BIOLOGIA PLANTARUM 41 (4): 601-606, 1998

BRIEF COMMUNICATION

Influence of Cd^{2+} on growth, chlorophyll content, and water relations in young barley plants

A. VASSILEV*, M. BEROVA and Z. ZLATEV

Department of Plant Physiology, Agricultural University, BG-4000 Plovdiv, Bulgaria

Abstract

Barley (*Hordeum vulgare* L., cv. Hemus) plants were grown in nutrient solution with or without 54 µM Cd^{2+} for 12 d. A treatment with Cd^{2+} inhibited the growth of young barley plants. The main factor limiting plant growth was net assimilation rate, due to decreased photosynthetic rate and accelerated dark respiration rate. One of the reasons for the reduced photosynthetic rate was the lower chlorophyll and carotenoid content. Cd^{2+} decreased water potential and transpiration rate, but relative water content in leaves of the treated plants was not significantly changed.

Additional key words: chlorophyll, growth analysis, *Hordeum vulgare*, transpiration rate, water potential.

The inhibitory effect of Cd^{2+} on plant growth is known for a long time, but the physiological nature of its negative effect is not fully understood. High internal Cd^{2+} concentrations in plants disturbed mineral nutrition (Siedlecka 1995), water relations (Barceló and Poschenrieder 1990), photosynthesis (Krupa and Baszyński 1995), enzyme activity (Van Assche and Clijsters 1990) and other processes.

A growth analysis is an usefull method for evaluation of the main factors limiting plant growth. In Cd^{2+}-treated plants this analysis was rarely made. Greger *et al.* (1991) and Landberg and Greger (1994) established that Cd^{2+} inhibited relative growth rate (RGR) in sugar beet plants. Abo-Kassem *et al.* (1995) reported that the

---

Received 12 July 1998, *accepted* 7 October 1998.

Abbreviations: E - transpiration rate; g_s - stomatal conductance; LAR - leaf area ratio; LMR - leaf mass ratio; NAR - net assimilation rate; P_N - net photosynthetic rate; R_D - dark respiration rate; RGR - relative growth rate; RMR - root mass ratio; RWC - relative water content; SLA - specific leaf area; SMR - stem mass ratio; Ψ_w - water potential

Acknowledgements: We thank the specialists Ms. M. Necheva and Ms. A. Trifonova for their techical assistance during the experiments.

* Corresponding author; fax: (+359) 32 265920; e-mail: vassilev@au-plovdiv.bg

toxic effect of Cd^{2+} on RGR of wheat plants is due to net assimilation rate (NAR) retardation rather than to leaf area ratio (LAR) inhibition.

Plastid pigments have offen been shown as one of the main sites of the toxic Cd^{2+} action. It was established that Cd^{2+} decreased chlorophyll content in many plant species, *e.g.*, tomatoes (Baszyński *et al.* 1980), wheat and cucumbers (Buczek 1984, Malik *et al.* 1992), maize (Stiborová *et al.* 1986, El-Enany 1995), beans (Barceló *et al.* 1988, Siedlecka and Krupa 1996). The reduced chlorophyll content in Cd^{2+}-treated plants are due both to inhibition of its biosynthesis (Stobart *et al.* 1985) and the activation of its enzymatic degradation (Somashekaraiah 1992). Lang *et al.* (1995) related the decreased chlorophyll content to the Cd^{2+}-induced iron deficiency in plants.

The negative effects of Cd^{2+} on water uptake, water movement and transpiration was observed (Barceló *et al.* 1986, Poschenrieder *et al.* 1989, Costa *et al.* 1994, Leita *et al.* 1995, Marchiol *et al.* 1996). The adaptation of Cd^{2+}-treated plants to disturbed water relations can be associated with osmotic adjustment as Kamenova-Yuchimenko *et al.* (1995) and Chen and Kao (1995) confirmed the accumulation of free proline in Cd^{2+}-treated plants. Alia and Saradhi (1991) established that Cd^{2+} was a strong inducer of proline accumulation, but without finding any relationship between this effect and osmotic adjustment. Shah and Dubey (1997/98) suggested that proline played role of enzyme protectant. The present report describes the main factors limiting plant growth in Cd^{2+}-treated young barley plants.

