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EFFECT OF CADMIUM STRESS ON GROWTH AND PHOTOSYNTHESIS OF YOUNG BARLEY (*H. vulgare* L.) PLANTS. 2. STRUCTURAL AND FUNCTIONAL CHANGES IN THE PHOTOSYNTHETIC APPARATUS

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Summary. The influence of Cd-stress on the structure and functional activity of the photosynthetic apparatus of 15-day-old barley plants cvs. Obzor and Hemus was investigated. Two concentrations, toxic for the growth of plants, namely 3 and 6 mg Cd/l 1/2 Knops' solution were used. The contact of plants with heavy metal lasted 12 days.
The following results were obtained: In Cd-treated plants was observed a tendency to decrease the photosynthetic rate. At the same time the changes in PS2 functional activity were insignificant, although a part of the chloroplasts were characterized by disturbed ultrastructural organization.
The data evaluating growth reaction, structural and functional changes in the photosynthetic apparatus suggest that at the initial stages of plant development, cv. Hemus is more susceptible to Cd-stress than cv. Obzor.

Key words: *Hordeum vulgare* L., cadmium stress, photosynthesis, chloroplast ultrastructure, chlorophyll fluorescence

Abbreviations: PA – photosyhthetic apparatus; PS1– photosystem 1; PS2 – photosystem 2; F_0 – initial chlorophyll fluorescence; F_v – variable chlorophyll fluorescence; F_m – maximal chlorophyll fluorescence; TEM – transmission electron microscopy; A – assimilation rate

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Introduction

The effect of cadmium on the photosynthetic process is a subject of intensive investigations. The attention of researchers is mainly focused on the possible sites of toxic cadmium effect – the pigment apparatus (Merakchiiska and Yordanov, 1983; Baszynski, 1986; Stiborova et al., 1986), electron transport (Singh and Singh, 1987; Becerril et al., 1988; Jian and Li, 1989; Greger and Ogren, 1991; Siedlieska and Baszynki, 1993) and photophosphorylation (Baszynski et al.,1980; Clijsters and van Assche, 1985; Weigel, 1985), chloroplast ultrastructure (Baszynski, 1986; Barcelo et al., 1988; Baszynski, 1989; Stoyanova and Chakalova, 1990), enzyme activities (Baszynski, 1986; Sheoran et al., 1990) etc.

Analysis of the integral photosynthetic response of *in vivo* treated plants is rather complex because of primary and secondary effects of the heavy metal and the existing system for self-regulation of the process.

There is an agreement among scientists about the effect of cadmium on chloroplast ultrastructure. In the experiments of Baszynski et al. (1980), Barcelo et al. (1988), Stoyanova and Chakalova (1990) it was established that cadmium, applied in toxic concentrations, disturbs the chloroplast envelope and the integrity of the membrane system and leads to increased plastoglobule number, changing the lipid composition and the ratios of the main structural components of thylakoid membranes. At the same time, no one-way data exist for the functional activity of the photosynthetic apparatus (PA) in plants, subjected to cadmium stress. Inhibition was mostly established, but in some cases stimulation of CO_2-fixation was also detected in plants treated *in vivo* with toxic cadmium concentrations (Baszynski et al., 1980; Merakchiiska and Yordanov, 1983; Sheoran et al., 1990; Greger and Ogren, 1991; Costa et al., 1994). The presence of stimulating effect on the integral photosynthetic process was explained by Merakchijska and Yordanov (1983) by the "concentrating cadmium effect" upon the PA, due to a stronger inhibition of plant growth as compared to that of the photosynthetic parameters in young bean plants. There are different opinions about the cadmium effect on membrane-bound photochemical reactions and their relative part in the integral process of inhibition. According to Baszynski et al. (1980), Becerril et al. (1988), Sheoran et al. (1990), cadmium, when applied *in vivo*, inhibits the electron transport of photosystem 2 (PS2) in tomatoes, clover, alfalfa and pea plants. In later studies of Siedlecka et al. (1993), evidence is given for electron transport inhibition of PS1, too, in 21-day-old maize plants. On the contrary, Greger et al. (1991) suggest that Cd does not affect the functional activity of either PS2 or PS1 and suppose that its inhibitory effect on CO_2-fixation is mainly connected with photophosphorylating and biochemical reactions.

