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Are calcium ions and calcium channels involved in the mechanisms of Cu^{2+} toxicity in bean plants? The influence of leaf age

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Abstract

The influence of calcium channel blockers and ionophore on Cu^{2+}-induced changes of the photosynthetic activity of runner bean plants (*Phaseolus coccineus* L.) was investigated. Excess Cu^{2+} was applied to leaves by injection or *via* the roots to examine a short/local or a long time/systemic effect of this metal, respectively. The changes in fluorescence parameters indicated that the mechanism of toxic action of Cu^{2+} ions on the photosynthetic apparatus was only partially connected with Ca^{2+} or Ca^{2+} channels. In young plants Ca^{2+} diminished especially photochemical and nonphotochemical dissipative processes induced by short- and long-term influence of excess Cu^{2+}. Blocking of Ca^{2+} channels did not change direct Cu^{2+} action on the photosynthetic activity, however, their opening distinctly intensified the inhibitory effect of the metal. After a longer accumulation period the effect of Cu^{2+} ions did not change significantly due to modified Ca^{2+} penetration through membranes (except that caused by La^{3+}). Copper directly introduced into older leaves diminished only at its highest concentration the activity both of the donor and acceptor sides of photosystem 2 (PS2) connected with Rfd decrease and increase of LNU. A similar effect was observed also after a long-term Cu^{2+} action, but disturbances on the acceptor side of PS2 were observed only at a higher Ca^{2+} content in the nutrient solution. Ca^{2+} ions, particularly after openning of channels, intensified direct inhibitory Cu^{2+} action on the photosynthetic activity expressed by decreased values

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Abbreviations: Chl - chlorophyll; F_m - maximal chlorophyll fluorescence in dark-acclimated leaves; F_0', F_m' - minimal and maximal chlorophyll fluorescence under steady-state irradiation, respectively; F_0 - minimal chlorophyll fluorescence when all dark-treated PS2 reaction centres are open; F_v - variable fluorescence (F_m - F_0); F_v/F_m - maximal photochemical yield of PS2 in dark-acclimated leaves; LNU - light energy not used for photochemistry; OEC - oxygen evolving complex; PPFD - photosynthetic photon flux density; PS - photosystem; Q_A - primary quinone electron acceptor of PS2; q_P - photochemical quenching of fluorescence; Rfd - chlorophyll fluorescence decrease ratio (vitality index); Ver - verapamil.

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of F_v/F_0 and Rfd. Lanthanum and verapamil, at a lower Ca^{2+} content in the medium, decreased the photosynthetic activity of Cu^{2+}-treated plants. This effect was also seen after additional Ca^{2+} supply to the leaves.

Additional key words: chlorophyll fluorescence; copper; lanthanum; *Phaseolus coccineus*; photosystem 2; senescence; toxicity; verapamil.