Seeds of barley (*Hordeum vulgare* L. cv. Hemus) were germinated for 2 d on a wet filter paper at 25 °C. The seedlings were transferred to a 1/2 strength Knop nutrient solution, enriched with microelements according to Hoagland, and with or without 54 μM Cd^{2+} in the form of $3\ CdSO_4 . 8\ H_2O$. Plants were grown for 12 d at photon flux density of 120 μmol $m^{-2}\ s^{-1}$ (PAR), day/night temperature 22 ± 2/18 ± 2 °C, and a 14-h photoperiod. The nutrient solution was aerated automatically and changed weekly. Dry mass of 15-d-old plants was determinated after drying of plant material at 105 °C to constant mass. The leaf area was measured using an electronic area meter *NEO-2* (*TU*, Sofia, Bulgaria). Growth analyses were made according to Beadle (1993). Chlorophyll and total carotenoid contents in leaves were estimated in 80 % acetone extracts according to Lichtenthaler and Wellburn 91983). The water potential (Ψ_w) in shoots was measured with a pressure chamber *EL540-305* (*ELE-International Ltd.*, Hertfordshire, England). The relative water content (RWC) in leaves was determined according to Morgan (1986). Transpiration rate (E) and stomatal conductance (g_s) were measured by *LI-6000* (*LI-Cor*, Lincoln, USA) at leaf temperature 26 - 28 °C, CO_2 concentration 400 μmol mol^{-1}, and photon flux density 800 μmol $m^{-2}\ s^{-1}$. For each measurement the first fully developed intact leaves of 5 plants were put in the leaf chamber giving a total leaf area of 10 cm^2.

The free amino acids were extracted from leaves in 80 % ethanol, the extract was passed through the ion-exchange resin *Dowex® 50X2-200* and eluated using 1 M NH_4OH. The content of free proline was determined using an automatical amino analyser *AAA-881* (*Mikrotechna*, Praha, Czechoslovakia). Cd and Fe content in plant organs was determined by inductively coupled plasma-atomic emission spectrophotometry (ICP - AES) using *Spectroflame* (*Spectro*, Kleve, Germany) after dry mineralization of samples. Three independent experiments were carried out. All parameters were measured in five replications. Significant differences were determined by the Student's *t*-test.

The Cd^{2+}-treated young barley plants were distinguished by their inhibited growth and the presence of obvious phytotoxic symptoms. An unspecific spot chlorosis, turning to yellowing and complete dying of leaf tips was observed. There was some browning of the root system and reduction in the number of primary root branches. These phytotoxicity symptoms correspond to those established by Arduini *et al.* (1944) and Vazquez *et al.* (1989) in other plant species subjected to heavy metal treatments.

Table 1. Cd^{2+} concentrations in roots and shoots and growth parameters of young barley plants, subjected to Cd^{2+} treatment (54 μM Cd^{2+} for 12 d). Means of 3 separate experiments ± SE ($n = 15$) and percentage of control. * - $P < 0.05$; *** - $P < 0.001$.

Parameters	Control plants	Cd^{2+}-treated plants	[%]
Cd^{2+} [mg kg^{-1}(d.m.)] in roots	0.15 ± 0.05	433.70 ± 16.5***	
Cd^{2+} [mg kg^{-1}(d.m.)] in shoots	0.08 ± 0.02	188.10 ± 8.40***	
Shoot height [cm]	19.15 ± 0.85	13.35 ± 0.42***	70
Root length [cm]	7.35 ± 0.41	4.40 ± 0.19***	60
Dry mass [mg per plant]	44.01 ± 1.70	30.68 ± 1.14***	70
Leaf area [cm^2]	15.60 ± 0.90	10.00 ± 0.70***	64
RGR [g kg^{-1} d^{-1}]	34.00 ± 0.20	5.10 ± 0.10***	15
NAR [g m^{-2} d^{-1}]	0.96 ± 0.01	0.15 ± 0.00***	16
LAR [m^2 kg^{-1}(plant)]	35.40 ± 1.40	32.60 ± 1.20	92
LMR [kg(leaf) kg^{-1}(plant)]	0.57 ± 0.01	0.59 ± 0.01	103
SMR [kg(stem) kg^{-1}(plant)]	0.26 ± 0.01	0.26 ± 0.01	101
RMR [kg (root) kg^{-1}(plant)]	0.17 ± 0.01	0.15 ± 0.01***	88
SLA [m^2 kg^{-1}(leaf)]	62.40 ± 2.50	55.60 ± 1.90*	89
Root/shoot mass	0.21 ± 0.01	0.18 ± 0.01*	86

The height of Cd^{2+}-treated plants and the length of their longest primary roots were inhibited by 30 and 40 %, respectively. A 30 % decrease in the dry mass accumulated and 36 % in the leaf area developed were established as compared to the respective control values. The Cd^{2+} concentrations in the leaves of control plants were within the background limits, while those in the treated plants were high, reaching 434 mg kg^{-1}(d.m.) in the roots. The heavy metal concentrations in roots were about 2 times higher than those in the shoots, which is an evidence for the barrier function of roots against Cd^{2+} uptake.

