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PHYSIOLOGICAL RESPONSE OF *Brassica napus* L. PLANTS TO Cu(II) TREATMENT

REAKCJA FIZJOLOGICZNA *Brassica napus* L. NA DZIAŁANIE MIEDZI(II)

Abstract: Rapeseed plants were exposed to seven different concentrations (0.5, 1, 3, 6, 12, 24, 60 µmol · dm^{-3}) of $CuSO_4 \cdot 5H_2O$ for 7 days. Within concentration range 0.5-3 µmol · dm^{-3} a significant increase of biomass (both plant organs) was observed. Decrease of biomass was notable after application of concentrations higher than 6 µmol · dm^{-3}. Considerable drop in content of chlorophylls as well as carotenoids was observed after application of 6 µmol · dm^{-3} Cu(II). Decline of protein content in leaves of plants was observed in concentration range 6-60 µmol · dm^{-3}. Lipid peroxidation expressed as a content of malondialdehyde in leaves was strong within concentration range 6-60 µmol · dm^{-3} Cu(II). Bioaccumulation factor values of roots were higher then those of shoots in the whole concentration range (0.5-60 µmol · dm^{-3} Cu). The portion of Cu allocated in shoots related to the total Cu amount accumulated by plant ranged from 27.6% (0.5 µmol · dm^{-3}) to 8.4% (60 µmol · dm^{-3}).

Keywords: copper accumulation, bioaccumulation factor, lipid peroxidation, rapeseed

Introduction

The content of Cu in the environment is usually low, but it is considerably increased in mining areas and in the vicinity of smelting plants. The usual content of Cu(II) ions in soils ranges from 10^{-9} to 10^{-4} mol · dm^{-3} [1, 2], but much of it is in forms which are not available to plants because of the strong binding of Cu by organic matter and other soil colloids [3].

Copper is an essential metal having important role in metabolic processes of plant cells. It is inevitable component of enzymes or of particular structural components of cells. At increased concentrations, Cu may have strong adverse effects on chromatin, the photosynthetic apparatus, growth and senescence processes [4]. Higher plant response to copper differs in mechanisms of its uptake and accumulation, and in the way of avoiding Cu-induced damage [5]. Visual symptoms of Cu toxicity depend strongly on plant growth stage at which the metal was applied. After a longer exposure to Cu (throughout the vegetation period) leaf area and content of chlorophylls and carotenoids is reduced [6, 7]. A common feature of the action of excess Cu in most plants is the decrease in root mass [8]. Copper impairs cellular transport processes and dramatically changes the biochemical metabolism [9]. Already at low concentrations, Cu inhibits photosynthesis by interaction with some constituents of photosystem (PS) II and by the formation of Cu-chlorophylls, which are unsuitable for photosynthesis [10, 11].

Being a redox-active metal, Cu catalyzes the production of *reactive oxygen species* (ROS), such as superoxide ($O^{\bullet 2-}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($OH^{\bullet}$), via Haber-Weiss and Fenton reactions [12]. ROS are damaging to essential cellular components such as DNA, proteins and lipids, therefore induction of ROS production results in oxidative stress affecting plant growth and alteration of antioxidant system [13].

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Plants have developed complex defence mechanisms by which they mediate the deleterious effects of the ROS. Such defence systems involve both enzymatic and non-enzymatic antioxidants. The enzymatic protective mechanism operates by sequential and simultaneous action of a number of antioxidant enzymes such as catalase, superoxide dismutase, ascorbate peroxidase, guaiacol peroxidase and glutathione reductase [14].

Rapeseed (*Brassica napus* L. *subsp. napus*) belonging to Brassicaceae family is known to be able to accumulate substantial amounts of metals; moreover, this plant has a high biomass and various genotypes are easily available [15].

The aim of this study is to investigate the effect of Cu(II) on plant biomass as well as Cu accumulation and translocation in plant organs of hydroponically cultivated *B. napus* plants (cv. Verona). Furthermore, content of soluble proteins, *malondialdehyde* (MDA), chlorophyll *a* and *b* as well as carotenoids in leaves was determined.

Material and methods

For experiments $CuSO_4 \cdot 5H_2O$ of analytical purity (Slavus, Bratislava) and seeds of *B. napus,* cv. Verona (Centre for Research of Crop Production, Research Institute of Crop Production, Piestany) were used. Twenty-two days old rapeseed plants were exposed in hydroponics for seven days in controlled conditions (mean air temperature: (25 ± 0.5)ºC, relative air humidity: 80% and photosynthetic active radiation: 80 $\mu mol \cdot m^{-2} s^{-1}$): control variant in Hoagland solution and metal-treated variants in Hoagland solution containing 0.5, 1, 3, 6, 12, 24 and 60 $\mu mol \cdot dm^{-3}$ $CuSO_4 \cdot 5H_2O$. Then the length and dry mass of shoots and roots were determined. *Flame atomic absorption spectroscopy* (AAS Perkin Elmer Model 1100, USA) was used for determination of Cu content in shoots and roots of rapeseed plants. Protein content in leaves was determined according to Bradford [16] and the content of malondialdehyde in leaves was determined as a content of *thiobarbituric acid reactive substances* (TBARS) [17] (the corresponding procedures are described in detail in [18]). Photosynthetic pigments were determined spectrophotometrically (Genesys 6, Thermo Scientific, USA)) after extraction into 80% acetone [19].

