BULG. J. PLANT PHYSIOL., 2003, 29(1–2), 33–43

Cu-INDUCED CHANGES IN CHLOROPLAST LIPIDS AND PHOTOSYSTEM 2 ACTIVITY IN BARLEY PLANTS

A. Vassilev* [1], F. Lidon[2], P. Scotti Campos[3], J. C. Ramalho[4], M. G. Barreiro[3], I. Yordanov[5]

[1]Agricultural University of Plovdiv, Dept. Plant Physiology and Biochemistry, 12 Mendeleev St., 4000 Plovdiv, Bulgaria
[2]Grupo de Disciplinas de Ecologia da Hidrosfera, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2825-114 Caparica, Portugal
[3]Plant Physiology Department, Estação Agronómica Nacional-INIA, Quinta do Marquês, Av. da Republica, 2784-505 Oeiras, Portugal
[4]Instituto de Investigação Científica Tropical - CEPTA, Tapada da Ajuda, Ap. 3014, P-1301-901 Lisboa, Portugal
[5]Acad. M. Popov Institute of Plant Physiology, Acad. G. Bonchev St., Bl.21, 1113, Sofia, Bulgaria

Received October 1, 2003

Summary. Twenty-day-old barley plants (*Hordeum vulgare* L., cv. CE9704) grown as a sand culture were treated 10 days with increasing concentrations of Cu 10, 15 and 20 mg/kg sand. Lipid peroxidation, thylakoidal fatty acid content and composition as well as photosynthetic electron transport and chlorophyll fluorescence were studied. It was established that excess Cu enhanced lipid peroxidation processes and decreased total fatty acids content as well as unsaturation level of the thylakoids. These negative effects had a great unfavourable impact on photosynthetic performance which resulted in both lower photosynthetic electron transport activities and efficiency of light utilisation.

***Key words*:** barley, copper, lipid peroxidation, ethylene production, photosynthetic electron transport, chlorophyll *a* fluorescence

***Abbreviations*:** OEC – oxygen evolving complex; DCPIP – 2,6-dichlorophenolindo-phenol; DPC – 1,5-diphenyl-carbohydrazide; MDA – malondialdehyde; TBA – 2-thiobarbituric acid; F_o – minimal fluorescence of antennae in dark adapted leaves; F_v/F_m – photochemical efficiency of PS2; q_P and q_{NP}

* Corresponding author, e-mail: vassilev@au-plovdiv.bg

– photochemical and non-photochemical quenching, q_E – energy-dependent component of q_{NP}; F'_v/F'_m – PS2 efficiency of energy conversion; ϕ_ε – quantum yield of photosynthetic non-cyclic electron transport

Introduction

Interactions between excess copper and photosynthesis has been studied for a long time (Sandmann and Bõger, 1980; Baszynski et al., 1982 Merakchiyska-Nikolova and Yordanov, 1983, Stoinova et al.1999), but the mechanisms of its *in vivo* toxic action are still a matter of debate (Maksymiec, 1997; Shainberg et al., 2001). Noticeably, the functioning of the photosynthetic apparatus of Cu-exposed plants is affected by both direct and indirect mechanisms. The excess Cu causes Rubisco and phosphoenol-pyruvat carboxilase (PEPC) inactivation by an interaction with SH-groups (Stiborova et al., 1986; Lidon and Henriques, 1991; Kamenova-Jouhimenko et al, 1999) and bio-production (Kamenova-Youchimenko et al., 2003). In addition, the redox active Cu ion induces membrane lipid peroxidation (Sandmann and Bõger, 1980; Weckx and Clijsters, 1996; Shainberg et al., 2001) that further results in serious damages of the thylakoids, namely disintegration of the lamellar system and increased unstacking of thylakoids (Angelov et al., 1993; Lidon et al., 1993 Stoynova and Merakchiyska-Nikolova, 1991). The excess Cu also decreases the photosynthetic pigments affecting both their synthesis and degradation (Merakchiyska-Nikolova and Yordanov, 1983; Lidon and Henriques, 1992a; Vangronsveld and Clijsters, 1994). Also, it alters plant source-sink relationships casing down-regulation of carbon metabolism and primary photochemistry (Ciscato et al., 1997), etc. There are also some data that two wheat *Aegelops ovata* hybrids show good tolerance at seedling stage to high Cu ion concentrations – 10^{-5} M and 10^{-6} M. However, he analysis of the photosynthetic response to excess Cu is rather complicated because of self-regulation processes, the difficulties in distinguishing the primary and the secondary effects as well as the dependence of the plant response to the experimental design used.