RGR in the Cd^{2+}-treated plants drastically decreased by about 85 %. RGR is determined by two indices: LAR and NAR. NAR was inhibited by 84 %, showing that the changes in that component were most important in RGR inhibition. The results obtained by us are in line with those of Abo-Kassem *et al.* (1995) concerning Cd-treated wheat plants. NAR depends on P_N, R_D and the relative ratio of non-photosynthesizing plant organs. This was in agreement with our earlier investigations showing that Cd^{2+} accelerated R_D and decreased P_N (Vassilev *et al.* 1994, 1995). We have already shown that Cd^{2+} causes disorders in chloroplast ultrastructure and functioning (Vassilev *et al.* 1997). Non-photosynthesizing organs in young barley plants are roots, whose relative mass ratio (RMR) decreased. Hence, NAR inhibition was due mainly to changes in P_N and R_D. Although to a lesser extent, the RGR inhibition was also related to changes of LAR. In the Cd^{2+}-treated plants, slight tendency to a LAR decrease of about 8 % was observed. LAR is determined by two components - leaf mass ratio (LMR) and specific leaf area (SLA). No LMR differences existed between the control and the Cd^{2+}-stressed plants, while SLA decreased by 11 %. The SLA decrease resulted from the stronger inhibition of leaf area formation as compared to dry mass accumulation (Table 1).

Table 2. Influence of Cd^{2+} on chlorophyll (Chl) *a* and *b*, carotenoid (Car), Fe, and proline contents, water potential (Ψ_w), relative water content (RWC), transpiration rate (E), and stomatal conductance (g_s) in young barley plants. Means of 3 separate experiments ± SE ($n = 15$); * - $P < 0.05$; ** - $P < 0.01$; *** - $P < 0.001$.

Parameter	Control plants	Cd^{2+}- treated plants	[%] of control
Chl *a* [mg g^{-1}(d.m.)]	7.895 ± 0.381	6.274 ± 0.395*	80
Chl *b* [mg g^{-1}(d.m.)]	4.428 ± 0.150	3.643 ± 0.178**	82
Car [mg g^{-1}(d.m.)]	3.085 ± 0.162	2.475 ± 0.317	80
Chl *a/b*	1.782 ± 0.090	1.722 ± 0.120	94
Chl *a+b*/Car	3.994 ± 0.135	4.001 ± 0.210	100
Fe [mg kg^{-1}(d.m.)]	77.40 ± 6.00	62.00 ± 5.70	80
proline [nmol g^{-1}(f.m.)]	108.60 ± 8.70	272.10 ± 14.2***	251
Ψ_w [kPa]	-280.0 ± 20	-630.0 ± 70***	225
E [mmol(H_2O) m^{-2} s^{-1}]	1.98 ± 0.09	1.73 ± 0.07*	87
g_s [mol m^{-2} s^{-1}]	0.123 ± 0.01	0.080 ± 0.01**	65
RWC [%]	93.61 ± 0.02	91.14 ± 0.46*	97

Cd^{2+} decreased the plastid pigment content by about 20 %. No considerable changes in their interrelations occurred. The negative effect of Cd^{2+}, might be due to the reduced concentrations of iron. We established that the Fe concentration in the Cd^{2+}-treated plants decreased by about 20 %, which did not lead to the development of Fe deficiency. According to Benton *et al.* (1991) the critical value of Fe in young barley leaves is 25 mg kg^{-1}(d.m.) Thus the reduced plastid pigment content was probably due to accelerated ageing of the photosynthetic apparatus in response to Cd^{2+} stress (Skorzyńska *et al.* 1991).

Water potential (Ψ_w) of leaves decreased by about 450 kPa, indicating that the Cd^{2+}-treated plants were in a state of mild water stress (Pil'shchikova 1993). This was confirmed by relatively small changes in RWC. The proline content increased by 151 %, but it is known that under strong water stress the proline accumulation can reach significantly higher values (Paleg and Aspinall 1981).

