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Photodestruction of Chlorophyll in *Zea mays* L. Leaves Under Different CO_2 Concentration

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Abstract. Photodestruction of chlorophyll (Chl) in *Zea mays* leaves, after their irradiation with high photon fluence rate (5000 μmol m^{-2} s^{-1}), was determined in fragments of whole leaves (WL) and also in fraction of mesophyll cells (MC) and bundle sheath cells (BSC) after their mechanical separation. The lag phase and the phase of photooxidation of Chl in MC chloroplasts were shorter than in BSC. Duration of both phases was reduced when the leaves were placed in 0 % CO_2 concentration in the atmosphere, while the increase of CO_2 concentration up to 0.3 % totally protected Chl against photodestruction in BSC within the 9 h experiment. During that period of time Chl was destructed by about 30 % in MC.

Irradiance considerably exceeding photosynthesis saturation induces destruction of the photosynthetic apparatus (Björkman 1981, Lidholm *et al.* 1987, Powles and Critchley 1980). The first effect of the destructive activity of radiant energy is photoinhibition of the photosynthetic process (Powles 1984). Photoinhibition of photosynthetic reaction begins when the energy of Chl activation is not entirely consumed in photochemical processes because of inadequate $NADP^+$ and ADP pools or when it is not restrained in other transmutations (Osmond 1981, Cleland *et al.* 1986). Simultaneous activity of high irradiance and some environment factors (CO_2 shortage, and water or thermal stresses) may considerably reduce the time of photodestruction of the photosynthetic apparatus (Lechowski and Białczyk 1989).

Long irradiating of organisms or photosynthetizing organs with high PFD may photodestruct photosynthetic pigments. It is preceded by a lag phase, during which partial or total inhibition of photosynthetic processes take place (Björkman 1981). Experiments on Chl photodestruction have been carried out on leaves of *Zea mays*, the NADP-ME species. MC and BSC of the C_4 plants show important differences of both cytological and biochemical nature. In *Z. mays* leaves these differences are connected, among others, with the occurrence of non-grana chloroplasts in BSC, and with a different Chl *a*/*b* ratio in both types of cells (Black and Mayne 1970, Woo *et al.* 1970). Chl *a*/*b* ratio is greater in BSC. Chl (*a*+*b*)/P 700 ratio is about 413 in MC chloroplasts, which is about

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the value in C_3 plants, while in BSC about 218 (Brown *et al.* 1974, Mayne *et al.* 1974). BSC chloroplasts of *Z. mays* do not posses active PS 2 (Woo *et al.* 1970, Bishop *et al.* 1971). Thick BSC cell walls containing suberine fuses are not passed for gas diffusion (Troughton 1972, Whelan *et al.* 1973). The above-mentioned differences, as well as a different uptake of CO_2 and the connected processes suggest the occurrence of different conditions promoting photodestruction, and at the same time, protecting photosynthetic apparatus against it. In our experiments we studied the influence of high PFD and different CO_2 concentration in the atmosphere surrounding the leaf on the kinetics of Chl photodestruction in MC and BSC.

MATERIALS AND METHODS

Plants

Seedlings of *Zea mays* L. were grown in controlled environment chambers in the garden soil of pH 6.8. A 12/12 h light/dark photoperiod was maintained. The radiation sources were 400 W mercury lamps and 100 W infra-red lamps (*LF-F,* Polamp, Poland). PFD during the growth reached 500 μmol m^{-2} s^{-1} (PAR). Irradiance was measured with a Li-Cor model LI-185 A PFD meter (Lambda Instruments, Lincoln, Nebraska, U.S.A.). Constant temperature during both phases of the photoperiod was maintained at 28 ± 1 °C, relative humidity was 65 ± 5 %. Fifths in fully expanded leaves of 30 d seedlings were normally harvested in the light phase and used for experiments.

Irradiation with High PFD

Irradiation was provided from a high pressure xenon lamp (2500 W, type *918,* Poland). Infra-red radiation emitted by the lamp was first eliminated by a reflecting mirror of the Bauer type and also by passing the obtained radiation through heat eliminating cut-off filters (5 cm thick water filter and 8 mm *BG-17* filter, *Schott,* Jena, Germany). The optical system was similar to that described by Lechowski (1973). Constant PAR irradiation of 5000 μmol m^{-2} s^{-1} for 0.5 h to 9 h was used. Detached leaves (bases of which were dipped in tiny vessels filled with water) were placed in a thermostat chamber with constant flow of normal air (about 0.03 % CO_2) or without CO_2 (after passing it through KOH solution) or in the enriched 0.3 % CO_2 concentration. In order to achieve lower transpiration, leaves were placed on damp filter paper in the chambers. The CO_2 concentration in the chamber was controlled by an infra-red analyzer. Infralyt III (Junkalor, Dessau, Germany). The temperature during the irradiation was 28 ± 1 °C and it was measured on the leaves surface with a constantan copper thermocouple (Burzyński and Lechowski 1983).

