PHOTOSYNTHETICA 45 (2): 239-244, 2007

Effects of copper and cadmium on photosynthesis in cucumber cotyledons

M. BURZYŃSKI and A. ŻUREK

Plant Physiology Department, Institute of Plant Biology, Wrocław University, Kanonia 6/8, 50-328, Wrocław, Poland

Abstract

The effects of 20 and 50 µM concentrations of Cu and Cd on photosynthesis in cucumber (*Cucumis sativus* L.) cotyledons were studied by the measurements of gas exchange characteristics, chlorophyll (Chl) fluorescence parameters, photosynthetic pigment contents, and two Calvin cycle enzymes activities: glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and 3-phosphoglyceric acid kinase (PGK). To minimize indirect metal action, seedlings were treated with metals in the stage of green, fully developed cotyledons. The metals reached the cotyledon tissue after 48 h of treatments, though symptoms of metal action were not visible at that time. The effect of metals on the light phase of the photosynthesis parameters such as potential efficiency of photosystem 2 (PS2; F_v/F_m), and photochemical and non-photochemical quenching of Chl fluorescence (q_P and q_{NP}) was negligible. In contrast, a decrease of PS2 quantum efficiency (Φ_{PS2}) was much more noticeable. Changes in the pigment contents were slight, as only 50 µM Cd decreased Chl *a* and *b* contents in small extent. On the contrary, metals in both concentrations drastically decreased (50 and more % of control) the net photosynthetic rate and the stomatal conductance, but not the internal CO_2 concentration. The activities of both GAPDH and PGK were also decreased by metals, although the effect on PGK was more prominent, particularly on its potential activity (dithiothreitol in extraction and incubation media). Hence Cu and Cd affected the synthesis of enzyme proteins rather than they influenced their modifications. The effects of both metals on most of the measured photosynthesis parameters were similar, but the accumulation of Cd in the cotyledons was significantly higher than Cu accumulation. Thus Cu was more toxic for the photosynthesis of cucumber cotyledons than Cd.

Additional key words: chlorophyll content and fluorescence; dithiothreitol; gas exchange; glyceraldehyde-3-phosphate dehydrogenase; 3-phosphoglyceric acid kinase; stomatal conductance.

Introduction

Photosynthetic organisms are highly sensitive to heavy metals. The effect of metal ions on higher plants includes disruption of many physiological functions such as water relations, respiration, mineral nutrient uptake, assimilation as well as photosynthesis. Copper in distinction to cadmium is an essential plant microelement. Beside other functions, Cu is also involved in the photosynthetic electron transport, being a component of plastocyanin, and it is also important in regulation of the function of PS2 (Barón *et al.* 1995). In excess, Cu can be frequently more toxic than cadmium.

Cd and Cu in toxic concentrations have multidirectional effects on photosynthesis (for reviews see Krupa and Baszyński 1995, Prasad and Strzałka 1999, and references therein). These metals may act on the different stages of the photosynthetic electron transport and also affect enzymes of the dark phase of photosynthesis. Cd also diminished the number of oxygen evolving complexes (Janeczko *et al.* 2005). A long-lasting action of Cd or Cu may have indirect character on the photosynthesis. The heavy metals disturb water uptake and water relations in plant tissue (Poschenrieder and Barcelo 1999) and in this way they decrease the growth of leaves and generally whole plant expansion. After the disruption of electron transport, they induce the synthesis of oxygen reactive forms and in consequence oxidative stress (Dietz *et al.* 1999). Heavy metals disturb the uptake and the distribution of essential nutrients including uptake and transport of Fe to leaves (Siedlecka and Krupa 1999, Krupa *et al.* 2002), which is necessary for chlorophyll (Chl) synthesis,

---

Received 14 August 2006, *accepted* 27 November 2006.