Transpiration rate (E) in the Cd-treated plants was inhibited by 13 %, and g_s was decreased by 35 %. The Cd^{2+} effect on the transpiration resulted from decreased root/shoot ratio (Table 1), leading to hampered water supply of shoots, and to the negative Cd^{2+} effects on the stomata functioning. According to Barceló and Poschenrieder (1990) Cd^{2+} may induce closing of stomata. The Cd^{2+} effects on water regime of barley plants were in agreement with the results obtained by Poschenrieder *et al.* (1989), Barceló *et al.* (1986), Costa *et al.* (1994) and Marchiol *et al.* (1996) for other plant species under Cd stress conditions.

Our results may be summarized as follows: 54 μM Cd^{2+} inhibited the growth of young barley plants. The main factor limiting plant growth was NAR inhibition, due to decreased P_N and increased R_D. One of the reasons for the reduced photosynthetic rate was the lower plastid pigment content. The Cd^{2+} treatment decreased both Ψ_w and E, but RWC in the treated plant leaves was not strongly changed.

References

Abo-Kassem, E., Sharaf-El-Din, A., Rozema, J., Foda, E.A.: Synergistic effects of cadmium and NaCl on the growth, photosynthesis and ion content in wheat plants. - Biol. Plant. **37**: 241-249, 1995.

Alia, Saradhi, P.: Proline accumulation under heavy metal stress. - J. Plant Physiol. **138**: 554-558, 1991.

Arduini, I., Godbold, D., Onnis, A.: Cadmium and copper change root growth and morphology of *Pinus pinea* and *Pinus piaster* seedlings. - Physiol. Plant. **92**: 675-680, 1994.

Barceló, J., Poschenrieder, C.: Plant water relations as affected by heavy metal stress: a review. - J. Plant Nutr. **13**: 1-37, 1990.

Barceló, J., Poschenrieder, C., Andreu, I., Gunse, B.: Cadmium-induced decrease of water stress resistance in bush bean plants (*Phaseolus vulgaris* L. cv. Contender). I. Effects of Cd on water potential, relative water content and cell wall elasticity. - J. Plant Physiol. **125**: 17-25, 1986.

Barceló, J., Vazquez, M., Poschenrieder, C.: Structural and ultrastructural disorders in cadmium-treated bush bean plants (*Phaseolus vulgaris* L.). - New Phytol. **108**: 37-49, 1988.

Baszynski, T., Wajda, L., Krol, M., Wolinska, D., Krupa, Z., Tukendorf, A.: Photosynthetic activities of cadmium-treated tomato plants. - Physiol. Plant. **48**: 365-370, 1980.

Beadle, C.: Growth analysis. - In: Hall, D., Scurlock, J., Bolhàr-Nordenkampf, H., Leegood, R., Long, S. (ed.): Photosynthesis and Production in a Changing Environment. A Field and Laboratory Manual. Pp. 36-45. Chapman & Hall, London 1993.

Benton, J., Wolf, J., Mills, H.: Plant Analysis Handbook. - Micro-Macro Publishing, New York 1991.

Buszek, J.: Effect of cadmium on chlorophyll content and dry matter yield in wheat and cucumber plants supplied either with nitrate or ammonium. - Acta Univ. wratislaviensis **31**: 11-21, 1984.

Chen, S., Kao, C.: Cd induced changes in proline level and peroxidase activity in roots of rice seedlings. - Plant Growth Regul. **17**: 67-71, 1995.

Costa, G., Michaut, J., Morel, J.: Influence of cadmium on water relations and gas exchange, in phosphorus deficient *Lupinus albus* L. - Plant Physiol. Biochem. **32**: 105-114, 1994.

El-Enany, A.: Alleviation of cadmium toxicity on maize seedlings by calcium. - Biol. Plant. **37**: 93-99, 1995.

Greger, M., Brammer, E., Lindberg, S., Larsson, G., Idestam-Almquist, J.: Uptake and physiological effects of cadmium in sugar beet (*Beta vulgaris*) related to mineral provision. - J. exp. Bot. **42**: 729-737, 1991.

Kamenova-Yuchimenko, S., Georgieva, G., Georgieva, N., Balabanova, M.: [Effect of Polystimulin-K on resistance of two pea cultivar to high cadmium concentrations.] - Bulg. J. Plant Sci. **32**: 48-50, 1995. [In Bulg.]

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

Landberg, T., Greger, M.: Influence of selenium on uptake and toxicity of copper and cadmium in pea (*Pisum sativum*) and wheat (*Triticum aestivum*). - Physiol. Plant. **90**: 637-644, 1994.

