 53; 31-001 Kraków, Poland

Układ chloroplastów jako czynnik fotosyntezy w liściu wielowarstwowym

Streszczenie

W pracy przedstawione zostały wyniki badań nad zależnością procesu fotosyntezy od układu chloroplastów dla dwóch gatunków roślin o różnej zdolności chloroplastów do przemieszczeń. Zmiany w układzie chloroplastów ustalono przez pomiar transmitowanego światła. Pomiar fotosyntezy dokonywany był w układzie zamkniętym przy pomocy gazowego analizatora podczerwieni typu Infralyt III. Do pomiarów wykorzystano specjalną komorę asymilacyjną umożliwiającą równoczesny pomiar transmisji światła przez liść i pomiar fotosyntezy.

Zależność fotosyntezy od układu chloroplastów stwierdzona została tylko w niskich natężeniach światła następujących bezpośrednio po działaniu światła silnego, kiedy chloroplasty znajdują się jeszcze w układzie profilowym. Przesunięcie się chloroplastów do układu płaskiego powoduje wzrost fotosyntezy w okresie do 25 min.

Średnia wartość redukcji fotosyntezy w profilowym układzie chloroplastów wynosi dla *Ajuga reptans* około 50,7 %. Obniżenie absorpcji w tym układzie jest około 6%. Wywierany efekt na fotosyntezę jest kilka razy większy aniżeli zachodzący spadek absorpcji światła.

Zależność taka nie była obserwowana u *Syringa vulgaris*, który nie wykazuje zdolności przemieszczeń chloroplastów.