In a previous study we found that excess Cu decreased equally both net photosynthetic rate (A) and photosynthetic capacity (oxygen evolution under saturating irradiance and CO_2 – A_{max}) of barley plants and concluded that A_{max} inhibition strongly related to mesophyll limitations (Vassilev et al., 2002). In order to clarify the toxic Cu effect on barley photosynthesis we further studied its effects on both chloroplast lipids and PS2 activity.

Materials and methods

Barley (*Hordeum vulgare* L. cv. CE9704) plants were grown in sand enriched by Hoagland's nutrient solution, in a greenhouse under natural conditions of light,

temperature and humidity as described elsewhere (Vassilev *et al*. 2002). Twenty-day-old plants were exposed for 10 days to Cu treatments by supplying an adequate volume of water Cu solution to final concentrations 0, 10, 15, and 20 mg $Cu.kg^{-1}$ sand. At the end of the exposure period plants were harvested and analysed.

Chlorophyll fluorescence parameters

Chlorophyll fluorescence parameters were measured with a PAM 2000 system (H. Walz, Effeltrich, Germany) on leaf discs (from undamaged areas) placed inside the LD2/2 O2 electrode, under CO_2 saturating conditions, at 25 ºC. Measurements of the initial fluorescence from the antennae, F_o, and photochemical efficiency of PS2, F_v/F_m, were taken from overnight dark-adapted leaves. The photochemical and non-photochemical quenching, q_P and q_{NP}, (Van Kooten and Snel, 1990), the PS2 efficiency of energy conversion, F'_v/F'_m, (Krupa et al., 1993), and the estimation of quantum yield of photosynthetic non-cyclic electron transport, ϕ_ε (Genty et al., 1989) were determined under photosynthetic steady-state conditions, using a photon flux density of 550 $\mu mol.m^{-2}.s^{-1}$ as actinic light and 4200 $\mu mol.m^{-2}.s^{-1}$ for the saturating flashes (with a duration of 0.8 s). For the measurement of the energy-dependent component of q_{NP} (q_E; Quick et al., 1989), a saturating flash was applied after the recovery of the fast component of the rise observed in F'_o.

Lipid peroxidation and fatty acids determination

The level of lipid peroxidation products in leaf samples was expressed as 2-thiobarbituric acid-reactive metabolites (mainly malondialdehyde, MDA) according to the method of Heath and Packer (1968). Chloroplast membranes were isolated using 2 g FW leaf tissue according to Droppa et al. (1987), with minor modifications, as described by Lidon and Henriques (1992b). The lipid fraction was then extracted from the pellet of chloroplast membranes according to Allen et al. (1966), in a mixture of chloroform/methanol/water (1:1:1, v/v/v). After evaporation of the chloroform layer, the dry residue was resuspended in 1 ml of a mixture of ethanol:toluene (1:4, v/v) and stored at –20 ºC, until analysis. After saponification with 0.5 M NaOH in methanol, the fatty acids of lipid extracts were methylated with BF3 (Merck) according to Metcalfe et al. (1966) and analyzed by gas liquid chromatography (UNICAM 610 Series Gas Chromatograph, Unicam Ltd., UK). Separation conditions and other details of the method are shown by Campos et al. (2003).