Introduction

Excess Cu^{2+} induced morphological disturbances in plants and also modified their biochemical processes (see Maksymiec 1997). This stress factor can specifically influence cellular processes through a signalling way connected with a strong increase of jasmonic acid synthesis (Rakwal *et al.* 1996), induce cytoskeletal modification (Fagotti *et al.* 1996), or affect primarily growth (Cook *et al.* 1997). An alternative way of receiving and transferring stress information are changes of Ca^{2+} ion concentrations and permeability of their channels (Ward *et al.* 1995, Webb *et al.* 1996). As yet no results are available which could explain to what extent Ca^{2+} participates in the mechanism of toxic action of Cu^{2+} ions. The recent studies *in vivo* have shown that excess Cu^{2+} can change Ca^{2+} content in plants and, on the other hand, Ca^{2+} can modify the range of Cu^{2+} influence (Ouzounidou 1994, Ouzounidou *et al.* 1995, Maksymiec and Baszyński 1998b), confirming the possibility of Cu-Ca interaction in higher plants. The main places of Cu^{2+} action are, among other things, membranes (Sandmann and Böger 1980, De Vos *et al.* 1991, Strange and Macnair 1991) and the photosynthetic apparatus (see Droppa and Horváth 1990, Maksymiec 1997). Leakage of ionic solutes observed on Cu^{2+} action (De Vos *et al.* 1989, Jensén and Adalsteinsson 1989) indicates that disturbances in membrane integrity take place. On the other hand, Ca^{2+} ions regulate the activity of photosynthetic complexes (Ghanotakis and Yocum 1990, Krieger and Weis 1993, Gilmore 1997) and stabilize the cell membranes. The investigations into Ca^{2+} depletion of PS2 complex showed inhibition of both the donor and acceptor sides of this photosystem (Tso *et al.* 1991). Previously (Maksymiec *et al.* 1994) we showed that excess Cu^{2+} in young and especially older plants partially released OEC proteins. In this case even local disturbances in Ca^{2+} concentration can modify the photosynthetic activity. More recently we (Maksymiec and Baszyński 1998a) showed that diminished content of Ca^{2+} in the nutrient solution can decrease some photochemical processes in younger plants. However, in older plants inhibition of the photosynthetic apparatus, connected with increased senescence processes and disturbances in water content (Maksymiec and Baszyński 1998b), was seen after Ca^{2+} addition. Abdel-Basset (1998) found that during drought stress the functioning of Ca^{2+} channels was significantly affected. The Ca^{2+} effect on elongation and senescence processes and photosynthetic activity is dependent on its cytosolic concentration, which is governed by the activity of channels in the plasma membranes.

It was tempting to assume fluctuations of Ca^{2+} concentrations are involved in mechanisms of Cu^{2+} toxic action, and to find whether modification of Ca^{2+} channels can influence a range of Cu^{2+}-stress actions observed in the photosynthetic apparatus.

Are these channels the way of Cu^{2+} influx into cells? Information about this phenomenon is lacking. Experiments made under a short (shorter than 1 h) and long (over 10 d) influence of excess Cu^{2+} should show whether the occurring changes are characterized by local and possibly reversible disturbances and whether they are changes of a wide range permanently modifying the metabolism of cells. We applied the chlorophyll (Chl) fluorescence measurements which serve as rapid indicator of stress in plants (Lichtenthaler *et al.* 1986, Lichtenthaler and Miehe 1997).

Materials and methods

Plants: Runner bean plants (*Phaseolus coccineus* L. cv. Piękny Jaś) after 5 d of germination were cultivated hydroponically in Knop nutrient solution (Ca^{2+} content in this solution was taken as a medium dose of the ion - MD) and its modification. The nutrient medium was composed of [g m^{-3}]: $Ca(NO_3)_2 \times 4\ H_2O$ 1000; KH_2PO_4 280; $MgSO_4 \times 7\ H_2O$ 250; KCl 120; H_3BO_3 0.2; $MnSO_4$ 0.5; $CuSO_4$ 0.05; $ZnSO_4 \times 7\ H_2O$ 0.1; $(NH_4)_6Mo_7O_{24} \times 4\ H_2O$ 0.025. To grow the plants at a high (HD) dose of Ca^{2+} in the nutrient solution, 235 g m^{-3} of $CaCl_2$ was added. In the case of a low (LD) Ca^{2+} dose the nutrient solution contained [g m^{-3}]: $Ca(NO_3)_2 \times 4\ H_2O$ 500; KH_2PO_4 280; $MgSO_4 \times 7\ H_2O$ 250; NH_4NO_3 105; KNO_3 160; H_3BO_3 0.2; $MnSO_4$ 0.5; $CuSO_4$ 0.05; $ZnSO_4 \times 7\ H_2O$ 0.1; $(NH_4)_6Mo_7O_{24} \times 4\ H_2O$ 0.025. In all nutrient solutions 25 g m^{-3} of Fe^{3+}-citrate was added. Therefore, in the LD, MD, and HD nutrient solutions the content of the macronutrient was: 85, 170, and 225 g m^{-3}, respectively. The plants were grown at 23/18 °C day/night and PPFD of 120 μmol m^{-2} s^{-1} under a 16 h photoperiod. Two ways of administering excess Cu^{2+} were used. Firstly, copper ($CuSO_4 \times 5\ H_2O$) at the final concentration of 20 g m^{-3} was added to the nutrient solution at different growth stages of primary leaves, *i.e.*, immediately after transferring the seedlings to the nutrient solution (young plants) and on day 12 (older plants) after this transfer. The primary leaves of control and the Cu^{2+}-treated plants were harvested for analyses 12 d after the treatment. Secondly, Cu^{2+} ions were introduced to leaves immediately using the infiltration method. In this case, control leaves of young or older plants growing at the MD of Ca^{2+} were poisoned by different Cu^{2+} doses.