Fax: 0713754118, e-mail: burzynsm@biol.uni.wroc.pl

Abbreviations: C_i – internal CO_2 concentration; Chl – chlorophyll; DM – dry mass; DTT – dithiothreitol; F_0 – minimal level of chlorophyll fluorescence; F_m, F_m' – maximum fluorescence of dark- and light-adapted leaves; F_v, F_v' – variable fluorescence of dark- and light-adapted state, respectively; F_v/F_m – potential efficiency of PS2; FM – fresh mass; g_s – stomatal conductance; GAPDH – glyceraldehyde-3-phosphate dehydrogenase; P_N – net photosynthetic rate; PGA – phosphoglyceric acid; PGK – 3-phosphoglyceric acid kinase; PPFD – photosynthetic photon flux density; PS2 – photosystem 2; q_P – photochemical quenching; q_{NP} – non-photochemical quenching of chlorophyll fluorescence; TPI – triosephosphate isomerase; Φ_{PS2} – light-adapted PS2 quantum efficiency.

and for normal function of all the processes connected with CO_2 assimilation. In consequence, a chronic contact of plants with Cd or Cu leads to the reduction of total photosynthetic area, degradations of Chl contents, and to premature senescence leading often to plant death. Changes in plants metabolism and photosynthetic parameters, after the heavy metal treatment, depend on the sort of metal pollution, duration of the metal stress, and the plant species.

To minimize the indirect effects of Cu and Cd on photosynthesis, we chose fully green and developed cotyledons for plant material. In young seedlings the transport of Cu and Cd through the roots to the photosynthetic organ (cotyledons) is faster. In addition, requirements of cotyledons for nutrients are relatively small and heavy metals arrest the uptake of essential elements, which hardly affects the results. Furthermore, CO_2 assimilation in this first photosynthetic organ is very important as it enables the continuation of growth and development of plants.

Materials and methods

Cucumber (*Cucumis sativus* L.) seeds germinated in darkness at 25 ºC were transferred to the modified Hoagland solution (four seedlings per 1 000 cm^3) containing [mM]: 5 $Ca(NO_3)_2$, 5 KNO_3, 1 KH_2PO_4, 1 $MgSO_4$; [µM]: 75 Fe-citrate, 10 $MnSO_4$, 5 H_3BO_3, 1 $CuSO_4$, 0.01 $ZnSO_4$, 0.05 Na_2MoO_4. Nutrients' pH was 5.5. On the 6th day (fully green and developed cotyledons), Cu or Cd were added to the nutrient solution (20 or 50 µM) in the form of chlorides, and after a 48-h treatment cotyledons were used for analyses. All seedlings were grown under a 16-h photoperiod, at the photosynthetic photon flux density (PPFD) of 180 µmol m^{-2} s^{-1}, at 25 ºC during the day and 22 ºC during the night.

Chl fluorescence was measured with a pulse amplitude modulated system (model *FMS2*, *Hansatech Instruments*, UK). Prior to the measurements, the cotyledons were dark-adapted for 30 min in order to maximize the oxidation of the primary quinone electron acceptor pool of photosystem 2 (PS2). Minimum Chl fluorescence (F_0) was measured using a 594 nm amber modulating beam, whereas maximum fluorescence (F_m) was induced by a 1-s pulse of saturating "white light" (10 800 µmol m^{-2} s^{-2}). Variable fluorescence (F_v) was calculated from the difference between F_m and F_0. The potential Chl fluorescence was calculated as F_v/F_m. The actual quantum efficiency of PS2 [$\Phi_{PS2} = (F_m' - F_s)/F_m'$] was determined after adaptation of the cotyledons to an actinic "white light" (500 µmol m^{-2} s^{-2}), when steady state of the fluorescence yield (F_s) was reached. In order to achieve F_m', a saturating pulse (10 800 µmol m^{-2} s^{-2}) was given. The photochemical and non-photochemical quenching (q_P and q_{NP}) was calculated using equations: $q_P = (F_m' - F_s)/F_m' - F_0)$, and $q_{NP} = (F_m - F_m')/(F_m - F_0)$.

P_N, stomatal conductance (g_s), and intercellular CO_2 concentration (C_i) in the cotyledons were measured by the infrared gas analyzer (*Ciras-1*, *PP System*, Hitchin, Herts, UK) with a leaf chamber (*PLC5*) controlling automatically the measurement conditions: irradiance of 300 µmol m^{-2} s^{-2}, 350 cm^3 m^{-3} CO_2 in air, and leaf temperature of 25 ºC.