Results and discussion

Chlorosis of leaves of three-weeks old *B. napus* plants submitted to Cu stress for 7 days was notable at moderate applied Cu(II) concentrations (12 and 24 $\mu mol \cdot dm^{-3}$). At the highest used concentration (60 $\mu mol \cdot dm^{-3}$) was chlorosis more pronounced and all plants were stunted, some leaves were wilted and desiccated. Two highest applied concentrations of Cu(II) (24 and 60 $\mu mol \cdot dm^{-3}$) caused that roots of plants were subtle and brownish.

Shoot and root dry mass as well as length of both plant organs of rapeseed plants treated with Cu(II) are summarized in Table 1. Within concentration range 0.5-3 $\mu mol \cdot dm^{-3}$ a significant increase of dry biomass (both plant organs) was observed. This positive effect was most pronounced after application of 0.5 $\mu mol \cdot dm^{-3}$ Cu(II) as the gain of biomass of leaves and roots related to the control was 17% and 25%, respectively. Lin et al [20] and Jiang et al [21] observed similar increase of biomass in their experiments with *Helianthus annuus* L. and *Zea mays* L. plants treated with low Cu(II) concentrations (10^{-5} $mol \cdot dm^{-3}$). Significant decrease of biomass of both plant organs was observed within

concentration range 6-60 μmol · dm^{-3}. In the concentration range of 6-24 μmol · dm^{-3} Cu(II) root length was affected by metal treatment to a greater extent than that of shoot. On the other hand, at the highest applied concentration (60 μmol · dm^{-3}) the length of shoots was affected more than that of roots. Although some authors consider that inhibition of the cell cycle is the basis for growth inhibition, the precise role of Cu in cell proliferation is as yet unknown and controversial. Copper may affect cell wall building by direct affecting the crosslinking of hydroxyproline-rich protein cell wall components [4] due to high reactivity of Cu(II) with amino acids. Some divalent ions (especially Mg(II) and Ca(II)), in relation to their concentration, inhibit or enhance plant growth. Mg and Ca have similar chemical ionic form as Cu and for this reason the competitive action of these elements may be possible in the phase of uptake and transport modifying in consequence plant growth [22].

Table 1

Shoot and root length and dry mass of rapeseed plants treated with different concentrations of Cu(II). Mean ± S.E., $n = 5$. Means followed by different letters are significantly different at the 0.05 probability level

c **[μmol · dm^{-3}]**	**Shoot dry mass [mg]**	**Root dry mass [mg]**	**Shoot length [cm]**	**Root length [cm]**
0	709.8 ± 9.4^{cd}	48.1 ± 2.5^{bc}	313.5 ± 3.9^{ab}	218.0 ± 6.3^{a}
0.5	828.0 ± 31.9^{a}	60.6 ± 2.0^{a}	325.3 ± 2.0^{a}	218.0 ± 6.1^{a}
1	745.0 ± 18.6^{bc}	49.5 ± 2.3^{b}	318.5 ± 5.4^{ab}	212.3 ± 5.2^{a}
3	784.3 ± 23.0^{ab}	49.9 ± 0.3^{b}	312.3 ± 9.8^{ab}	212.5 ± 4.2^{a}
6	655.5 ± 17.3^{de}	44.2 ± 1.3^{c}	304.0 ± 4.6^{b}	192.5 ± 2.4^{b}
12	624.8 ± 18.4^{e}	37.8 ± 2.2^{d}	284.0 ± 8.6^{c}	189.5 ± 4.0^{b}
24	457.5 ± 10.8^{f}	29.5 ± 2.2^{e}	271.8 ± 8.4^{c}	186.8 ± 6.4^{b}
60	282.0 ± 22.9^{g}	16.0 ± 1.5^{f}	217.5 ± 5.6^{d}	179.0 ± 3.6^{b}

Contents of chlorophyll *a, b* and carotenoids in leaves of rapeseed plants are summarized in Table 2. Significant decrease in the content of chlorophylls and carotenoids was notable after application of 6 μmol · dm^{-3} Cu(II). Within concentration range 3-60 μmol · dm^{-3} was the content of chlorophyll *b* affected the most. Effect of copper on chlorophyll synthesis may be caused by inhibitory influence of Cu at the stage of δ-aminolevunilic acid formation. Also a direct Cu effect is possible, through inhibition of Fe uptake which is indispensable for chlorophyll formation [4, 8].