Lang, F., Sarvari, E., Szigeti, Z., Fodor, F., Cseh, E.: Effects of heavy metals on the photosynthetic apparatus in cucumber. - In: Mathis, P. (ed.): Photosynthesis: from Light to Biosphere. Vol. IV. Pp. 533-536. Kluwer Academic Publishers, Dordrecht - Boston - London 1995.

Leita, L., Marchiol, L., Martin, M., Peressotti, A., Delle Vedove, G., Zerbi, G.: Transpiration dynamics in cadmium-treated soybean (*Glycine max* L.) plants. - J. Agron. Crop Sci. **175**: 153-156, 1995.

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

Malik, D., Sheoran, I. S., Singh, R.: Carbon metabolism in leaves of cadmium treated wheat seedlings - Plant Physiol. Biochem. **30**: 223-229, 1992.

Marchiol, L., Martin, M., Peressoti, A., Zerbi, G.: Physiological responses of two soybean cultivars to cadmium - J. Environ. Qual. **25**: 562-566, 1996.

Morgan, J.A.: The effect of N nutrition on the water relations and gas exchange characteristics of wheat (*Triticum aestivum* L.). - Plant Physiol. **80**: 52-58, 1986.

Paleg, L., Aspinall, D.: The Physiology and Biochemistry of Drought Resistance in Plants. - Academic Press, Sydney 1981.

Pil'shchikova, N.B.: Vodnyĭ Obmen Sel'skokhozyaĭstvennykh Kul'tur. [Water Regime of Crop Plants.] - MSKhA, Moskva 1993. [In Russ.]

Poschenrieder, C., Gunse, B., Barceló, J.: Influence of cadmium on water relations, stomatal resistance, and abscisic acid content in expanding bean leaves. - Plant Physiol. **90**: 1365-1371, 1989.

Shah, K., Dubey, R.S.: Effect of cadmium on proline accumulation and ribonuclease activity in rice seedlings: role of proline as a possible enzyme protectant. - Biol. Plant. **40**: 121-130, 1997/98.

Siedlecka, A.: Some aspects of interactions between heavy metals and plant mineral nutrients. - Acta Soc. Bot. Pol. **64**: 265-272, 1995.

Siedlecka, A., Krupa, Z.: Interaction between cadmium and iron and its effects on photosynthetic capacity of primary leaves of *Phaseolus vulgaris*. - Plant Physiol. Biochem. **34**: 833 - 841, 1996.

Skórzyńska, E., Urbanik-Sypniewska, T., Russa, R., Baszyński, T.: Galactolipase activity of chloroplasts in cadmium-treated runner bean plants. - J. Plant. Physiol. **138**: 454-459, 1991.

Somashekaraiah, B., Padmaja, K., Prasad, A.: Phytotoxicity of cadmium ions on germinating seedlings of mung bean (*Phaseolus vulgaris*): Involvement of lipid peroxides in chlorophyll degradation. - Physiol. Plant. **85**: 85-89, 1992.

Stiborová, M., Hromádková, R., Léblová, S.: Effect of ions of heavy metals on the photosynthetic characteristics of maize (*Zea mays* L.). - Biol. Plant. **41**: 1221-1228, 1986.

Stobart, A., Griffiths, W., Ameen-Bukhari, I., Sherwood, R.: The effect of Cd^{2+} on the biosynthesis of chlorophyll in leaves of barley. - Physiol. Plant. **63**: 293-298, 1985.

Van Assche, F., Clijsters, H.: Effect of metals on enzyme activity in plants. - Plant Cell Environ. **13**: 195-206, 1990.

Vassilev, A., Yordanov, I., Tsonev, T.: Effects of Cd^{2+} on the physiological state and photosynthetic activity of youn barley plants. - Photosynthetica **34**: 293-302, 1997.

Vassilev, A., Yordanov, I., Chakalova, E., Kerin, V.: Effect of cadmium stress on growth and photosynthesis of young barley (*H. vulgare* L.) plants. 2. Structural and functional changes in photosynthetic apparatus. - Bulg. J. Plant Physiol. **21**: 12-21, 1995.

Vassilev, A., Zlatev, Z., Kerin, V.: [Physiological response of two barley cultivars (*H. vulgare* L.) to cadmium toxicity.] - Agr. Univ. Plovdiv sci. Works **39**: 295-300, 1994. [In Bulg.]

Vazquez, M., Poschenrieder, C., Barceló, J.: Pulvinus structure and leaf abscission in cadmium-treated bean plants (*Phaseolus vulgaris*). - Can. J. Bot. **67**: 2756-2764, 1989.