Infiltration experiments: Leaf discs of bean plants were infiltrated in a partial vacuum by aqueous solution of calcium channel blockers (50 μM verapamil or 30 μM $LaCl_3$) or the ionophore A23187 (1 μM) during 15 min. Control leaf discs were infiltrated by pure bidistilled water. In several cases 10 mM $CaCl_2$ or $CuSO_4$ (in concentrations of 50, 200, and 600 μM) was added. After the next 10 min of incubation at atmospheric pressure, the samples were tapped for fluorescence measurements. The time from the beginning of the infiltration procedure to Chl fluorescence measurements (including the dark-adapted period) was 55 min.

Chlorophyll fluorescence induction kinetics was measured at 20 °C using a *PAM 101* Chlorophyll Fluorometer (*H. Walz*, Effeltrich, Germany) equipped with a *PAM 103* trigger control unit and *Schott KL 1500* lamps (*FL 101* and *FL 103*). Before

measurements of the Chl fluorescence, the leaf discs of bean plants were dark adapted for at least 30 min, and air was blown over the leaf surface to minimize the buildup of a boundary layer of CO_2. Fluorescence yields were determined on the upper surface of the primary leaves by first measuring the ground Chl fluorescence intensity F_0 with a weak, 1.6 kHz measuring beam (10 nmol m^{-2} s^{-1}). The maximal Chl fluorescence intensity, F_m, was then determined by applying one pulse of strong saturating "white light" (8400 μmol m^{-2} s^{-1}), while simultaneously switching the *PAM* measuring beam to 100 kHz. Short pulses of saturating radiation (8400 μmol m^{-2} s^{-1}), supplied by *Schott* lamp flashes every 30 s, were used to obtain F_m'. The F_0' was obtained by simultaneously switching off the actinic radiation (290 μmol m^{-2} s^{-1}) and applying a far-red radiation (*Balzers 710*, Gesenheim/Rhein, Germany). Photochemical quenching coefficient (q_P) was calculated according to Van Kooten and Snel (1990), LNU (light energy not used for photochemistry) according to Cornic (1994) calculated as follows, $LNU = 1 - (q_P F_v'/F_m')/(F_v/F_m)$. The Chl fluorescence decrease ratios, the Rfd values (so-called vitality indexes), were calculated according to Lichtenthaler and Rinderle (1988) and Lichtenthaler and Miehe (1997) as the ratio of Chl fluorescence decrease F_d (from F_m) to the steady state fluorescence F_s.

Statistical analysis: The estimated values are means of samples from three independent experiments, each with at least 3 replicates. The statistical calculations were done using the computer programme *GraphPad InStat tm.*

Results

Direct insertion of Cu^{2+} into leaves

Young leaves: $CuSO_4$ solution was directly infiltrated into young leaves. With its increasing concentration some primary parameters of Chl fluorescence gradually decreased, *i.e.*, F_v/F_0 to about 80 %, q_P and Rfd to 71 and 62 %, respectively, of the control level. LNU increased (at 600 μM $CuSO_4$) by 44 % of the control. The Ca^{2+} channel blockers (Ver or La^{2+}) did not modify this effect. Ionophore A23187 increased the inhibitory effect of Cu^{2+} ions, especially expressed by LNU, q_P, and Rfd (Fig. 1). Additionally applied Ca^{2+} ions to Cu^{2+} infiltrated young leaves partially reversed the q_P decrease and the LNU increase. Ca^{2+} did not modify the other investigated Chl fluorescence parameters.