The objective of this investigation was to study the effect of cadmium stress (evaluated by growth parameters, published earlier, Vassilev et al., 1993) on the photosynthetic rate in young barley plants and to try to analyze their photosynthetic

response on the basis of data about the chloroplast ultrastructure and the functional activity of PS2.

Material and Methods

Fifteen-day-old barley plants were grown as water cultures in a climatic room. The plants of two cultivars, Obzor and Hemus, were included in the experiments. The method and experimental conditions are described in detail elsewhere (Vassilev et al., 1993).

Samples for electron microscopy analyses were taken from the middle part of the first whole expanded leaf of control plants and plants subjected to moderate (3 mg $Cd.l^{-1}$) and strong (6 mg $Cd.l^{-1}$) cadmium stress. The material was fixed in the cold (4°C) in 3% glutar aldehyde and 1% potassium permanganate for two hours. Embedding of the fixed and dehydrated tissue in Durcupan and contrasting were made by lead citrate. The sections (700–900 Å) were made by ultramicrotome (Tesla BS 49OA). The observations and micrographs were performed on a transmission electron microscope (PEM-100, Ukraine).

By means of PAM-fluorimetry (Pulse Amplitude Modulation Fluorimeter, H. Walz, Germany) in similar parts of the same leaves, the parameters of fast chlorophyll fluorescence: F_0, F_v, F_v/F_0 and F_v/F_m, were determined at 25°C. The measurements were made on leaf discs (3 discs with diameters of 8 mm in each measurement) after 3 min dark adaptation. The intensity of actinic white light was 3000 µmol $m^{-2} s^{-1}$.

The rate of steady-state photosynthesis was determined by means of a closed gas-analytical system LI 6000 (Li-Cor, USA). For each measurement, intact leaves (about 10 cm^2) of 5–6 plants, put in a chamber at 800 µmol $m^{-2} s^{-1}$ (PAR) were used. The leaf temperature was 28–30°C and the CO_2 concentration in the system - ca. 400 ppm.

Cadmium content in the thylakoid membranes, isolated by the method of Whatly and Arnon (1963), was determined by ICP emission spectrophotometry (H. Walz, Germany).

Three independent experiments were conducted. The variants were analyzed in five replications at a base of 6 plants per pot. The results shown are mean values ± (SE). The significance of differences was determined by Student's criterion (Zaprjanov and Marinov, 1978).

Results and Discussion

Results presented in Table 1 show that the rate of photosynthesis in barley plants, subjected to moderate (3 mg. l^{-1}) cadmium stress for 12 days, is not significantly affected. A tendency to decreasing is observed at the action of a 6 mg $Cd.l^{-1}$ nutrient solution.

The tendency established for decrease in the photosynthetic rate was better expressed in plants of cv. Hemus, treated with 6 mg $Cd.l^{-1}$, which corresponded to its higher accumulation in plant organs (Vassilev et al., 1993). Our previous results (Vassilev et al., 1995) received by means of ^{14}C incorporation showed that at saturated CO_2 concentration, where the stomatal limitation of photosynthesis was avoid, the rate of this process in cv. Hemus was slightly stimulated.

This means that the degree of photosynthetic rate inhibition is lower than that established for plant growth (Vassilev et al., 1993).

Table 1. The effect of cadmium stress on the rate of photosynthesis in the first leaf of barley plants

Cultivar	Variant (mg $Cd.l^{-1}$)	A (mg $CO_2.m^{-2}.s^{-1}$)	%
Obzor	0	0.2235 ± 0.0192	100
	3	0.2064 ± 0.0246	92
	6	0.1955 ± 0.0227	87
Hemus	0	0.2366 ± 0.0935	100
	3	0.2376 ± 0.0136	100
	6	0.1888 ± 0.0128	80

These results are in accordance with the standpoint of Merakchiiska and Yordanov (1983) about the different sensitivity of growth and photosynthesis to cadmium stress at the initial stages of plant development. In our opinion, that may be a consequence of the fact that on one hand growth as physiological process is more integrated, with higher degree of regulation than photosynthesis, and on the other hand that the role of heterotrophic nutrition in young plants is significant.

The higher photosynthetic resistance of barley to the toxic effect of cadmium as compared to that in a number of other plant species, such as peas, alfalfa, tomatoes, etc. should be noted (Baszynski et al., 1980; Becerrel et al., 1988; Sheoran et al., 1990).

Suppression of photosynthetic rate was accompanied by structural and functional changes in PA of the treated plants.