Ethylene production

Ethylene production was measured in 500 µl of the obtained chloroplast membranes incubated in 2 ml flasks at a light intensity of 500–600 $\mu mol.m^{-2}.s^{-1}$, provided by a Björkman lamp. After two hours of incubation a 1 ml gas sample was withdrawn from

the headspace gas of the incubating flask using a gas-tight syringe. Ethylene concentration in this gas sample was assayed by a Pye Unicam Series 204 gas chromatograph equipped with a Porapak Q column and a flame ionization detector (FID). Nitrogen, at a flow rate of 30 ml.min^{-1}, was the carrier gas. The temperatures were set to 90 ºC for the oven, room temperature for the injection port, and 150 ºC for the detector. Ethylene was identified and quantified by comparison with the peak area from the gas samples containing a known concentration (29 µmol.mol^{-1}) of ethylene standard.

Photosynthetic electron transport

Determination of photosynthetic activities coupled to PS2 were measured with a Clark-type oxygen electrode (LW2, Hansatech, Kings Lynn, UK), using the chloroplast membranes obtained as described by Droppa *et al.* (1987), with minor modifications, described by Lidon and Henriques (1992b).The electron transport rates were determined according to Droppa *et al*. (1987) in 1 ml of reaction mixture containing 100–150 µg chl, at 25 ºC and with PPFR of 3000 µmol.m^{-2}.s^{-1}, given by a Bjorkman lamp (Hansatech).

Statistical analysis was performed using a one way ANOVA (for $P < 0.05$).

Results

We measured the peroxidation status of Cu-exposed barley plants by two different methods: by the commonly used test based on thiobarbituric acid reactive metabolites (mainly MDA), and by ethylene production associated with extracted thylakoids. The level of lipid peroxidation products in the leaves of Cu-exposed barley plants, measured as thiobarbituric acid reactive metabolites, was similar to that of control plants at the first two treatments (10 and 15 mg Cu.kg^{-1}), but sharply increased (almost 3-fold) at the highest treatment 20 mg Cu.kg^{-1} (Fig. 1A). On the other hand, the ethylene production associated with the extracted thylakoids was significantly higher in all Cu treatments (Fig. 1B). Since ethylene production associated with thylakoid membrane degradation and mediated by oxy radicals and H_2O_2 is a final product of acyl lipid peroxidation (Lidon and Henriques, 1993), the obtained results showed that excess Cu triggered lipid peroxidation processes in the chloroplasts. Cu treatment clearly resulted in a reduction of total fatty acids (TFA) content of the chloroplast membranes. The decrease in total fatty acids (TFA) was about 57% for 20 mg Cu.kg^{-1} (Table 1). The strongest decrease was detected in the content of linolenic (C18:3) and *trans* hexadecenoic (C16:1*t*) fatty acids, being lower as compared with the control plants by 65% and 68%, respectively. Cu treatments slightly changed fatty acids percentage, but the level of fatty acid unsaturation tended to decrease as indicated by the double bond index (DBI) although it was not significantly different (Table 1).