The cotyledons used for the gas exchange and Chl fluorescence measurements were subsequently removed from plants and used for the determination of pigments and enzyme activities. In another part of the cotyledons, fresh mass (FM), dry mass (DM), and metal accumulation were measured. The amounts of Chl and carotenoids were determined in 80 % acetone solution according to the procedures of Arnon (1949) and Lichtenthaler and Wellburn (1983).

The activities of 3-phosphoglyceric acid kinase (PGK) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were determined according to Hatch and Kagawa (1973) and Leegood and Walker (1981), respectively. The cotyledons were removed and frozen in liquid N_2 and maintained at –70 ºC. 1-g portions of the cotyledon sections were pre-cooled with liquid nitrogen, ground in a mortar, and subsequently pestle-minced. Next, 6 cm^3 of the extraction buffer was added to the mixture. The medium contained 100 mM Hepes-NaOH (pH 8.0), 10 mM $MgCl_2$, 0.4 mM EDTA, 1 % polyvinylpyrrolidone, 100 mM sodium ascorbate, and 0.1 % bovine serum albumin. 50 mM dithiothreitol (DTT) was added into the extraction media when potential activity was measured. A homogenate was centrifuged 10 min at 15 000×*g* and the supernatant was subsequently used for the enzyme activity measurements (0.1 cm per 1.5 cm^3 of incubation mixture). The enzyme activities were measured spectrophotometrically with coupled reactions monitoring NADH or NADPH oxidation at 340 nm. Reaction mixture for GAPDH measurement contained: 31 mM Hepes-KOH (pH 8.0), 10 mM $MgCl_2$, 1 mM KH_2PO_4, 1 mM NaF, 4 mM PGA, 5 mM DTT (in case of the total activity measurement; when initial activity was measured, DTT was omitted), 4 units per cm^3 TPI, and 20 units per cm^3 PGK. Reactions were started by the addition of 5 mM ATP and 0.12 mM NADPH. Reaction mixture for PGK measurements contained: Hepes-KOH (pH 7.8), $MgCl_2$, NaF, KH_2PO_4, PGA, DTT (total activity measurement only), and TPI, all in the before mentioned concentrations and 4 units per cm^3 GAPDH. Reactions were initiated by the addition of 2 mM ATP and 0.1 mM NADH.

For the measurement of metal concentration, the cotyledons were digested with $HClO_4/HNO_3$ mixture in the microwave system after drying at 70 ºC. The contents of Cu, Cd, and Fe were determined by the atomic absorption spectrophotometer (*Perkin-Elmer 3300*).

Results

After 48 h of metal treatment no visible symptoms of metal toxicity were observed. However, a decrease in fresh (FM) and dry (DM) masses was detected (Fig. 1), nevertheless, the differences were statistically significant only for 50 µM Cu concentration.

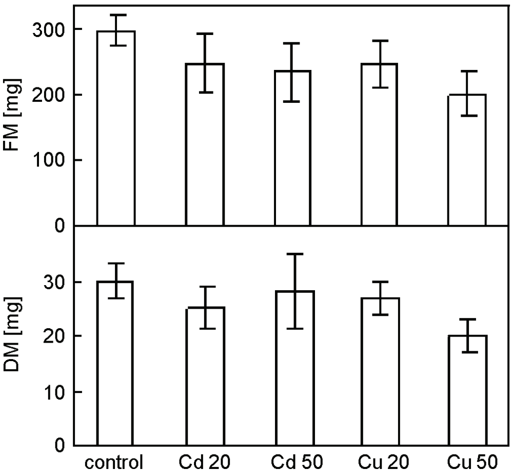

Fig. 1. Fresh (FM) and dry (DM) masses of one cotyledon after seedling treatment with Cu or Cd (20 and 50 µM) for 48 h.

Accumulation of Cd in the cotyledons was nearly 3 times higher for 20 µM and 1.5 times higher for 50 µM than the accumulation of Cu. After the addition of Cd or Cu no changes were observed in Fe content in cotyledon tissues (Table 1). Only Cd in the concentration of 50 µM lowered the contents of Chl *a* and *b* by 15 and 25 %, respectively, in comparison to control. The contents of carotenoids in the cotyledons were similar to those observed in control in all the treatments (Table 1).