Older leaves: Control older leaves in comparison to the young ones were characterized, after water infiltration, by a diminution of the primary photosynthetic processes in PS2 as expressed by a decrease of F_v/F_0, Rfd, and especially q_P, and a significant increase of LNU (see Figs. 1 and 2). At two lower Cu^{2+} doses a small increase of the Chl fluorescence indices was observed. Only LNU decreased to about 86 % of the control. At the highest Cu^{2+} concentration, F_v/F_0, q_P, and Rfd significantly decreased (by about 15-35 %). LNU increase did not exceed 15 % above the control level. La^{3+} and especially Ver and also excess Ca^{2+} abolished the positive effect exerted by two lower doses of Cu^{2+} on the photosynthetic apparatus. At the highest dose of Cu^{2+}, Ca^{2+} ions and La^{3+} did not modify the Chl fluorescence

parameters. In contrast, in the presence of Ver a decrease of F_v/F_0 and Rfd (to about 70 and 50 % of the control values, respectively) took place. A similar effect was observed at an ionophore A23187 injection into leaves.

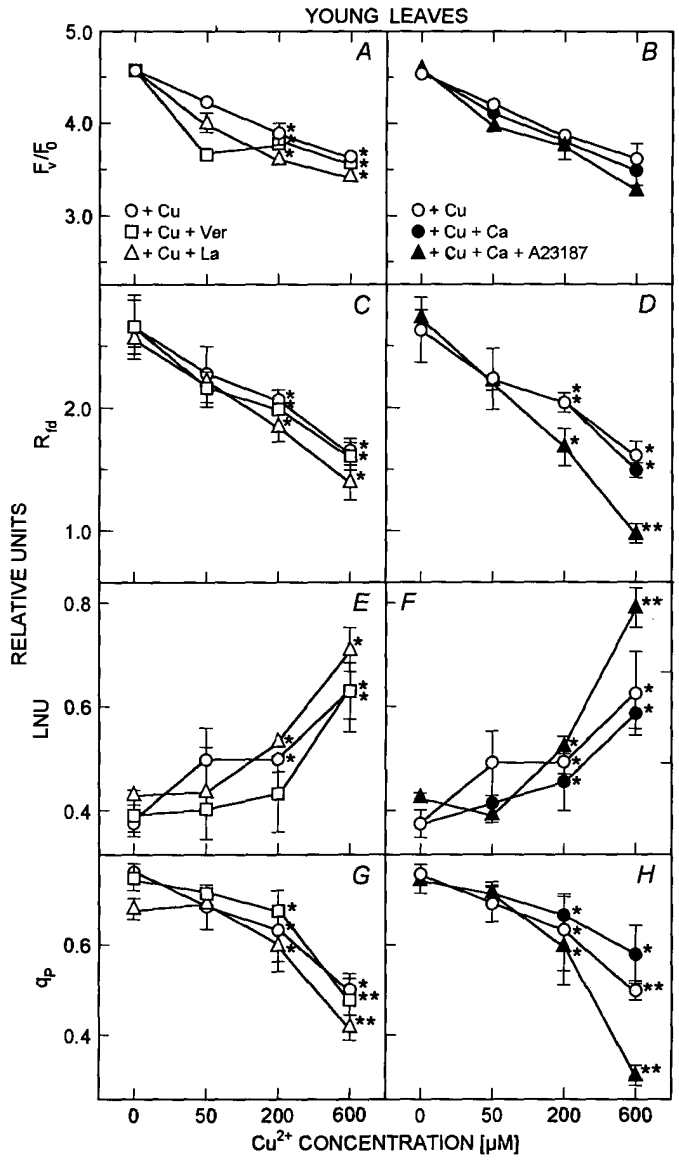

Fig. 1. The chlorophyll fluorescence parameters of young bean leaves after direct insertion of Cu^{2+} in relation to excess Ca^{2+}, calcium channel blockers, or the ionophore A23187. The values are the mean ± SE of 3 experiments. Where indicated, differences from control were significant at $^{*}p<0.05$ and $^{**}p<0.01$.