Abbreviations used: BSC = bundle sheath cell; Chl = chlorophyll; MC = mesophyll cell; PFD = photon flux density; PS = photosystem; WL = whole leaf.

Separation of MC and BSC Chloroplasts

MC and BSC chloroplasts were obtained after mechanical separation using a modification of methods described by Woo *et al.* (1970) and Farineau (1975). 5 g of leaf tissue was sliced (1–2 mm) and suspended in 50 cm^3 of medium A (0.35 mM $MgCl_2$, 0.5 mM K_2HPO_4, 2 mM $NaNO_3$, 0.35 M sorbitol, 4 mM sodium pyrophosphate, 0.8 mM EDTA, 0.5 % (m/v) bovine serum albumin were resuspended in 30 mM Na-tricine buffer at pH 7.6) at 2 °C. Leaf tissue was homogenized for 5 s in a Unipan type 302 omnimix (Poland) at 60 % of line voltage, and the obtained homogenate was rapidly filtered through two layers of Miracloth. The filtrate, containing predominantly MC chloroplasts, was centrifuged for 5 min at 1000 × *g*. The chloroplast pellet was washed with medium A and centrifuged (1000 × *g* for 10 min), and then used for determining Chl. The BSC strands which adhered to the Miracloth were resuspended in 50 cm^3 of medium B (0.35 M sorbitol, 4 mM $MgCl_2$, 2 mM KH_2PO_4, 10 mM isoascorbate resuspended in 20 mM HEPES-buffer at pH 8.0). This tissue was homogenized three times for 20 s at 60 % of line voltage. Then, the homogenate was filtered through one layer of Miracloth. The BSC strands which were left on the Miracloth underwent a future resuspension in the medium A and were homogenized for 30 s at 100 % of line voltage. The remaining unbroken BSC strands were resuspended in 50 cm^3 of the medium A and homogenized for 90 s at 100 % line voltage. The filtrate from these homogenization contained predominantly chloroplasts of BSC (it was checked by microscopic examination for MC contamination and the degree of BSC intactness). The BSC chloroplasts were washed twice in the medium A and centrifuged at 10 000 × *g* for 10 min and then used for determining Chl. Cross-contamination between the homogenates used for the chloroplast preparation was assessed by using phosphoenolpyruvate carboxylase (EC 4.1.1.31) as an MC enzyme marker and ribulose-1,5-bisphosphate carboxylase (EC 4.1.1.39), and NADP-malic enzyme (EC 1.1.40) as enzyme markers for BSC as described by Powles *et al.* (1980). On this basis, MC chloroplasts were contaminated with maximum 8 % BSC chloroplasts, and BSC chloroplasts were contaminated with maximum 4 % MC chloroplasts.

Chlorophyll Determination

Chl determination was done by the method of Arnon (1949), using a Zeiss (Jena, Germany) UV-VIS spectrophotometer. Chl recovery was measured in WL tissues and estimated per fresh matter unit. Chl *a*/*b* ratio for WL, MC, and BSC fractions was a basis for establishing Chl concentration in MC and BSC by using equations suggested by Woo *et al.* (1970). Data presented in this work are average results from 10 independent experiments.

RESULTS AND DISCUSSION

Microscopic observation of water infiltrated maize leaf fragments, after a 3 h irradiation with high PFD, showed photobleaching in MC, but no changes in BSC colour (data not shown). For the experiments determining Chl concentration in WL, MC and BSC fractions after mechanical separation of leaves, the purity of the fractions was checked both by using marker enzymes and Chl *a*/*b* ratio. The Chl *a*/*b* ratios 3.84 ± 0.12, 3.11 ± 0.05 and 5.39 ± 0.08 for the WL, MC, and BSC fractions that contained relative Chl (*a*+*b*) amounts 100.00 ± 1.23, 57.19 ± 1.02 and 42.71 ± 0.58, respectively were similar to the values presented by Kanai and Edwards (1973). Photodestruction kinetic of Chl in WL, MC, and BSC depended on irradiation time in normal air (Fig. 1).