Metals did not change the potential efficiency of PS2 (F_v/F_m). Light-adapted PS2 quantum efficiency (Φ_{PS2}) was diminished only after metal treatment in 50 µM concentrations. In particular, the difference was noticeable after Cu action (decrease by 24 %) (Table 2). The utilization of the excitation energy to drive the photosynthetic electron transport (q_P) was similar as in control, with exception of the seedlings treated with 50 µM Cu where it was decreased to 88 %. In this combination the non-photochemical quenching (q_{NP}) showed that the heat dissipation of excitation energy was slightly higher than in the control (Table 2).

Both metal concentrations used drastically decreased the net photosynthetic rate (P_N). 20 µM Cu and Cd diminished P_N to 55 % and 50 µM to 27 % of the control value (Fig. 2*A*). After treatment with both metals, a decrease of the stomatal conductance (g_s) to 43 % (20 µM Cd and Cu) and to 23 % (50 µM Cd and Cu) was detected (Fig. 2*B*). Changes in P_N and g_s did not affect the internal CO_2 concentrations (C_i) in cotyledon tissues (Fig. 2*C*).

Table 1. Contents of Cu, Cd, Fe, and photosynthetic pigments in the cotyledons of seedlings treated for 48 h with Cu or Cd. SE were calculated for twelve replications for three independent experiments. *Different letters* represent significant differences ($p<0.05$) using one-way ANOVA

Nutrient solution [µM]	Cotyledons Cu [$mol\ kg^{-1}(DM)$]	Cd	Fe	Chl *a* [$g\ kg^{-1}(FM)$]	Chl *b*	Carotenoids
Control	0.18±0.03		5.9±0.8 a	5.90±0.3 a	2.25±0.1 a	1.05±0.05 a
Cd 20	0.12±0.04	2.2±0.10	5.3±0.7 a	5.72±0.2 a	2.20±0.2 a	1.10±0.06 a
50	0.11±0.03	2.7±0.12	5.7±0.5 a	5.08±0.3 b	1.70±0.2 a	1.10±0.05 a
Cu 20	0.75±0.05		5.8±0.7 a	5.90±0.4 a	2.25±0.2 a	1.00±0.07 a
50	1.80±0.07		5.3±0.9 a	5.90±0.3 a	2.25±0.3 a	1.05±0.05 a

Table 2. Photosynthetic fluorescence parameters in the cotyledons of seedlings treated with 20 or 50 µM Cd or Cu for 48 h. The parameter was measured in 10 cotyledons for each combination. Experiment was repeated three times. Means±SE. *Different letters* represent significant differences ($p<0.05$) using one-way ANOVA.

Metal content [µM]	F_v/F_m	[%]	Φ_{PS2}	[%]	q_P	[%]	q_{NP}	[%]
Control	0.830±0.019 a	100	0.550±0.020 a	100	0.725±0.021 a	100	0.425±0.022 a	100
Cd 20	0.821±0.015 a	99	0.512±0.015 a	93	0.706±0.025 a	97	0.412±0.020 a	97
50	0.817±0.014 a	98	0.480±0.017 b	87	0.700±0.031 a	96	0.445±0.019 a	105
Cu 20	0.831±0.009 a	100	0.520±0.018 a	94	0.723±0.028 a	99	0.427±0.021 a	100
50	0.814±0.010 a	98	0.419±0.015 c	76	0.641±0.019 b	88	0.490±0.019 b	115

In the control cotyledons both the PGK and NADPH-dependent GAPDH activities were about three times higher when DTT was added to the extraction and incubation media (potential activity), compared to the activity without DTT (actual activity) (Fig. 3). The metals decreased activities of both enzymes, yet the effect was more prominent in the case of PGK, especially as concerns its potential activity. The potential activity of PGK was decreased by Cd and Cu to 50–30 % in both concentrations. The actual activity was suppressed only by the higher metal concentrations (50 µM) (Fig. 3*A*). In contrast to PGK, the actual activity of GADPH was more decreased (50 % of the control in 50 µM Cd and Cu concentrations). Statistically significant decrease of the potential activity of this enzyme was detected only for 50 µM Cu (Fig. 3*B*).