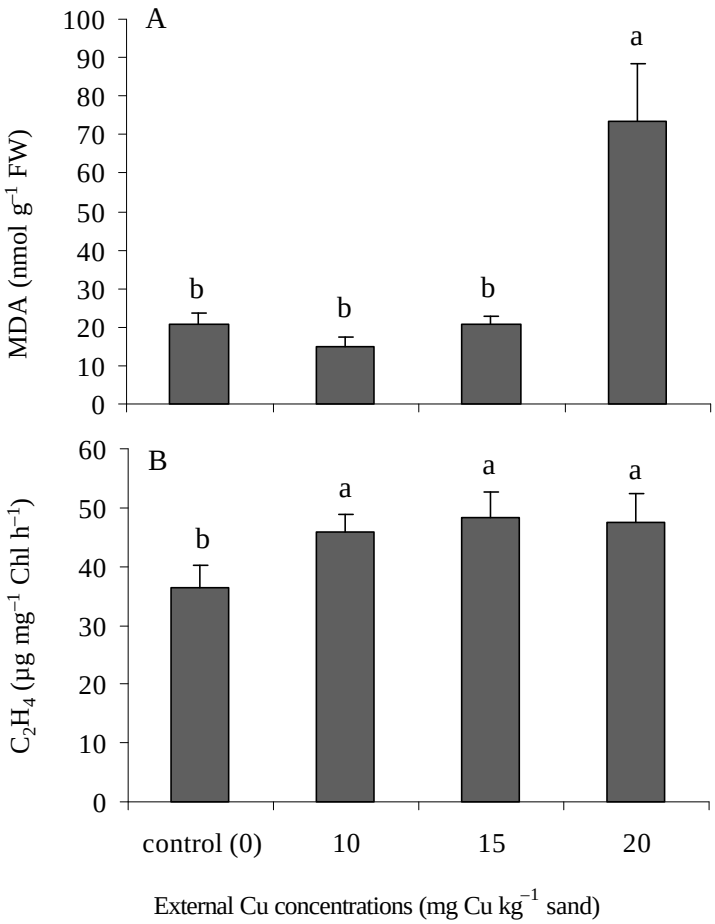

Fig. 1. Leaf lipid peroxidation (A) and ethylene production (B) of photosynthetic membranes isolated from barley plants grown for 10 days in Cu-contaminated sand. Means ±SE (n = 3). Within the same parameter, values followed by the same letters are not significantly different ($P \leq 0.05$).

The photosynthetic performance of Cu-exposed barley plants was evaluated by the activity of photosynthetic electron transport and by chlorophyll fluorescence measurements. The results obtained by both techniques showed that excess Cu inhibited photosynthetic functioning. The activity of photosynthetic electron transport involving PS2 was significantly lowered (Fig. 2). The inhibiting effect of Cu increased gradually with the Cu concentration applied. After a 10 days exposure to 20 mg Cu.kg^{-1}, the decrease in PS2 activity with OEC was about 45% and without OEC – 49%. Chlorophyll fluorescence response of Cu-exposed plants also showed some negative changes (Table 2). Significant reductions of q_P and ϕ_ε values as well as increases in q_{NP} and q_E values have been detected in the last two Cu treatments (15 and 20 mg Cu.kg^{-1}).

Table 1. Total (TFA) fatty acids content (mg.g DW^{-1}), composition (mol %) and unsaturation (DBI) of thylakoid membrane lipids isolated from barley plants grown for 10 days in Cu-contaminated sand. The determined fatty acids were palmitic (16:0), *cis* and *trans* hexadecenoic (16:1), stearic (18:0), oleic (18:1), linoleic (18:2) and linolenic (18:3). The values represent the mean of triplicates. Within each row, data followed with the same letters are not statistically different ($P \leq 0.05$).

Parameter	control (0)	10 mg Cu kg^{-1}	15 mg Cu kg^{-1}	20 mg Cu kg^{-1}
	($mg.g^{-1}$ DW)			
TFA	164.2 a	154.3 a	138.2 a	69.9 b
C18:3	36.8 a	35.7 a	31.6 a	12.7 b
C16:1*t*	2.5 a	1.8 a	1.2 b	0.8 b
	(mol %)			
< C16:0	10.19 a	12.98 a	11.89 a	15.88 a
C16:0	18.36 ab	16.11 b	17.71 ab	22.37 a
C16:1*c*	3.96 ab	3.92 b	4.97 ab	5.51 a
C16:1*t*	3.74 a	2.92 ab	2.15 b	2.69 a
C18:0	0.64 b	0.57 b	0.69 ab	0.96 a
C18:1	0.73 ab	0.58 b	0.65 b	1.11 a
C18:2	6.22 a	5.02 a	4.94 a	6.64 a
C18:3	56.13 ab	57.88 a	56.95 ab	45.53 a
DBI	6.68 a	6.53 a	6.24 a	4.16 a