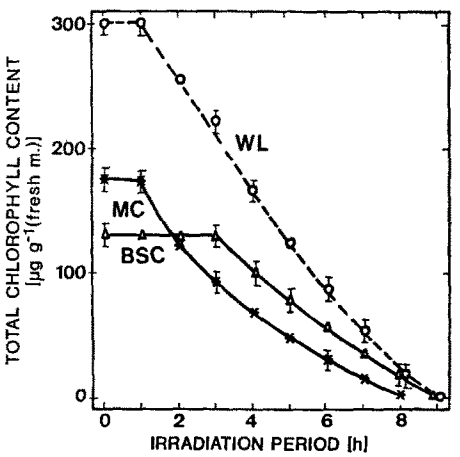

Fig. 1. Chlorophyll photodestruction in WL, MC, and BSC fractions of *Zea mays* L. leaves during the irradiation with high PFD (5 000 μmol m^{-2} s^{-1}) in normal air. Bars represent ± SE higher than 5 %.

In MC chloroplasts the lag phase was about 1 h and then the Chl concentration declined more or less linear by till about the 8th hour. In BSC chloroplasts the lag phase was about 3 h and the Chl photodestruction was completed after 9 h irradiation. Chl concentration in detached leaves left under growth irradiance did not show considerable differences during the time of experiment (data not shown). Chl *b* underwent far slower photodestruction than Chl *a* (Table 1) which was consistent with the results of Thomas and Nijhus (1968) and van Rensen (1975). Chl photodestruction showed some correlations with microscopic observations of the irradiated leaves: a well visible BSC decolourization encircling small conductive bundles was observed after 5 h, while in the medium conductive bundles after 7 h, and in the big ones even later. Numerical ratio of conductive bundles of the small type to the medium and big ones in *Z. mays* leaves were 7:1 and 10:1, respectively (Evert *et al.* 1977). Correlation between

TABLE 1

Percentage of chlorophyll concentration in photosynthetic tissue of *Zea mays* L. leaf during continuous irradiation with high rate of PFD (5000 μmol m^{-2} s^{-1}).

Time	MC			BSC		
h	Chl *a*	Chl *b*	Chl (*a*+*b*)	Chl *a*	Chl *b*	Chl (*a*+*b*)
0	43.27 ± 2.10	13.92 ± 1.51	57.19 ± 2.72	36.01 ± 1.28	6.68 ± 0.62	42.71 ± 1.90
1	43.27 ± 2.35	13.92 ± 1.72	57.19 ± 2.07	36.01 ± 1.52	6.68 ± 0.72	42.71 ± 2.24
2	30.12 ± 1.05	11.03 ± 0.50	41.15 ± 1.55	36.01 ± 1.15	6.68 ± 0.91	42.71 ± 2.06
3	24.52 ± 0.80	10.08 ± 0.38	34.60 ± 1.28	36.03 ± 0.95	6.68 ± 0.82	42.71 ± 1.77
5	16.80 ± 0.68	7.47 ± 0.15	24.27 ± 0.83	20.39 ± 0.58	4.93 ± 0.35	25.32 ± 0.93
7	2.09 ± 0.22	1.90 ± 0.15	3.99 ± 0.37	11.60 ± 0.45	3.59 ± 0.38	15.90 ± 0.83
8	0.00	0.00	0.00	4.21 ± 0.45	1.73 ± 0.28	5.94 ± 0.73
9	0.00	0.00	0.00	0.00	0.00	0.00

the rate of Chl photodestruction and the size of a conductive bundle may have a certain connection with the thickness of the BSC cell walls encircling its given type, and due to this it can modify gas relations inside the cells.

The lag phase and the phase of Chl photodestruction were reduced when the leaves were placed in an atmosphere without CO_2 (Fig. 2). The total decay of Chl in MC chloroplasts occurred under these conditions after about 4 h, while in the BSC chloroplasts after about 6 h. The modifying influence of CO_2 on

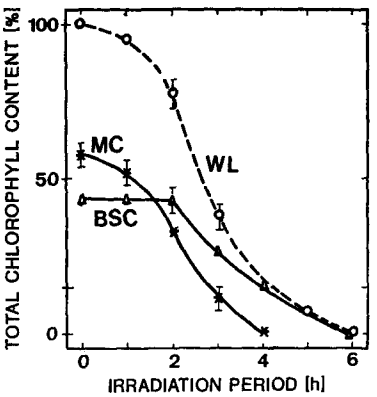

Fig. 2. Chlorophyll concentration in *Zea mays* L. leaves during irradiation with high PFD (5 000 μmol m^{-2} s^{-1}) in gas atmosphere without CO_2.