Cu^{2+} insertion through the root system

Young plants: After 12 d of Cu^{2+}-treatment of young bean plants a distinct decrease of q_P and increase of LNU at a low (LD) Ca^{2+} dose were observed (Table 1). Only small changes occurred in other fluorescence parameters. La^{3+} intensified the

inhibitory effect of Cu^{2+} expressed by additional decrease in q_P and Rfd values. Also, a significant LNU increase took place. The additionally injected Ca^{2+} (in the presence of La^{3+}) into leaves partially abolished the effect of La^{3+}.

MD and HD in the nutrient solution (especially after infiltration with Ver) diminished the inhibitory or stimulatory effect of excess Cu^{2+} on q_P and LNU, respectively. Partially decreasing F_v/F_0 ratio at LD and MD increased above that of control (plants not treated by Cu^{2+}) at HD. At MD and HD, La^{3+} did not change the effect of excess Cu^{2+} on the Chl fluorescence parameters. The additional supply

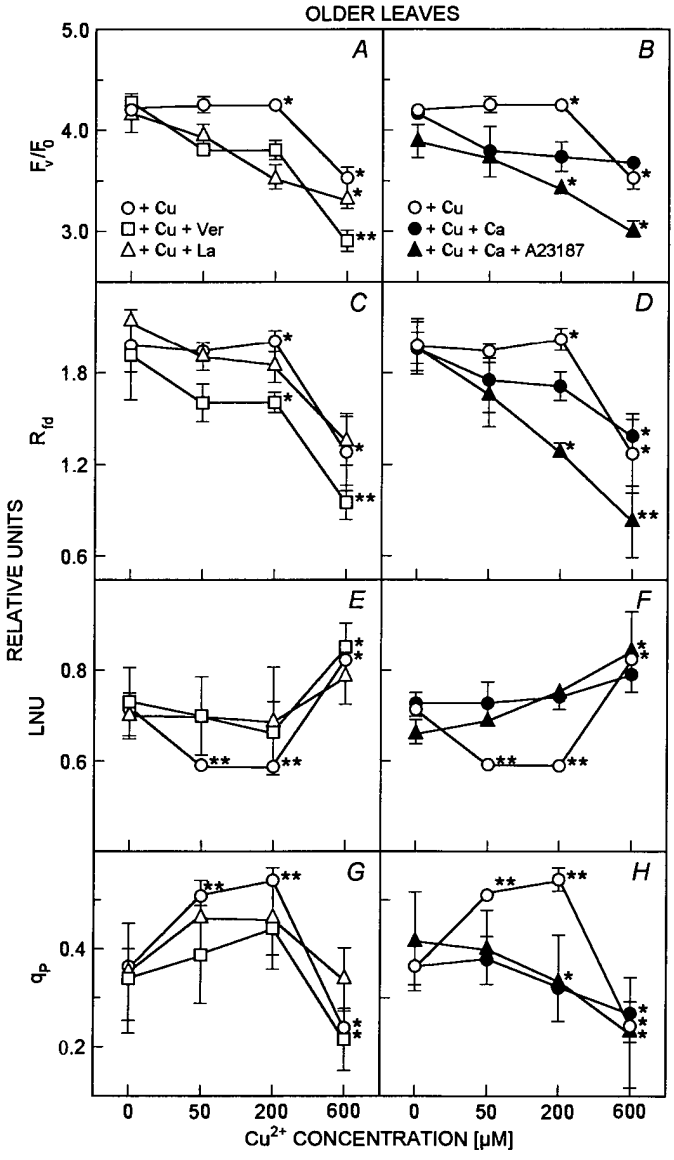

Fig. 2. The chlorophyll fluorescence parameters of older bean leaves after direct insertion of Cu^{2+} in relation to excess Ca^{2+}, calcium channel blockers, or the ionophore A23187. The values are the mean ± SE of 3 experiments. Where indicated, differences from control were significant at $^{*}p<0.05$ and $^{**}p<0.01$.

of ionophore to Cu^{2+}-treated plants, at MD and HD, did not significantly influence the Chl fluorescence parameters (in comparison to control plants supplied with A23187).