Double bond index (DBI)=[(C16:1*c*+C16:1*t*+C18:1+2xC18:2+3xC18:3) / (<C16:0+C16:0+C18:0)]

Discussion

The obtained results generally confirmed our suggestion that Cu-induced decrease of net photosynthetic rate (A) in barley plants was related to mesophyll limitations (Vassilev et al., 2002). We identified as a reason for these constrains the ability of Cu to act as an efficient generator of toxic oxygen species that might initiate lipid peroxidation processes (Sandmann and Bõger, 1980). Both tests applied gave us an evidence for enhanced lipid peroxidation in Cu-exposed barley plants. The established higher level of thiobarbituric acid reactive metabolites in leaves of the plants at 20 mg $Cu.kg^{-1}$ treatment probably are due to the oxidizing properties of Cu itself (Weckx and Clijsters, 1996) as well as Cu-provoked stimulation of lipoxygenase activity (Gora and Clijsters, 1989). Cu-induced ethylene production in the thylakoids corresponds with the finding of Lidon et al. (1993) in Cu-exposed rice plants. These authors suggested that it is due to lipid peroxidation processes along with the concurrent formation of linolenate hidroperoxides. Further, excess of Cu can reduce these peroxides via Fenton-type reaction to alkoxy radical, spliting an ethyl radical which in a reaction with Cu is able to produce ethylene (Lidon, 1999).

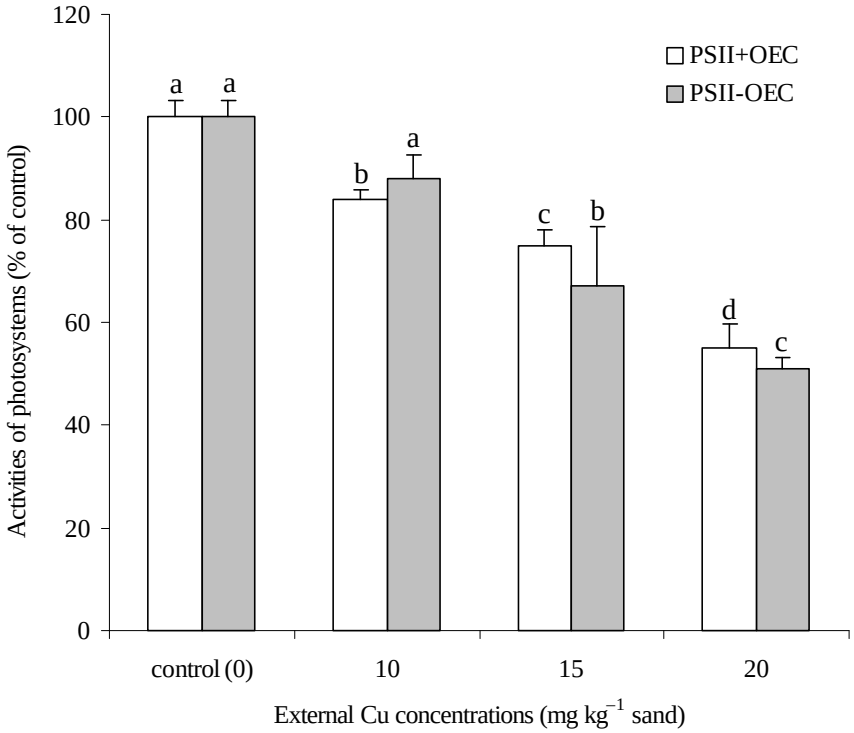

Fig. 2. Rates of photosynthetic electron transport between H_2O and 2,6-dichlorophenol-indo-phenol (DCPIP) (PSII+ oxygen evolving complex - OEC) and 1,5-diphenyl-carbo-hydrazide (DPC) and DCPIP (PSII-OEC) in thylakoid membranes isolated from leaves of barley plants grown for 10 days in Cu-contaminated sand. Control values (representing 100%) were 63.6 and 52.4 µmol O_2 mg.chl^{-1}.h^{-1} for PSII+OEC and PSII-OEC, respectively. Values are the mean ±SE (n = 3). Within the same parameter, values followed by the same letters are not significantly different ($P \leq 0.05$).