The electron micrographs of chloroplasts of control barley plants, given in Fig. 1A,B were typical for the species. Their shape was elongated/elliptical. The internal membrane system, occupying the greatest part of their volume, was well developed and oriented along the longitudinal axis. The number of grana stacks were normal with no starch grains.

Under moderate cadmium stress (3 mg $Cd.l^{-1}$), changes occurred in the shape of leaf cells in the treated plants of cv. Obzor (Fig. 2A). Folding of cellular envelope was observed. This caused changes in the typical location of chloroplasts – fast by the cellular wall to make the diffusion path shorter. They were located in groups away from

A B

Fig.1. TEM micrographs of chloroplasts of the first leaf from control barley plants. A – cv. Obzor, ×15000; B – cv. Hemus, ×10000

the plasmalemma. Besides in the distribution, changes were established in the shape of the chloroplast ultrastructure, too (Fig. 2B). Increase in the short axis was observed. Grana were slightly disorganized, granal and stromal thylakoids were swollen. On the double-membrane envelope of some chloroplasts a small-grain deposition was established, which is probably related to the adsorption of the heavy metal. It could be mentioned that the negative cadmium effect was not equally expressed in all chloroplasts, this fact being reported by other authors, too (Barcelo et al., 1988). The disturbed organization of the chloroplast ultrastructure led to formation of structure-functional associations between the chloroplasts and mitochondria. Increase in the contact surface between them was observed (Fig. 2C).

The changes in the ultrastructural organization of chloroplasts in the plants of cv. Hemus, that had experienced a moderate cadmium stress, were similar but more significant. Here, the negative cadmium effect on the swelling of granal thylakoids was considerably more clearly expressed (Fig. 2D). The link between stromal thylakoids and grana was disturbed. Thinning and partial tearing of the chloroplast envelope was observed. A stage of significant damage in the mesophyll chloroplasts of the Cd-treated plants from cv. Hemus was present.

Strong cadmium stress led to still more significant changes in the shape of cells in cv. Obzor. The shape of chloroplasts was almost spherical, and the grana therein were distributed in concentric circles (Fig. 3B). A large part of the chloroplasts were with disturbed envelopes. Here, the cadmium effect on thylakoid swelling was more obvious than that at moderate stress.

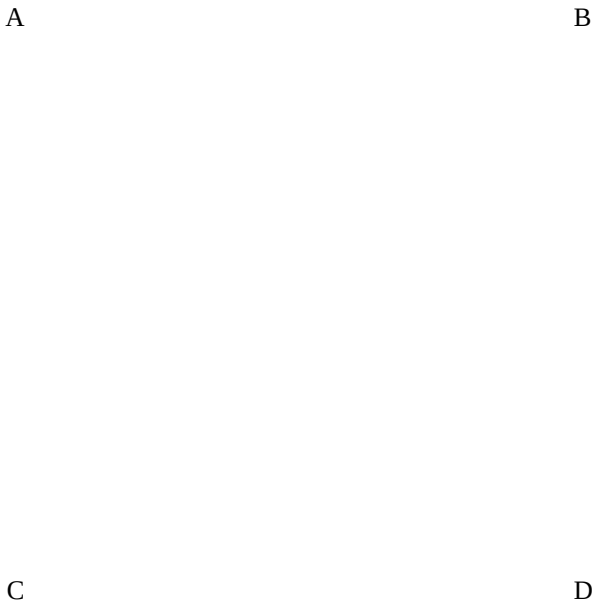

Fig. 2. TEM micrographs of chloroplasts of the first leaf from treated with 3 mg Cd. l^{-1} barley plants. A – cv. Obzor, ×2000; B – cv. Obzor, ×10000; C – cv. Obzor, ×8000; D – cv. Hemus, ×15000

In plants of cv. Hemus, treated with 6 mg $Cd.l^{-1}$, the swollen thylakoids were highly disorganized. There was a tendency for reduction in the number of grana and thylakoids therein. In the chloroplast stroma, vesicular structures were observed, which were probably related to stromal thylakoid lysis. The chloroplast envelope was broken and increased contact between damaged chloroplasts were present (Fig. 3D). Such a tendency was observed by Stoyanova et al. (1990), who interpreted it as a compensatory energetic mechanism. The observations corresponded to the data for

Fig.3. TEM micrographs of chloroplasts of thefirst leaf from treated with 6 mg $Cd.l^{-1}$ barley plants. A – cv. Obzor, ×10000; B – cv. Hemus, ×8000

the higher cadmium accumulation in the thylakoid membranes of cv. Hemus (2.107 ± 0.020 ppm) in comparison with cv. Obzor (1.622 ± 0.014 ppm).