Older plants: At LD a small effect of excess Cu^{2+} on the photosynthetic activity of bean plants was observed (Table 1). The F_v/F_0 decreased (to about 85 % of control) and q_P increased. In the presence of Ca^{2+} channel blockers, q_P and Rfd decreased in Cu^{2+}-treated plants to the level below that of control. Excess Ca^{2+}, infiltrated into leaves together with La^{3+}, mostly decreased the photosynthetic activity as shown by a decrease of q_P and Rfd and an LNU increase.

Table 1. Chlorophyll fluorescence parameters of primary leaves of control and Cu-treated young and older bean plants in relation to Ca^{2+} concentration (low - LD, medium - MD, high - HD) in the nutrient solution and to a short treatment with calcium channel blockers or the ionophore A23187. Means of 3 experiments. The SE values were less than 9 % of the values presented. Significant differences at $^{*}p<0.05$ or $^{**}p<0.01$.

Plant type, added substance	Chlorophyll fluorescence parameter											
	F_v/F_0			q_P			LNU			Rfd		
	LD	MD	HD	LD	MD	HD	LD	MD	HD	LD	MD	HD
Young plants												
control	4.03	4.54	3.58	0.62	0.79	0.54	0.52	0.43	0.60	2.27	2.65	2.11
Cu	4.00	4.37	3.90	0.49*	0.61	0.56	0.67*	0.52	0.64	2.37	2.60	2.32
Ver	3.20*	4.57	3.71	0.72*	0.70	0.55	0.40	0.45	0.60	2.31	2.77	2.00
Cu+Ver	3.21*	4.77	3.10	0.46*	0.80	0.70	0.66*	0.35	0.40	2.00	3.24	2.68
La	3.40	4.30	3.69	0.64	0.66	0.57	0.48	0.48	0.50	2.30	2.58	2.12
Cu+La	4.03	4.34	3.77	0.22**	0.52	0.64	0.87**	0.62	0.50	1.23**	2.40	2.40
Cu+La+Ca	3.85	3.90*	3.14	0.46	0.49*	0.66	0.69*	0.62	0.48	2.03	1.86*	2.60
A23187	3.75	4.60	3.42	0.65	0.78	0.63	0.43	0.39	0.51	2.30	2.80	2.10
Cu+A23187	4.07	4.40	3.23	0.46*	0.67	0.53	0.62	0.50	0.61	2.03	2.89	2.00
Cu+A23187+Ca	3.75	4.66	3.25	0.49	0.49	0.64	0.66*	0.42	0.50	2.33	3.10	2.33
Older plants												
control	4.02	4.24	4.46	0.40	0.41	0.42	0.72	0.70	0.68	2.45	2.42	2.42
Cu	3.40*	2.60*	3.50*	0.44	0.32*	0.28*	0.72	0.78*	0.82*	2.45	1.75*	1.95*
Ver	3.74	4.21	3.65*	0.53	0.38	0.41	0.63	0.70	0.71	2.44	2.43	2.28
Cu+Ver	3.60*	2.43**	3.64*	0.37	0.35*	0.34*	0.74	0.80*	0.76	1.86*	1.65*	1.86*
La	3.86	3.85	4.00	0.52	0.39	0.44	0.64	0.70	0.66	2.22	2.40	2.49
Cu+La	2.90*	2.26**	3.29*	0.39	0.26*	0.39	0.75	0.81*	0.68	2.00	1.44**	1.89*
Cu+La+Ca	3.53	1.80*	3.48*	0.28*	0.36	0.32	0.80*	0.79*	0.73	1.22**	1.79*	1.85*
A23187	3.69	4.23	4.67	0.38	0.43	0.47	0.73	0.67	0.64	1.92	2.17	2.50
Cu+A23187	3.44	3.75*	3.74	0.31*	0.33*	0.35*	0.80*	0.83*	0.75	2.00	1.39*	1.78*
Cu+A23187+Ca	3.00**	3.27*	3.36*	0.43	0.24*	0.36*	0.72	0.82*	0.74	2.10	1.54*	1.99

