SOME ASPECTS OF RUNNER BEAN PLANT RESPONSE TO CADMIUM AT DIFFERENT STAGES OF THE PRIMARY LEAF GROWTH

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ABSTRACT

Changes in morphology of primary leaves and ultrastructure of chloroplasts have been studied in runner bean plants (*Phaseolus coccineus* L., cv. Piękny Jaś) grown on Knop nutrient solution either without Cd or supplied with $2.5 \cdot 10^{-5}$ M Cd (in the form of $3\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$) in different stages of growth. The metal applied to the nutrient solution during the early stages of growth resulted in a reduction of the leaf area, chlorophyll accumulation increase and small decrease in PSII activity. Conversely to it Cd added at the end of primary leaf growth revealed almost unchanged leaf area but a significant decrease of chlorophyll level and PSII activity. Changes in the leaf morphology (large intercellular spaces in palisade as well as spongy mesophyll) and ultrastructure of chloroplasts (degradation of intergranal thylakoids and appearance of numerous plastoglobuli) indicating their disorganization were observed only in plants treated with Cd at the end of primary leaf growth. Morphological and ultrastructural disturbances related to the growth stages are discussed in relation to functional changes of the photosynthetic apparatus.

KEY WORDS: *Phaseolus coccineus*, cadmium, carotenoids, chlorophyll, chloroplast ultrastructure, leaf anatomy, leaf area.

INTRODUCTION

Inhibitory effect of Cd on light reactions of photosynthesis has been well documented in *in vitro* as well as *in vivo* experiments. PSII was found to be the primary site action of Cd for photosynthetic electron transport (Clijsters and van Assche 1985, Baszyński 1986 and references therein, Becerril et al. 1988). Release of fatty acids from thylakoid membranes and dissociation of polypeptides of oxygen evolving complex in parallel with a low PSII activity in leaves of plants cultured on a medium supplemented with Cd indicate a degradation mechanism of the metal action on the photosynthetic apparatus (Krupa and Baszyński 1989, Skórzyńska et al. 1991, Skórzyńska and Baszyński 1993). This is supported by enhanced activity of galactolipase in Cd-treated plants (Skórzyńska et al. 1991).

Significant changes in thylakoid membrane composition are usually correlated with ultrastructural disorders in the photosynthetic apparatus. Cd inducing premature senescence of leaves altered the chloroplast organization and photochemical functions of the thylakoid system (Baszyński et al. 1980, Lindsey and Lineberger 1981, Barceló et al. 1988, Skórzyńska and Baszyński 1993). Heavy damage of the photosynthetic apparatus in mature leaves requires an adequate Cd concentration and a long exposure to the metal (Rascio et al. 1993). Little information is also available concerning delayed

formation of thylakoids, decrease in biosynthesis of the membrane components and, in consequence, limited photochemical competition of developing chloroplasts in Cd-treated seedlings (Krupa et al. 1987, Ghoshroy and Nadakavukaren 1990).

Recently, we have shown a response of the photosynthetic apparatus activity to Cd, depending on the growth stage of primary leaves in which runner bean plants were treated with Cd (Skórzyńska and Baszyński 1994). Taking it into account the aim of the present paper was to study Cd-induced alterations of leaf morphology and chloroplast inner organization related to the stage of primary leaf growth in which the runner bean plants were exposed to Cd.