The destructive changes established in chloroplast ultrastructure corresponded to the general idea about chloroplast response to cadmium stress, reported by a number of authors (Baszynski et al., 1980; Barcelo et al., 1988; Stoyanova and Chakalova, 1990). It is considered that the ultrastructural disorders observed are similar to those occurring at leaf ageing. This logical opinion, which we accept, is based on the data for increased ethylene production (Fuhrer, 1982) and activity of galactolipase (Skorsinska et al., 1991) in Cd-treated plants.

Absence of plastoglobules in the disordered chloroplasts in our experiments was probably connected with the use of potassium permanganate in post-fixation of the experimental material.

The photosynthetic apparatus structure disorders, resulting from strong cadmium stress are in correspondence with the established CO_2-fixation suppression (Table 1) and are a prerequisite for changes in the membrane-bound photochemical reactions.

Chlorophyll fluorescence is a sensitive indicator for the status of photochemical reactions. At physiological temperatures, fluorescence mainly originates from PS2 chlorophyll (Papageorgiou, 1975). That is why the chlorophyll fluorescence parameters could be an indicator of the status of photochemical processes in PS2, considered to be more sensitive to stress.

Functionally, ground (F_0) fluorescence differs from variable, F_v, fluorescence. F_0 does not depend on photochemical phenomena and is affected when structural changes occur in the pigment apparatus. F_0 fluorescence describes excitation energy

losses during its transfer from pigment bed to reaction centre. The parameters F_v/F_0, at saturated light, and F_v/F_m are indicators for the functional activity and quantum efficiency of PS2. Table 2 shows the effect of strong Cd^{2+} stress on the main chlorophyll fluorescence parameters in barley plants. It can be seen that after Cd-treatment the values of F_0 in cv. Hemus increase and those of the variable, F_v, decrease. This shows that Cd affects the structure of photosynthetic units and the electron transport, which corresponds to the established ultrastructural disorders in chloroplasts. The F_v/F_0 and F_v/F_m ratios decrease but noticeably in the Cd-treated variant of cv. Hemus. At the same time, this decrease in the photosynthetic quantum efficiency according to Schreiber and Bilger's opinion (1987) is insignificant. Thus, the values in this range do not significantly influence the photosynthetic rate and probably are not the reason for the observed photosynthesis decrease.

Our results are in accordance with those of Greger et al. (1991) and confirm their opinion. We also think that under the existing disorders in thylakoid membranes and chloroplasts as a whole, it is possible other metabolic units to be also affected besides the primary photochemical reactions.

Table 2. Effect of strong Cd stress (6 mg.l^{-1}) on the parameters of fast chlorophyll fluorescence in barley plants

Cultivar	Variant (mgCdl^{-1})	F_0	F_v	F_v/F_0	F_v/F_m
Obzor	0	773±19 (100)	3604±54 (100)	4.7±0.06 (100)	0.82±0.002 (100)
	6	808±26 (104)	3422±106 (94)	4.2±0.02 (89)	0.81±0.001 (98)
Hemus	0	785±27 (100)	3375±70 (100)	4.3±0.1 (100)	0.81±0.004 (100)
	6	905±11 (115)*	2927±143 (86)*	3.2±0.17 (74)**	0.76±0.001 (93)**

* $P < 0.05$ ** $P < 0.01$

Weigel's suggestion (1985) that after Cd treatment, photosynthesis is inhibited *in vivo* mainly at the level of dark reactions from Calvin's cycle, is also probable. Further information on the matter will be obtained from the analyses undertaken on quenching chlorophyll fluorescence and on tracing the ^{14}C path in photosynthetic products.

Conclusions

The results of our investigation substantiate the following conclusions:

The changes in functional activity of PS2 in Cd-treated barley plants are insignificant. They are within the limits of the norm, and obviously at this stage of plant development are not the reason for the established tendency of decrease in the photosynthetic rate.

The photosynthetic apparatus of barley is characterized by comparatively high tolerance to cadmium. The data evaluating the growth reaction, the structural and functional changes in the photosynthetic apparatus allow the suggestion that at the initial stages of plant development, cv. Hemus is more susceptible to cadmium stress than cv. Obzor.

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