MD and HD intensified the inhibitory effect of excess Cu^{2+} on the photosynthetic apparatus. The values of q_P, F_v/F_0, and Rfd decreased, but LNU increased at MD and HD. Verapamil did not block the inhibitory effect of Cu^{2+} on the photosynthetic apparatus, but La^{3+} increased it at MD. Additionally introduced Ca^{2+} diminished La^{3+} action on q_P and Rfd, but the F_v/F_0 ratio decreased. At MD, the ionophore A23187 intensified Cu^{2+}-induced q_P and the Rfd decrease and LNU increase, but diminished the F_v/F_0 decrease. At HD, A23187 did not cause any clear changes in the fluorescence parameters of Cu^{2+}-treated plants. Additional Ca^{2+} supply increased the Cu^{2+} effect (under presence of A23187) on F_v/F_0 ratio, but decreased it in the case of q_P and Rfd.

Discussion

Earlier studies (Renganathan and Bose 1989, Renger *et al.* 1993, Maksymiec *et al.* 1994, Maksymiec and Baszyński 1996) showed a particularly high sensitivity of PS2 and of elongation and senescence processes of leaves to excess Cu^{2+}. Further studies showed that increased Ca^{2+} concentration in the medium decreased Cu^{2+} action on morphological phenomena of young bean plants, but increased it in older ones (Maksymiec and Baszyński 1998b). In younger leaves of plants treated with Cu^{2+}, a decreased Ca^{2+} content was observed at the same time, whereas in the older ones its increase was progressing. This showed that interaction of these elements could modify the mechanism of excess Cu^{2+} action. Over a fairly short period of Cu^{2+} action, which occurred after its direct injection into leaves (Fig. 1), inhibition of the activity of the whole photosynthetic apparatus (Rfd decrease) as well as diminishing electron flow activity to Q_A (q_P and F_v/F_0 decrease) were observed, as a result of decreased fraction of the absorbed radiant energy used for photochemistry (LNU increase). Infiltration techniques did not change the physiological state of leaves (values not shown). Protective Ca^{2+} effect on the activity of photosynthetic apparatus, abolished after adding its ionophore, showed that excess Cu^{2+} can penetrate into the cell interior and then to chloroplasts through Ca^{2+} channels or structures similar to them. However, both La^{3+} and Ver did not inhibit Cu^{2+}, indicating that in young leaves Cu^{2+} can operate through Ca^{2+} channels only to a limited extent. Probably there exists another way of Cu^{2+}-penetration into cells. It is also possible that calcium channel blockers do not form an adequate barrier for Cu^{2+} ions. Under stress, they can modify the structure of plasmatic membranes to such an extent that their permeability increases also for Ca^{2+} ions (Abdel-Basset 1998).

In a long term Cu^{2+} action (Cu^{2+} applied through the root system), Ca^{2+} ionophore and Ver during the incubation period did not modify the toxic effect exerted by excess Cu^{2+} only at low Ca^{2+} doses. On the other hand, La^{3+} intensified it. These inhibitors vary in their mode of action (Tsien *et al.* 1987, Soumelidou *et al.* 1994, Reiss and Beale 1995). There also exists a differentiated sensitivity of Ca^{2+} channels to channel-blocking agents in different plants (Graziana *et al.* 1988, Rengel and Elliot 1992, Marshall *et al.* 1994). We used Ver and La^{3+} at low concentrations, which, according to Cho and Hong (1996) and Terry *et al.* (1992), significantly