Dependence of protein (Fig. 1A) and MDA (Fig. 1B) content in leaves of rapeseed plants on the applied Cu(II) concentration has bi-linear course. A rapid decline of protein content was observed in the concentration range 6-24 μmol · dm^{-3}, application of higher Cu concentrations resulted in less sharp decrease. After application of 6 μmol · dm^{-3} Cu(II) the protein content in leaves dropped only by 6% in comparison with the control, while application of the highest Cu(II) concentration (60 μmol · dm^{-3}) caused about 48% decrease in protein content.

Copper induced oxidative stress in rapeseed plants was evident from the increased lipid peroxidation (content of MDA, Fig. 1 B) in leaves. In the concentration range of 0-3 μmol · dm^{-3} Cu(II) only mild increase in MDA content was observable but further increase of Cu(II) concentration caused sharp gain of malondialdehyde in leaves of rapeseed plants. At the concentration 60 μmol · dm^{-3} Cu(II) the content of this substance three times exceeded

that of the control. This finding is in agreement with the results of other studies carried out with rice plants cultivated in hydroponics [23, 24].

Excess Cu can induce a number of free radical processes in protein and lipid cell membrane components, causing destabilization of membranes and increase of their permeability. Polypeptide components of membranes can also be modified through Cu action on genetic material [4].

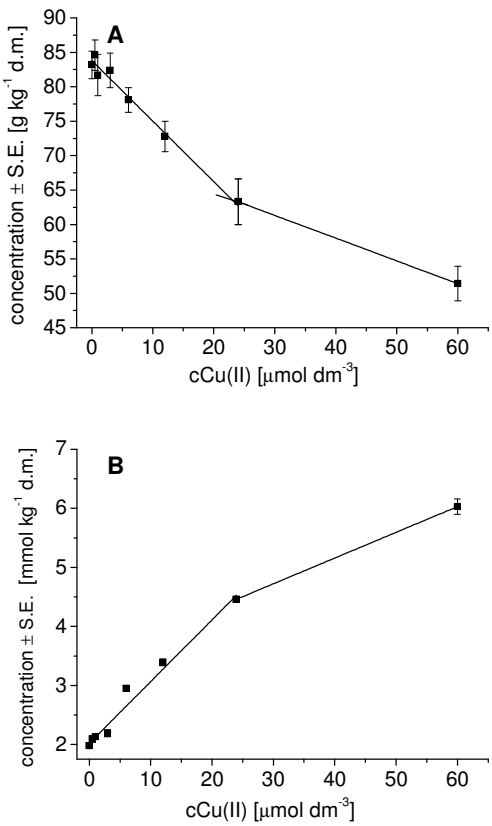

Fig. 1. Effect of applied Cu(II) concentration on protein (A) and MDA (B) content in leaves of rapeseed plants. Mean ± S.E.; n = 5; d.m. - dry mass; S.E. - standard error

Heavy metal toxicity is considered to induce the production of reactive oxygen species and may result in significant damage to cellular constituents. Membrane lipids and proteins are especially sensitive to attack by free radicals and they are considered to be reliable indicators of oxidative stress in plants [25, 26].

Copper is taken up by higher plants largely in the form of Cu(II) ions due to the action of still not well-known mechanisms. For its absorption from rhizosphere probably the

functioning of Fe-dependent reductase is necessary. In its further transport phase, a significant role may by played by nicotinamide [4].

Table 2

Chlorophyll *a*, chlorophyll *b* and carotenoids contents in leaves of rapeseed plants treated with different concentrations of Cu(II). Mean ± S.E., n = 5. Means followed by different letters are significantly different at the 0.05 probability level; d.m. - dry mass

Cu(II) conc. [µmol · dm^{-3}]	**Chl *a* conc. [g · kg^{-1} d.m.]**	**Chl *b* conc. [g · kg^{-1} d.m.]**	**Carot. conc. [g · kg^{-1} d.m.]**
0	17.6 ± 0.7^{a}	5.2 ± 0.4^{a}	5.7 ± 0.1^{a}
0.5	17.8 ± 0.2^{a}	5.3 ± 0.5^{a}	5.6 ± 0.1^{a}
1	17.6 ± 0.2^{a}	5.3 ± 0.4^{a}	5.5 ± 0.1^{a}
3	17.0 ± 0.5^{a}	5.0 ± 0.4^{a}	5.1 ± 0.1^{b}
6	15.9 ± 0.5^{b}	4.5 ± 0.2^{ab}	5.0 ± 0.3^{b}
12	12.5 ± 0.2^{c}	3.6 ± 0.3^{bc}	3.9 ± 0.1^{c}
24	10.0 ± 0.1^{d}	2.9 ± 0.3^{c}	3.3 ± 0.1^{d}
60	6.6 ± 0.2^{e}	1.8 ± 0.1^{d}	2.1 ± 0^{e}

Content of Cu in shoots and roots of rapeseed plants treated with different metal concentrations, the corresponding bioaccumulation (BAF) and translocation (TF) factors as well as the fraction of Cu allocated in shoots related to the total amount of Cu accumulated by plants are summarized in Table 3.