MATERIAL AND METHODS

Plant material

Runner bean plants (*Phaseolus coccineus* L., cv. Piękny Jaś) were germinated from seeds on wet filter paper in a thermostated darkened chamber (23°C, 95% relative humidity). From day 6 the plants were cultivated hydroponically in aerated Knop nutrient solution at 20°C and PPFD of $100 \mu\text{mol m}^{-2}\text{s}^{-1}$ at a day/night regime of 16/8 hours. Cadmium in the form of $3\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$ at a final concentration of $2.5 \cdot 10^{-5}$ M was added to the nutrient solution at different stages of primary leaves

Figs 1-6. Light micrographs of primary leaf section from control (Fig. 1, 3, 5) and Cd-treated (Figs 2, 4, 6) runner bean plants. Cd-treated plants were supplemented with Cd on day 0 (Fig. 2), 6 (Fig. 4) and 10 (Fig. 6) after their transfer to the nutrient solution and analysed 10 days later. These plants were compared with control (Figs 1, 3, 5) being of the same age, respectively.

growth *i.e.* immediately after seedlings transfer to the nutrient solution (0), and on day 6 and 10, respectively, after this transfer. The primary leaves of control and Cd-treated plants were harvested for analyses 10 days after the treatment.

Photochemical activity of chloroplasts

For measurements of PSII activity intact chloroplasts were isolated according to Muñoz-Rueda et al. (1986). Photosynthetic O_2 evolution ($H_2O \rightarrow FeCN_6$) was determined using the basic medium described by Maksymiec and Baszyński (1988) and a Hansatech DW2 oxygen electrode coupled to a Hansatech IF1/2 computer interface board and DRS1 software.

Estimation of chloroplast pigments

Chlorophyll and carotenoids were estimated in 80% acetone leaf extracts according to Lichtenthaler and Wellburn (1983).

Measurement of leaf area

The leaf areas were measured using GeniScan GS-4500 scanner (Genius, Taiwan) and dedicated area measuring computer software manufactured by Witra (Warsaw, Poland).

Microscopic studies

For microscopy studies the material was fixed two times. In both experiments three leaves of both control and Cd-treated plants were examined. The leaf tissue samples were cut from the same area of lamina and fixed with 3.5% glutaraldehyde in 0.5 M sodium cacodylate buffer, pH 6.9, for 12 h at room temperature. The specimens were postfixed in 2% OsO_4 , dehydrated in an acetone series and embedded in epoxy resin (Spurr 1969).

For light microscopy semithin 1.0-1.5 μm sections were cut and stained with 1% toluidine blue. The leaves thickness was measured on transverse sections, ignoring natural tissue contraction during the embedding proces. For electron microscopy, ultrathin 50-60 nm sections were obtained with the Reinhardt Ultratom operating with a glass or diamond knife and contrasted with uranyl acetate for 15 min and lead citrate for 15 min. Examinations were performed with JEM 100B transmission electron microscope.

RESULTS

Runner bean plants response to Cd depended on the leaf growth stages (Table 1). Seedlings treated with Cd immediately after their transfer to the nutrient solution showed after 10 days of growth a reduction of the primary leaf area to 39% of control. Cd applied in the later growth stages (on day 6 and 10 after transfer) showed a smaller effect on the leaf area size despite the same time of the metal action.

Some differences in the leaf anatomy were observed in semithin sections of control and Cd-treated plants. Leaves of Cd-treated plants were thinner than control, which was more distinct in younger individuals. Moreover, Cd treatment of plants during the early growth stage resulted in a smaller size of mesophyll cells and reduction of intercellular spaces. In older leaves treated with Cd on day 10 after plant transfer to the nutrient solution yellow and brown necrotic spots were observed. In such leaves the intercellular spaces in spongy as well as palisade mesophyll were extremely large (Fig. 6). The anatomical details were not statistically analysed, but the differences are distinct and reliable. In each individual case several leaves were compared.

Figs 7-12 show electron micrographs of chloroplasts from primary leaves of control and Cd-treated plants. The chloroplasts from control leaves were bigger and had a typical ultrastructure with grana stacks and a regular system of intergranal membranes (Figs 7, 9, 11). These control chloroplasts independently of their age (in the range examined) were similar though not identical. Differences in the degree of thylakoid stacking in grana and plastoglobuli accumulation were seen. For example, in younger plants chloroplast grana with 7-10 thylakoids were found (Fig. 7). In older plants grana were more developed, they consisted of 20-30 thylakoids and typical intergranal membrane connections. Sporadic and small plastoglobuli visible in younger leaves (Fig. 7) were bigger and more numerous in older ones (Fig. 11).