Table 2. Selected chlorophyll fluorescence parameters and quenching analysis coefficients in leaves of barley plants grown for 10 days in Cu-contaminated sand. Within each column, data followed with the same letters are not statistically different ($P \leq 0.05$).

Parameter	F_o	F_v/F_m	F'_v/F'_m	q_P	q_{NP}	q_E	ϕ_ε
control (0)	44.8 a	0.797 a	0.488 a	0.497 a	0.782 a	0.472 a	0.242 a
SE	0.3	0.004	0.019	0.012	0.011	0.033	0.013
10 mg Cu kg^{-1}	44 a	0.801 a	0.473 a	0.489 a	0.813 a	0.5 a	0.233 a
SE	1.1	0.007	0.014	0.034	0.011	0.016	0.023
15 mg Cu kg^{-1}	42.4 a	0.811 a	0.508 a	0.426 b	0.798 a	0.565 b	0.216 b
SE	0.7	0.001	0.008	0.017	0.005	0.005	0.01
20 mg Cu kg^{-1}	41.5 a	0.808 a	0.473 a	0.442 b	0.824 b	0.556 b	0.208 b
SE	1.1	0.001	0.014	0.033	0.008	0.036	0.011

Drastically reduced TFA content of the barley thylakoids at 20 mg Cu.kg^{-1} treatment as a result of the enhanced lipid peroxidation may further lead to increased membrane leakness and permeability. The decreased membrane unsaturation level (expressed in a lower DBI value) may be explained by the strong decrease of the linolenic acid (C18:3) in the same treatment. An evidence for the altered fatty acid desaturation process in Cu-exposed plants has been already reported (Lanaras et al., 1993; Quariti et al., 1997). The established lower content of *trans* hexadecenoic (C16:1*t*) may decrease the oligomerization of the light-harvesting complex 2 (LHC2). According to Krupa and Baszynski (1995) the level of C16:1*t* fatty acid is positively correlated with LHC2 oligomerization, because of its specifically binding in *sn*-2 position in the chloroplastic phosphatidylglycerol. Thus, the decreased C16:1*t* content may diminish the content of LHC2 oligomer that means less efficient energy collection and distribution between photosystems.

The inhibiting Cu effect on photosynthetic electron transport in barley plants corresponds with the results of Baszynski et al. (1982) and Lidon and Henriques (1991) found in spinach and rice plants, respectively. The lower f_e observed in plants at 15 and 20 mg Cu.kg^{-1} treatment, clearly indicated lower efficiency of light utilisation (Table 2). Obviously, it was due to a decrease in q_P as F'_v/F'_m, a measure of PSII photochemical efficiency under steady-state light conditions (Krupa et al., 1993), was not depressed considerably. The smaller fraction of the open PS2 reaction centers (q_P) together with a q_{NP} and q_E tendency to increase supports the suggestion of Maksymec (1997) for feedback control due to reduced demand for ATP and NADPH. In this way, Cu-induced alterations in primary carbon metabolism (Stiborova et al., 1986; Lidon and Henriques, 1991; Angelov et al., 1993) may lead to down-regulation of PS2 activity. To some extend this may be also due to an alteration of source-sink relationship with a consequently diminished requirement for products of photosynthesis as was suggested by Ciscato et al. (1997).

In conclusion, the 10 days exposure of barley plants to excess Cu induced serious disturbances of the chloroplast membranes and PS2 activity. The observed increase of the thiobarbituric acid reactive metabolites as well as the ethylene production associated with the thylakoids gave some evidences for Cu-enhanced lipid peroxidation processes. These negative effects had a great impact on photosynthetic performance presented by both lower electron transport activities and efficiency of light utilisation.