Discussion

Heavy metals such as Cu or Cd are toxic for plant metabolism, including photosynthesis. Some authors observed negative Cu and Cd action on different stages of the light phase of photosynthesis (electron transport) (Maksymiec *et al.* 1994, Maksymiec and Baszyński 1996, Myśliwa-Kurdziel *et al.* 2002, and references therein). Other authors claim that in the first place heavy metals attack Calvin-cycle enzymes, localized in chloroplast stroma (Weigel 1985, Sheoran *et al.* 1990). On the other hand, heavy metals can also act indirectly on the photosynthetic processes. Toxic Cu or Cd concentrations in plant tissues usually induce oxidative stress, disturb metabolism of water and minerals or normal Chl synthesis (Dietz *et al.* 1999, Krupa *et al.* 2002, Myśliwa-Kurdziel and Strzałka 2002). General perturbation of plant metabolism after metal treatment is the main reason of inhibition of leaf and root expansion and diminishing of yields. In order to minimize indirect metal influence on the photosynthesis process, we chose fully developed and green cotyledons for plant material. During 48 h of metal treatments both metals reached cotyledon tissues, though almost no changes were observed in Fe accumulation and in contents of photosynthetic pigments. Slight decrease in Chl *a* and *b* contents was detected only after Cd treatment when 50 µM concentration was used. Cd accumulation in the cotyledons was higher than that of Cu. The influence of metals on DM and FM of cotyledons was rather mild. Statistically significant decrease in DM and FM was observed only after the 50 µM Cu treatment. In the present experiments, light phase of photosynthesis was less sensitive to Cu and Cd actions than the dark one. Similarly as in our earlier study (Burzyński and Kłobus 2004), where cucumber leaves were used for experiments, also in the present work no changes were

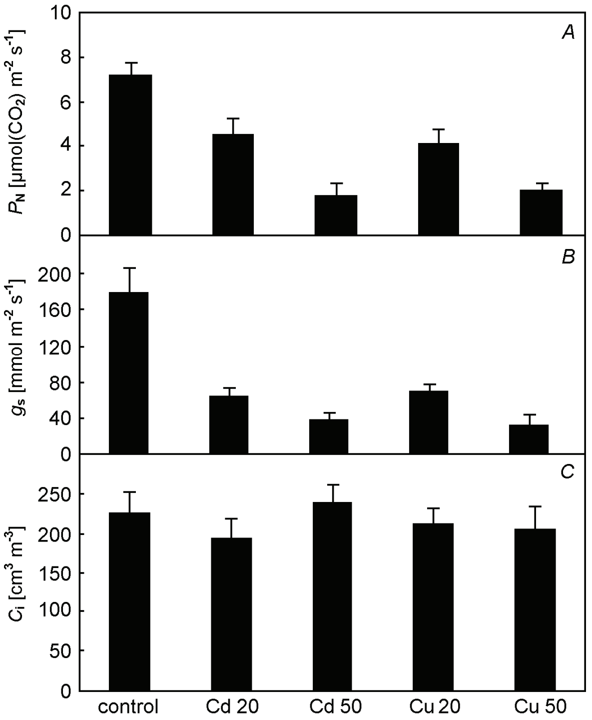

Fig. 2. Net photosynthetic rate, P_N (*A*), stomatal conductance, g_S (*B*), and internal CO$_2$ concentration, C_i (*C*) in cotyledons of cucumber seedlings treated with Cu or Cd (20 and 50 µM) for 48 h. Measured in 10 cotyledons for each combination, experiment was repeated three times. Means±SE.