The chloroplasts of Cd-treated plants were smaller and poorly differentiated in their ultrastructure. The number of intergranal thylakoids was lower and the arrangement of the membrane was loose (Fig. 8, 10). In chloroplasts, (Fig. 10 mag 45000x), fragments of disintegrated intergranal lamellae were observed. The chloroplasts of plants treated with the metal at the end of leaf growth stage were characterized by partial degeneration of intergranal thylakoids and more numerous plastoglobuli (Fig. 12).

This ultrastructural differentiation was related to the level of chloroplast pigments (Table 1). Leaves of older plants showed a considerably smaller chlorophyll accumulation and a significant decrease of PSII activity than control. However, the leaf area reduction in plants treated with Cd during the early stage of growth was accompanied by a high level of the pigment (which became higher the bigger the leaf area reduction was) and slight decrease in photochemical activity. A higher level of carotenoids in Cd-treated plants than in control was seen in all growth stages studied.

TABLE 1. Characteristics of primary leaves of runner bean plants treated with Cd in relation to the growth stage in which the element was applied to the nutrient solution. The plants were analyzed 10 days after the metal application. The values are expressed in % referred to the control plants = 100%. Plastid pigments were calculated on the leaf area basis and then expressed in %. The values represent the mean $\pm$ SE (n=7). Percentage of control in parenthesis.

Parameter	Control	Cd-treated		
		0*	6*	10*
leaf area	100	39 $\pm$ 3	66 $\pm$ 2	85 $\pm$ 4
chlorophyll a + b	100	135 $\pm$ 2	117 $\pm$ 4	76 $\pm$ 5
carotenoids	100	136 $\pm$ 2	114 $\pm$ 5	81 $\pm$ 4
photosynthetic O_2 evolution ($H_2O \rightarrow FeCN_6$)	100	84 $\pm$ 9	82 $\pm$ 2	63 $\pm$ 7

*day of plant growth in the nutrient solution after which Cd was applied

DISCUSSION

Low activity of the photosynthetic electron transport chain induced by Cd treatment of plants commonly known for a long time has suggested Cd-induced alterations in the structure of the photosynthetic apparatus. A few papers dealing with this problem have informed about disruption of the chloroplast envelope, reduction in the number and size of grana, ab-

Figs 7-12. Comparison of chloroplast organization in primary leaves of runner bean plants untreated (Fig. 7, 9, 11) and treated with Cd (Figs 8, 10, 12) at different stages of growth. Mag. x 26000 except Fig 10, x 45000. Explanations as in Figs 1-6.

normal stacking and intrathylakoidal spacing as well as increase in the number and size of plastoglobuli (Baszyński et al. 1980, Lindsey and Lineberger 1981, Balceró et al. 1988, Stroiński et al. 1990, Skórzyńska and Baszyński 1993). These observations were made on different plant species treated with Cd concentrations required to reveal symptoms of toxic and long-term action of the metal on plants. However, in the mentioned papers plant growth stages were not taken into consideration.

The presented results show that primary leaf of plants treated with Cd during the early stage of their development did not result in any ultrastructural disorders of chloroplasts. The leaf area reduction in this growth stage indicating Cd-affected leaf expansion was the only visible symptom of the metal action (Table 1). A high chlorophyll level and a small decrease of PSII activity, resulting probably from a high pigment accumulation, and lack of changes in the inner chloroplast structure account for a considerable resistance of plants to Cd in this growth stage. Limited increase in the leaf area in the early growth stage mentioned elsewhere (Balceró et al. 1988, Sheoran et al. 1991) and simultaneously increasing chlorophyll accumulation may suggest a lower sensitivity of the pigment to Cd than the processes of leaf growth.