Acknowledgments. The authors thank Tech. Eng. Carlos S. Carvalho and Ana P. Ramos from EAN, Oeiras, Portugal for technical assistance as well as the Dept. Plant Physiology in EAN for the working facilities provided.

References

Allen, C. F., P. Good, H. S. Davis, P. Chisum, S. D. Fowler, 1966. Methodology for separation of plant lipids and application to spinach leaf and chloroplast lamellae. J. Amer. Oil Chem. Soc., 43, 223–230.

Angelov, M., T. Tsonev, A. Uzunova, K. Gaidardjieva, 1993. Cu^{2+} effects upon photosynthesis, chloroplast structure, RNA and protein synthesis of pea plants. Photosynthetica, 28(3), 341–350.

Baszynski, T., M. Krol, Z. Krupa, M. Ruszkowska, U. Wojcieska, D. Wolinska, 1982. Photosynthetic apparatus of spinach exposed to excess copper. Z. Pflanzenphysiol., 108, 385–395.

Campos, P. S., V. L Quartin, J. C. Ramalho, M. A. Nunes, 2003. Electrolyte leakage and lipid degradation account for cold sensitivity in leaves of *Coffea sp*. plants. J. Plant Physiol., 160, 283–292.

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

Droppa, M., J. Masojidek, Z. Rosza, A. Wolak, L. Horvath, I. Farkas, E. Horvath, 1987. Characteristics of Cu deficiency-induced inhibition of photosynthetic electron transport in spinach chloroplasts. Biochim. Biophys. Acta, 891, 75–84.

Genty, B., J. Briantais, N. Baker, 1989. The relation between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim. Biophys. Acta, 990, 87–92.

Gora, L., H. Clijsters, 1989. Effects of copper and zinc on the ethylene metabolism in *Phaseolus vulgaris* L. In: Biochemical and physiological aspects of ethylene production in lower and higher plants. Eds. H. Clijsters, M. De Proft, R. Marcelle, M. Van Poucke, Kluwer Acad. Publ., Dordrecht, 219–228.

Heath, R., L. Packer, 1968. Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Arch. Biochem. Biophys., 125, 189–190.

Kamenova-Jouhimenko, S., V. Georgieva, J. Markovska N. Georgieva, M. Boichinova, 1999. Changes in the bioproductivity and enzyme activity at C-3 type of plants, grown under conditions of Cd and Cu toxicity. In: Achievements and perspectives of mineral nutrition and water relations of plants in Bulgaria, 1999, 133–135.

Kamenova-Jouhimenko, S., V. Georgieva, Tz. Tzonev, V. Gecheva, 2003 Kamenova-Jouhimenko, S., V. Georgieva, The influence of surplace of Cd and Cu on photosynthesis, transpiration and bioproductivity of pea. Ecology and future,. 2(1), 25–28.

Krupa, Z., G. Öquist, N. Huner, 1993. The effects of cadmium on photosynthesis of *Phaseolus vulgaris* – a fluorescence analysis. Phisiol. Plant., 88, 626–630.

Krupa, Z., T. Baszynski, 1995. Some aspects of heavy metals toxicity towards photosynthetic apparatus – direct and indirect effects on light and dark reactions. Acta Physiol. Plant., 7, 55–64.

Lanaras, T., M. Moustakas, L. Symeonidis, S. Diamantoglou, S. Karataglis, 1993. Plant metal content, growth responses and some photosynthetic measurements on field-cultivated wheat growing on ore bodies enriched in Cu. Physiol. Plant., 88, 307–314

Landjeva, S., M. Merakchiyska, and G. Ganeva, 1998. Seedlings copper tolerance and cytogenrtic haracterization of wheat–*Aegelops ovata* hybrid lines. Wheat information. No 87, 27–30

Merakchiyska-Nikolova, M., I. Yordanov, 1983. Influence of some heavi metals on the growth, pigment contents and photosynthetic activity of bean plants. In: Third Natl. Botanical Conference , Plovdiv, Bulgaria, pp. 848– 853 (in. Bulg.)