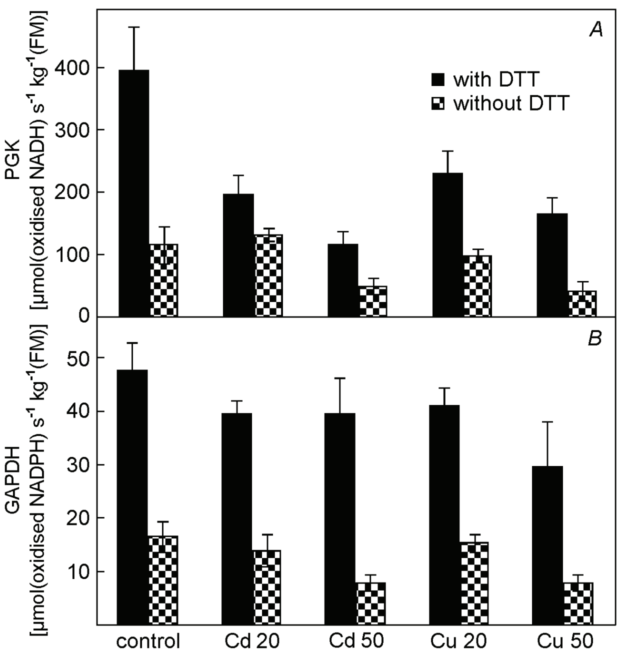

Fig. 3. Potential (DTT added to extraction and incubation media) and actual (without DTT) 3-phosphoglyceric acid kinase, PGK (*A*) and glyceraldehyde-3-phosphate dehydrogenase, GAPDH (*B*) activities in cotyledons of seedlings treated for 48 h with Cd or Cu (20 or 50 µM). Means of 10 replicates, four independent experiments.

observed in the potential efficiency of PS2 after metal treatment. The very small influence of heavy metals on F_v/F_m was also observed by Ciscato *et al.* (1997) in *Triticum durum* and by Sgardelis *et al.* (1994) in *Sonchus* spp. and *Taraxacum* spp. Slight decrease was observed for q_P and moderate increase was documented for q_{NP}, which showed level of the heat dissipation of excitation energy only after 50 µM Cu treatment. Changes in these fluorescence parameters in plants growing under heavy metal stress were usually more drastic (Skórzyńska-Polit and Baszyński 1997, Krupa and Moniak 1998) which might indicate that in our experiment Cu and Cd did not interrupt significantly the photosynthetic electron transport in cucumber cotyledons.

The only fluorescence parameter that was noticeably diminished by Cu and Cd was Φ_{PS2}. The reduction of this fluorescence parameter might be explained by a low utilization of ATP and NADPH in the dark phase of the photosynthesis (Kitao *et al.* 1997, Subrahmanyam and Rathore 2000). Despite the fact that the influence on fluorescence parameters was negligible, drastic inhibition of P_N and g_s was observed after the metal treatments. Presence of 50 µM Cu [1.8 mmol kg^{-1}(DM)] and 50 µM Cd [2.7 mmol kg^{-1}(DM)] in solutions decreased P_N by 75 % and 20 µM concentrations [Cu 0.75 and Cd 2.20 mmol kg^{-1}(DM)] decreased P_N almost by 50 %. Lower g_s in the cotyledons treated with metals did not influence P_N as C_i remained unchanged, and was high. The independence of P_N on g_s was suggested by Sheoran *et al.* (1990). Decrease of the assimilation of CO_2 by Cu and Cd can be caused by the influence of these metals on the activities of photosynthetic carbon reduction cycle enzymes. Therefore, PGK and GAPDH, enzymes necessary in the reduction phase of Calvin-cycle, were tested. Both enzymes were inhibited by Cu and Cd to some extent. Metals additionally decreased the potential activity of PGK, which suggests that they can influence not only the activity of the enzyme but also the synthesis of this protein. The negative effect of Cd on PGK was observed by Sheoran *et al.* (1990) in the pigeon pea leaves, whereas both metals affected this enzyme in the experiments of Stiborová *et al.* (1986) on maize leaves. Sheoran *et al.* (1990) additionally showed that Cd also decreases the activity of GAPDH.

The examination of the Cu and Cd action on the photosynthesis processes, after relatively short periods of time, allowed vastly exclude their indirect action (*e.g.* deficiency of Fe, a decrease in the Chl contents, and general cell metabolism destruction).

Our results, the present and the previous (Burzyński and Kłobus 2004), suggest that Cu and Cd initially destroy the photosynthetic carbon reduction cycle and subsequently influence the photosynthetic electron transport.

References

Arnon, D.I.: Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. – Plant Physiol. **24**: 1-15, 1949.