A high accumulation of carotenoids in all growth stages of primary leaves examined supports the opinion about a higher resistance of these pigments than chlorophyll to Cd (Tukendorf and Baszyński 1991). It also confirms the view about the increase of carotenogenesis observed in *Dunaliella salina* grown in stress conditions (Visviki and Rachlin 1994).

Leaf area reduction became smaller and smaller when the metal was added to the nutrient solution the more leaf growth was advanced. In the final growth stage of leaves Cd did effect significantly their area, however, it evoked senescence symptoms. This is demonstrated by chlorophyll level decrease, alterations in leaf morphology and the ultrastructure of chloroplasts, particularly by the increase of the number and size of plastoglobuli, indicating disorganization of thylakoid membranes. These disorders account for a low PSII activity. The changes in the thylakoid membrane composition caused by degradation of acyl lipids, release of both fatty acid and polypeptides of oxygen evolving complex, which were found earlier in older mature leaves exposed to Cd (Baszyński et al. 1980, Baszyński and Krupa 1989, Skórzyńska et al. 1991), are in agreement with the changes in the organization of the photosynthetic apparatus presented above. This higher sensitivity of older leaves to Cd seems to be reasonable in the light of the recent studies of Rascio et al. (1993). Studying the chloroplast architecture of Cd-treated maize leaves in which along the main axis tissues are of different age, the authors found ultrastructural alteration indicating a higher sensitivity of mature leaf tissues to toxic effect of Cd.

Summing up, it should be stressed that changes of the leaf morphology and ultrastructure of chloroplasts and a low photochemical activity as a response to long-term action of Cd are related to the stages of leaf growth at which the plants were exposed to Cd. These results supplement our earlier data concerning the photochemical activity of chloroplasts in Cd-treated plants as a function of leaf growth stages (Skórzyńska and Baszyński 1994). Such a response of the photosynthetic apparatus to Cd may apply to other heavy metals because similar relationships between the structure and activity of chloroplasts were also found in plants treated with excess Cu at different stages of their growth (Maksymiec et al. 1994).

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NIEKTÓRE ASPEKTY ODPOWIEDZI FASOLI WIELOKWIATOWEJ NA DZIAŁANIE KADMU W RÓŻNYCH FAZACH WZROSTU PIERWSZEGO LIŚCIA

STRESZCZENIE

Badano zmiany morfologii pierwszego liścia i ultrastruktury chloroplastów fasoli wielokwiatowej (*Phaseolus coccineus* L., cv. Piękny Jaś) rosnącej na pożywce Knopa, do której dodawano $2.5 \cdot 10^{-5}$ M Cd (w formie $3\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$) w różnych fazach wzrostu i analizowano po 10 dniach działania metalu. Cd dodany do pożywki we wczesnej fazie wzrostu powodował redukcję powierzchni liścia, znaczną akumulację chlorofilu oraz niewielkie obniżenie aktywności PSII. Przeciwnie, Cd dodany do pożywki w końcowej fazie wzrostu nie powodował istotnych zmian w powierzchni liścia, natomiast znacznie obniżał poziom chlorofilu i aktywności PSII. Znaczniejsze zmiany morfologii liścia (duże przestrzenie międzykomórkowe w miękiszu palisadowym i gąbczastym) i ultrastruktury chloroplastu (degradacja tylakoidów intergranowych i pojawienie się licznych plastoglobul), wskazujące na ich dezorganizację obserwowano jedynie u roślin traktowanych Cd w końcowej fazie wzrostu liścia. Zakłócenia morfologiczne i ultrastrukturalne liścia zależne od fazy wzrostu dyskutowano w aspekcie funkcjonalnych zmian aparatu fotosyntetycznego.

SŁOWA KLUCZOWE: *Phaseolus coccineus*, anatomia liścia, chlorofil, kadm, karotenoidy, powierzchnia liścia, ultrastruktura chloroplastu.