Lidon, F. C., 1999. An overview of the effects of excess copper on the photosynthesis of rice pants. Agronomia Lusitana, 47, 69–88.

Lidon, F., F. S. Henriques, 1991. Limiting step on photosynthesis of rice plants treated with varying copper levels. J. Plant Physiol., 138, 115–118.

Lidon, F., F. S. Henriques, 1992a. Effects of excess copper on the photosynthetic pigments in rice plants. Bot. Bull. Academia Sinica, 33, 141–149.

Lidon, F., F. S. Henriques, 1992b. Changes in the contents of photosynthetic electron carriers, RNAse activity and membrane permeability. Photosynthetica, 26, 371–380.

Lidon, F., F. S. Henriques, 1993. Oxygen metabolism in higher plant chloroplasts. Photosynthetica, 29, 249–279.

Lidon, F. C., J. C. Ramalho, F. Henriques, 1993. Copper inhibition of rice photosynthesis. J. Plant Physiol., 142, 12–17.

Maksymiec, W., 1997. Effects of copper on cellular processes in higher plants. Photosynthetica, 34 (3), 321–342.

Metcalfe, L. D., A. A. Schemitz, J. R. Pelka, 1966. Rapid preparation of fatty-acid esters from lipids for gas chromatographic analysis. Anal. Chem. 38, 514–515.

Quariti, O., N. Boussama, M. Zarrouk, A. Cherif, M. H. Ghorbal, 1997. cadmium- and copper-induced changes in tomato membrane lipids. Phytochemistry, 45(7), 1343–1350.

Quick, W.P., M. Stitt, 1989. An examination of factors contributing to non-photochemical quenching of chlorophyll fluorescence in barley leaves. Biochim. Biophys. Acta, 997, 287–296.

Sandmann, G., O. Bõger, 1980. Copper-mediated lipid peroxidation processes in photosynthetic membranes. Plant Physiol., 66, 797–800.

Shainberg, O., B. Rubin, H. D. Rabinowitch, E. Tel-Or, 2001. Loading beans with sublethal levels of copper enhances conditioning to oxidative stress. J. Plant Physiol., 158(11), 1415–1421.

Stiborova, M., M. Doubravova, S. Leblova, 1986. A comparative study of the effect of heavy metal ions on ribulose-1,5-biphosphate carboxylase and phosphoenolpyruvate carboxylase. Biochem. Physiol. Pflanz., 181, 373–379

Stoynova, E., M. Merakchiyska-Nikolova, 1991. Ultrastructure of the formating photosynthetic apparatus of bean (*Phaseolus vulgaris* L.) under the action of $CuSO_4$ and kinetin. Compt. Rend. Bulb. Acad. Sci., 44(12), 93–96

Stoinova, J., M. Merakchiyska, Z. Sabeva, S. Phileva, S. Paunova. 1998/1999. Productivity and sensibility to heavi metals of new lines of triticale. Genetics breedings, 29(4), 25–31

Van Kooten, O., J. F. H. Snell, 1990. The use of chlorophyll fluorescence nomenclature in plant stress physiology. Photosynth. Res., 25, 147–150.

Vangronsveld, J. H. Clijsters, 1994. Toxic effects of metals. In: Plants and the chemical elements. Biochemistry, uptake, tolerance and toxicity, Ed. M. E. Farago, VCH Publishers, Weinheim, Germany, 150–177.

Vassilev, A., F. C. Lidon, M. do Céu Matos, J. C. Ramalho, I. Yordanov, 2002. Photosynthetic performance and some nutrients content in cadmium– and copper–treated barley plants. J. Plant Nutr., 25(11), 2343–2360.

Weckx, J. E. J., H. Clijsters, 1996. Oxidative damage and defense mechanisms in primary leaves of *Phaseolus vulgaris* as a result of root assimilation of toxic amounts of copper. Physiol. Plant., 96, 506–512.