Barón, M., Arellano, J.B., López Gorgé, J.: Copper and photosystem II: A controversial relationship. – Physiol. Plant. **94**: 174-180, 1995.

Burzyński, M., Kłobus, G.: Changes of photosynthetic parameters in cucumber leaves under Cu, Cd, and Pb stress. – Photosynthetica **42**: 505-510, 2004.

Ciscato, M., Valcke, R., Van Loven, K., Clijsters, H., Navari-Izzo, F.: Effect of in vivo copper treatment on the photosynthetic apparatus of two *Triticum durum* cultivars with different stress sensitivity. – Physiol. Plant. **100**: 901-908, 1997.

Dietz, K.J., Baier, M., Krämer, U.: Free radicals and reactive oxygen species as mediators of heavy metal toxicity. – In: Prasad, M.N.V., Hagemayer, J. (ed.): Heavy Metal Stress in Plants. From Molecules to Ecosystems. Pp. 73-97. Springer-Verlag, Berlin – Heidelberg – New York 1999.

Hatch, M.D., Kagawa, T.: Enzymes and functional capacities of mesophyll chloroplasts from plants with C_4-pathway photosynthesis. – Arch. Biochem. Biophys. **159**: 842-853, 1973.

Janeczko, A., Kościelniak, J., Pilipowicz, M., Szarek-Łukaszewska, G., Skoczowski, A.: Protection of winter rape photosystem 2 by 24-*epi*brassinolide under cadmium stress. – Photosynthetica **43**: 293-298, 2005.

Kitao, M., Lei, T.T., Koike, T.: Effects of manganase toxicity on photosynthesis of white birch (*Betula platyphylla* var. *japonica*) seedlings. – Physiol. Plant. **101**: 249-256, 1997.

Krupa, Z., Baszyński, T.: Some aspects of heavy metals toxicity towards photosynthetic apparatus – direct and indirect effects on light and dark reaction. – Acta Physiol. Plant. **17**: 177-190, 1995.

Krupa, Z., Moniak, M.: The stage of leaf maturity implicates the response of the photosynthetic apparatus to cadmium toxicity. – Plant Sci. **138**: 149-156, 1998.

Krupa, Z., Siedlecka, A., Skórzynska-Polit, E., Maksymiec, W.: Heavy metal influence on the light phase of photosynthesis. – In: Prasad, M.N.V., Strzałka, K. (ed.): Physiology and Biochemistry of Metal Toxicity and Tolerance in Plants. Pp. 287-303. Kluwer Academic Publishers, Dordrecht 2002.

Leegood, R.C., Walker, D.A.: Photosynthetic induction in wheat protoplasts and chloroplasts. Autocatalysis and light activation of enzymes. – Plant Cell Environ. **4**: 59-66, 1981.

Lichtenthaler, H.K., Wellburn, A.R.: Determinations of total carotenoids and chlorophylls *a* and *b* of leaf extracts in different solvents. – Biochem. Soc. Trans. **603**: 591-592, 1983

Maksymiec, W., Baszyński, T.: Different susceptibility of runner bean plants to excess copper as a function of the growth stages of primary leaves. – J. Plant Physiol. **149**: 217-221, 1996.

Maksymiec, W., Russa, R., Urbanik-Sypniewska, T., Baszyński, T.: Effect of excess Cu on the photosynthetic apparatus of runner bean leaves treated at two different growth stages. – Physiol. Plant. **91**: 715-721, 1994.

Myśliwa-Kurdziel, B., Prasad, M.N.V., Strzałka, K.: Heavy metal influence on the light phase of photosynthesis. – In: Prasad, M.N.V., Strzałka, K. (ed.): Physiology and Biochemistry of Metal Toxicity and Tolerance in Plants. Pp. 229-257. Kluwer Academic Publ., Dordrecht 2002.

Myśliwa-Kurdziel, B., Strzałka, K.: Influence of metals on the biosynthesis of photosynthetic pigments. – In: Prasad, M.N.V., Strzałka, K. (ed.): Physiology and Biochemistry of Metal Toxicity and Tolerance in Plants. Pp. 201-229. Kluwer Academic Publ., Dordrecht 2002.