influence cell processes of higher plants. It is possible that La^{3+} can increase the toxic effect of Cu^{2+} ions similar to Cd^{2+} ions used at μM concentrations as shown in *Pinus* seedlings (Arduini *et al.* 1994). These results indicated that in the first place Cu^{2+} ions partially and reversibly change Ca^{2+} gradient in the cells and in photosynthetic apparatus. After a longer time more stabile changes occur, probably partially connected with changes in polypeptide and lipid fractions of thylakoid membranes found earlier (Sandmann and Böger 1980, Lidon and Henriques 1993, Maksymiec *et al.* 1994). If Cu^{2+} significantly changes the q_P and LNU fluorescence parameters only at a low Ca^{2+} concentration, it can be assumed that the inhibition action of excess Cu^{2+} on the photosynthetic apparatus is based on its competition with Ca^{2+} ions, the effect of which is inhibition of the donor and acceptor side of PS2 (Tso *et al.* 1991). A similar effect was also shown in noninfiltrated leaves (Maksymiec and Baszyński 1999). This supports suggestion of Šeršeň *et al.* (1997) that Cu substitutes Ca^{2+} in OEC. However, a durable effect of Cu^{2+} on photochemical and nonphotochemical processes indicated that another place of Ca/Cu interactions are probably Ca^{2+} gates in the chloroplast coupling factor marked in the model of Chiang and Dilley (1989) or cyclic electron transport around PS2 (see Prasil *et al.* 1996).

Older plants were more resistant to directly introduced Cu^{2+} than the young ones. However, after Cu^{2+} introduction through the root system the older plants were more sensitive to heavy metal toxicity, especially after a higher Ca^{2+} supply. This indicated that in the older plants rather long-term processes are connected with Cu^{2+}-toxicity, which confirms the results of Huang *et al.* (1997), who showed intensification of senescence processes at increased Ca^{2+}. In older Cu-treated leaves at a higher Ca^{2+} content in the soil solution a strikingly high Ca^{2+} concentration, more than 60 g kg^{-1}(DM) (for classification of Ca^{2+} indispensability see Ramalho *et al.* 1995), was observed (Maksymiec and Baszyński 1998b). Similarly to young plants, La^{3+} increased the q_P and q_N after a long-term action of Cu^{2+}, especially at a low Ca^{2+} content in the nutrient solution. Additional Ca^{2+} supply did not reverse this effect, supporting the idea that La^{3+} in the presence of Cu^{2+} can induce durable disturbances of membranes. Energy dissipation intensified by ionophore within the photosynthetic apparatus at LD indicates that Ca^{2+} channels can only in certain conditions be the place of facilitated Cu^{2+} infiltration also in older plants.

Comparison of long- and short-term Cu^{2+} action indicates that in younger plants the initial changes, distinctly stimulated by Ca^{2+} ionophore, are strongest at the dark phase of photosynthesis (especially strong Rfd decrease). Further changes concern non-photochemical energy dissipation (LNU increase), reoxidation Q_A decrease (q_P decrease), and stabilization of the PS2 complex, particularly on its donor side. After a longer exposure to the metal, activity of the dark phase of photosynthesis and PS2 donor side are equalized to the control level (similar Rfd value and F_v/F_0 of control and Cu-treated plants, respectively). However, processees of photochemical and nonphotochemical fluorescence quenching are significant and increase with Ca^{2+} deficiency. Also the sensitivity of membranes to the modifying effect of Ca^{2+} channel blockers is increased. In older plants it cannot be pointed out which site of the photosynthetic apparatus is more sensitive to excess Cu^{2+}. The inhibition of the

whole photosynthetic apparatus may occur simultaneously, but electron transport processes within PS2 presented by q_P and F_v/F_0 undergo a stronger inhibition after a longer time of Cu^{2+} action.

These results indicate that Ca^{2+} channels can partially constitute the way of Cu^{2+} infiltration in the initial period of exposure to the metal, particularly in younger plants. After a longer Cu^{2+} action, opening of the Ca^{2+} channels does not change the possibility of the metal exploration in the photosynthetic apparatus, indicating durable changes independent on partial changes of the Ca^{2+} concentration gradient. Modification of the structure of channels causing their dosing in control conditions, particularly by the cation blocker, can be the cause of a distinct increase of toxic excess Cu^{2+} action.

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