Table 3

Copper concentrations in shoots and roots of rapeseed plants treated with different concentrations of Cu(II), corresponding values of bioaccumulation (BAF) and translocation (TF) factors and fraction of Cu allocated in shoots related to the total amount of Cu accumulated by plants (in [%]); d.m. - dry mass

c **[µmol · dm^{-3}]**	**Cu conc. [mg · kg^{-1} d.m.] BAF**		**TF [% Cu in shoot]**
	Shoot	**Root**	
0.5	6.2 **195.1**	222.7 **7009.1**	0.028 **27.6**
1	8.2 **129.1**	311.9 **4908.3**	0.026 **28.4**
3	9.2 **48.3**	421.8 **2212.6**	0.022 **25.5**
6	13.6 **35.7**	635.4 **1666.5**	0.021 **19.5**
12	18.5 **24.3**	1255 **1645.8**	0,015 **19.6**
24	19.8 **13**	2870 **1881.8**	0,007 **9.7**
60	47.9 **12.6**	9249 **2425.8**	0,005 **8.4**

Bioaccumulation factor (BAF) express the ratio of the metal concentration in the biological material [µmol · g^{-1} or µg · g^{-1} dry mass] to the metal concentration in external solution in [µmol · dm^{-3} or µg · dm^{-3}]. In the whole concentration range 0.5-60 µmol · dm^{-3} Cu BAF values of roots were higher than those of shoots. Low values of BAFs determined for shoots reflect less effective mobility of Cu within the plant. The translocation factor

corresponds to the ratio of accumulated Cu amount in shoots and roots. Translocation factor values lower than 1 correspond to lower Cu concentration (mg · kg^{-1} dry mass) in the shoots than in the roots.

The total Cu content occurring in individual plant organs is affected not only by Cu concentrations in shoots and roots but also by actual dry mass of these plant organs. After application of 0.5, 1 and 3 μmol · dm^{-3} Cu(II) portion of Cu allocated in shoots was over 20%.

Treatment with higher Cu(II) concentrations (24 and 60 μmol · dm^{-3}) caused that this portion was under 10% (Table 3). The dependence of accumulated Cu content in shoots and roots on the applied Cu(II) concentration showed linear increase. The amount of Cu accumulated in roots was 36- to 193-times higher than in shoots. Greater Cu content in the roots than in the shoots of rapeseed plants may indicate adoption of exclusion mechanism to tolerate the copper-induced toxicity in which the roots accumulate the metal, preventing its subsequent transport into the shoots [13].

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REAKCJA FIZJOLOGICZNA *Brassica napus* L. NA DZIAŁANIE MIEDZI(II)

Abstrakt: Rośliny rzepaku poddawano działaniu $CuSO_4 \cdot 5H_2O$ w siedmiu różnych stężeniach (0,5, 1, 3, 6, 12, 24, 60 µmol · dm^{-3}) przez 7 dni. W zakresie stężeń 0,5-3 µmol·dm^{-3} zaobserwowano znaczny wzrost biomasy (obie części roślin). Zmniejszenie biomasy zauważono po zastosowaniu wyższych stężeń niż 6 µmol ·dm^{-3}. Znaczny spadek zawartości chlorofili oraz karotenoidów stwierdzono po zastosowaniu 6 µmoli · dm^{-3} Cu(II). Spadek zawartości białka w liściach roślin zaobserwowano w zakresie stężeń 3-60 µmol · dm^{-3}. Peroksydacja lipidów wyrażona zawartością dialdehydu malonowego w liściach była silna w zakresie stężeń 6-60 µmol · dm^{-3} Cu(II). Wartości współczynnika bioakumulacji w korzeniach była większe niż w pędach w całym zakresie stężeń (0,5-60 µmol · dm^{-3} Cu). Stosunek Cu zakumulowanej w pędach do całkowitej ilości miedzi zakumulowanej przez rośliny mieścił się w zakresie od 27,6% (0,5 µmol · dm^{-3}) do 8,4% (60 µmol · dm^{-3}).

Słowa kluczowe: akumulacja miedzi, współczynnik bioakumulacji, peroksydacja lipidów, rzepak