Chl photodestruction was also shown in experiments with 0.3 % CO_2 in the air flowing through the chamber (Fig. 3): the lag phase was lenghtened to about 6 h in MC chloroplasts, and after 9 h irradiation with high PFD the decrease of Chl ($a+b$) was about 30 %. In BSC chloroplasts the concentration of Chl did not change even after 9 h irradiation (Fig. 3). The duration of the lag phase mirrored all the protective mechanisms responsible for photostabilization of the photosynthetic apparatus (van Hasselt 1972), that ensure undisturbed functioning of a chain of electron transport (van Hasselt 1974). The presence of CO_2 allows to preserve carboxylation processes, and due to this consumption of the light phase products. In MC of *Z. mays* leaves the action of high PFD at a simultaneous lack of CO_2 decreases the activity of the enzymes of the C_4 cycle, malic dehydrogenase and pyruvate kinase (Powles *et al.* 1982). Activity of these enzymes *in vivo* was not lower under the minimum CO_2 pool, which confirms their activity with undisturbed chain of electron transport (Powles *et al.* 1980, 1982). In BSC, the CO_2 attainability influences the degree of activity of the Calvin-Benson cycle due to which it modifies the intensity of photodestructive processes (Powles *et al.* 1980). Restraining the Calvin-Benson cycle in experiments under low temperature lead to photodestruction of photosynthetic pigments in *Cucumis sativus* leaves (van Hasselt 1972, 1974). The CO_2 concentration in BSC of *Z. mays* leaves is about 50 times higher than in MC

(Hatch 1976). Such difference in CO_2 concentration may be one of the essential causes of the observed difference in the velocity of Chl photodestruction in chloroplasts of MC and BSC. Destructive processes in MC take place gradually, which does not exclude the possibility of partial CO_2 uptake and transformation into phosphoenolpyruvate. The produced C_4 acids are transported to BSC, and the CO_2 created, after their decarboxylation sustains the operative Calvin-Benson cycle. In natural environment, in C_4 type plants the direct CO_2 uptake to the Calvin-Benson cycle is rather small and equals about 5–10 % of the total pool of the CO_2 uptake in the photosynthetic processes. On the basis of

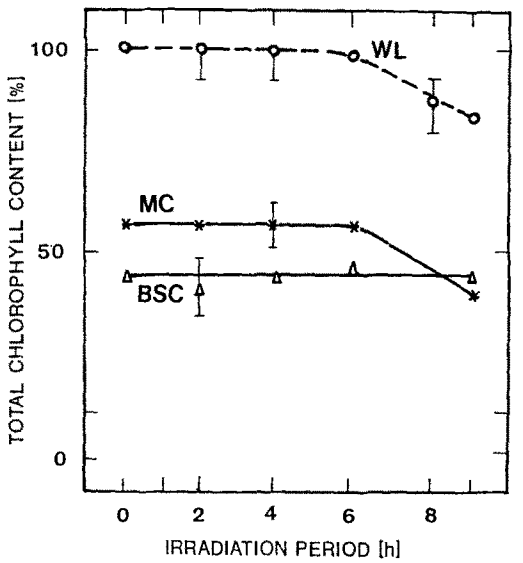

Fig. 3. Chlorophyll concentration in *Zea mays* L. leaves during irradiation with high PFD (5 000 μmol m^{-2} s^{-1}) at 0.3 % CO_2 in gas atmosphere.

theoretical calculations, Hatch (1976) thinks that the potential ability is greater, and can account for up to one third of the total photosynthetic pool of the CO_2 uptake. Such a possibility can occur at a rather low activity of the Calvin-Benson cycle, and even after a considerable increase of the photoinhibition process in MC. A certain CO_2 pool, orginated from the dissimilation process, can also for a certain period of time maintain to a small degree the Calvin-Benson cycle functioning.

The other cause can be connected with smaller concentration of oxygen in BSC. The increase of the oxygen concentration as well as the PFD increase reduce the duration of the lag phase (Sironwal and Kandler 1958, van Hasselt 1972). However, there are no quantitative data stating the oxygen concentration in BSC. Calculation of its concentration, possible in the case of CO_2, however, is not possible here. Hatch (1976) accepts that oxygen concentration in BSC comparable to the concentration in the air. Nevertheless, some data may suggest

a low O_2 concentration in BSC, which may be connected with: (a) the lack of active PS 2 (Woo *et al.* 1970, Bazzaz and Gonvinjee 1973, Ku *et al.* 1974), (b) the actual use of oxygen in photorespiration processes occuring in BSC (Osmond 1981), and (c) thick cell wall, poorly permeable for gas diffusion (Troughton 1972, Whelan *et al.* 1973). The differences in the kinetics of Chl photodestruction between MC and BSC may have some physiological importance. C_4 plants occur in a highly insolated environment, where this kind of damages may occur. The destructive activity of irradiation may be, to a greater degree, limited to MC, while in BSC the damage is considerably slower and thus the assimilation organ may survive.

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