Poschenrieder, C., Barcelo, J.: Water relation in heavy metals stressed plants. – In: Prasad, M.N.V., Hagemayer, J. (ed.): Heavy Metal Stress in Plants. From Molecules to Ecosystems. Pp. 207-231. Springer-Verlag, Berlin – Heidelberg – New York 1999.

Prasad, M.N.V., Strzałka, K.: Impact of heavy metals on photosynthesis. – In: Prasad, M.N.V., Hagemayer, J. (ed.): Heavy Metal Stress in Plants. From Molecules to Ecosystems. Pp. 117-139. Springer-Verlag, Berlin – Heidelberg – New York 1999.

Sgardelis, S., Cook, C.M., Pantis, J.D., Lanaras, T.: Comparison of chlorophyll fluorescence and some heavy metal concentrations in *Sonchus* spp. and *Taraxacum* spp. along an urban pollution gradient. – Sci. total Environ. **158**: 157-164. 1994.

Sheoran, I.S., Singhal, H.R., Singh, R.: Effect of cadmium and nickel on photosynthesis and the enzymes of the photosynthetic carbon reduction cycle in pigeonpea (*Cajanus cajan* L.). – Photosynth. Res. **23**: 345-351, 1990.

Siedlecka, A., Krupa, Z.: Cd/Fe interaction in higher plants – its consequences for the photosynthetic apparatus. – Photosynthetica **36**: 321-331, 1999.

Skórzyńska-Polit, E., Baszyński, T.: Differences in sensitivity of photosynthetic apparatus in Cd-stressed runner bean plants in relation to their age. – Plant Sci. **128**: 11-21. 1997.

Stiborová, M., Doubravová, M., Leblová, S.: A comparative study of the effect of heavy metal ions on ribulose-1,5-bisphosphate carboxylase and phosphoenolpyruvate carboxylase. – Biochem. Physiol. Pflanz. **181**: 373-379, 1986.

Subrahmanyam, D., Rathore, V.S.: Influence of manganese toxicity on photosynthesis in ricebean (*Vigna umbellata)* seedlings. – Photosynthetica **38**: 449-453, 2000.

Weigel, H.J.: The effect of Cd^{2+} on photosynthetic reactions of mesophyll protoplasts. – Physiol. Plant. **63**: 192-200, 1985.

Cassells, A.C., Gahan, P.B.: **Dictionary of Plant Tissue Culture.** – Food Products Press, an Imprint of the Haworth Press, New York – London – Oxford 2006. ISBN 1-56022-919-3 (soft). 565 pp., USD 29.95.

This book is a very practical guide to the basic technical terms of plant cell, tissue, and organ culture. The guide is organized as an alphabetical list of terms. The dictionary entries are well selected and cover all fields of plant cell tissue and organ culture. The book contains 29 figures and schemes of key concepts on 256 pages. The extensive list of references covers recent reviews, textbooks, and research papers that can be good introduction to the topic of plant tissue culture. It is also an excellent tool for good orientation in the plant tissue culture systems.

The reasons of guide publication are defined by the authors, distinguished scientists in the field, in the preface: "Similar to other fields of scientific research, plant tissue culture has developed its own technical terms. The value of a dictionary such as this lies in providing definition of these terms, thereby helping the reader understand the literature of the field. It is anticipated that this dictionary will find its readership among teachers, researchers, and undergraduate and postgraduate students in basic and applied plant tissue culture."

This dictionary covers the field of plant tissue cultures comprehensively. It provides clear, concise, and broad overview in plant tissue cultures. Most entries include literature citations. The citations point mainly to general textbooks in the background areas of plant anatomy, biochemistry, developmental biology, genetics, microbiology, micropropagation, plant breeding, plant biotechnology, plant pathology, and plant tissue culture. The key concepts are illustrated by clear and well arranged figures. They can be used as teaching material as well as other clear explanation of the basic terms in plant biotechnology.

I recommend this dictionary as an excellent handy book for all new practitioners of plant biotechnology. It can be used by students, teachers, and researchers. It is essential for anyone using tissue culture for plant breeding, plant pathology, or genetic manipulations.

Z. VONDRÁKOVÁ (*Praha*)