EFFECT OF INCREASING Cu^{2+} CONCENTRATIONS ON GROWTH AND CONTENT OF FREE PHENOLS IN TWO LINES OF WHEAT *(TRITICUM AESTIVUM)* WITH DIFFERENT TOLERANCE

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Summary. In the present work, we described the effects of three different concentrations of Cu^{2+} (10^{-6}, 10^{-5}, 10^{-4}M) on the growth and accumulation of free phenols in the roots and the above-ground parts of 16-day-old plants of two different lines (LTL1 and TL19) of wheat (*Triticum aestivum* L.) The lines were isolated from hybrids of *Avrora* cultivar and its genome replaced form *Avrosis* (AABBSshSsh, 2n=41-42) in which the wheat D-genome was replaced by the Ssh-genome of *Aegilops sharonesis* Eig. TL19 line was tolerant whereas LTL1 line showed lower tolerance to Cu^{2+} excess. Differences in the growth parameters of the two lines were observed mainly at a concentration of 10^{-5}M Cu^{2+} which was inhibitory for LTL1 and stimulating for TL19. The content of free phenols was found to increase in both lines with increasing Cu^{2+} concentration in the medium. In the roots free phenols increased in plants grown at 10^{-5} M $CuSO_4$ whereas in the above-ground parts 10^{-4} M $CuSO_4$ was the most effective concentration. The higher

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tolerance of TL19 line to Cu^{2+} toxicity correlated with higher ability for accumulation of free phenols.

***Key words*:** Cu^{2+} toxicity, free phenols content, tolerance, *Triticum aestivum*.

INTRODUCTION

Cu is a microelement with important physiological functions in plants, however, at higher concentrations it can become toxic, thus leading to physiological and morphological disturbances and, eventually to decreased yield (Nicholls and Tarun, 2003; Agrawal and Sharma, 2006). Generation of reactive oxygen species (ROS) is the major response of plants to toxic concentrations of heavy metals including Cu (Shützendübel and Polle, 2002; Maksymiec and Krupa, 2003). ROS levels are controlled by the antioxidant system which includes enzymes and secondary metabolites (Schützendübel and Polle, 2002; Imaculada, 2005). Phenolic compounds are powerful antioxidants and it has been proved that the decrease in the effect of stress as well as genetic resistance of plants to fungal diseases and abiotic factors correlate with the amount of total free phenols (Beta et al., 2005; Olichenko and Zagoskina, 2005; Zagoskina et al., 2005).

To form defense mechanisms for survival under conditions of high concentrations of different chemical elements plants have to undergo natural selection either in conditions of pollution or in a simulating selective medium (Macnair, 1993). Our previous results have also shown that upon selection of plants whose root growth in conditions of heavy metal stress is not seriously depressed, their tolerance to Cu toxicity is increased in the next hybrid generations (Ganeva et al., 2005).

The aim of the present work was to study the effects of increased Cu^{2+} concentrations on the growth and accumulation of phenols in two lines of wheat differing in their tolerance to Cu^{2+} excess.

MATERIALS AND METHODS

Plant material

Two wheat lines showing different tolerance to increased Cu^{2+} concentrations in an aqueous solution of $CuSO_4$ (the low tolerant line LTL1 and the tolerant line TL19) were used throughout the experiments. The lines were created by selection based on the tolerance to Cu^{2+} excess of hybrids of *Avrora* cultivar and its genome replaced form *Avrosis* (AABBSshSsh, 2n=41-42) in which the wheat D-genome was replaced by the Ssh-genome of *Aegilops sharonesis* Eig (Ganeva et al., 2005). Measurements were done on the 16th day from the onset of germination.

Embryo culture

For sterilization seeds were treated with detergents (70% ethanol and 0.1 % $HgCl_2$). Immature embryos were isolated and cultured on hormone-free Murashige and Skoog nutrient medium (Murashige and Skoog, 1962) supplemented with Cu^{2+} applied as $CuSO_4.5H_2O$ at different concentrations (10^{-6}, 10^{-5}, 10^{-4} M). Sucrose and agar-agar contents were constant (6 % and 0.7%, respectively).

Tolerance test

The test was performed on *in vitro* obtained seedlings. Root number, root and shoot lengths were measured on the 16th day from the onset of germination. The results are the mean values of three different experiments.

Determination of free phenols (FP)

Free phenolic compounds were extracted from the plant material with hot 70 % ethanol and determined spectrophotometrically using Folin reactive (Swein and Hills, 1959). Chlorogenic acid was used to draw a calibration curve. The results are the mean values of three different experiments.

The data presented are expressed as means ± SD. Statistical evaluation of the data was carried out using Student's t-test.

RESULTS AND DISCUSSION

The responses of the two wheat lines to the increasing Cu^{2+} concentrations in the medium were similar to our previous results established during cultivation on an aqueous solution of $CuSO_4$ (Ganeva et al., 2005). The comparison between the two experiments showed that at 10^{-6} M Cu^{2+} the growth of roots in plants grown on an aqueous solution of $CuSO_4$ was inhibited. On the other hand, cultivation on a nutrient medium at the same concentration of Cu^{2+} resulted in enhanced growth of the plants of both lines which was expressed to a greater extent in LTL1 line showing accelerated

Lines	Roots		Shoots		Plants	
$[Cu^{2+}]$	(mg g $^{-1}$ FW)	%	(mg g $^{-1}$ FW)	%	(mg g $^{-1}$ FW)	%
LTL1						
Control	0.42±0.015	100.0	1.13±0.021	100.0	1.55±0.04	100.0
10^{-6}	0.36±0.010*	85.7	1.06±0.025***	93.8	1.42±0.03	91.6
10^{-5}	0.69±0.012***	164.3	1.12±0.010	99.1	1.81±0.03	116.8
10^{-4}	0.51±0.012**	121.4	1.54±0.010**	136.3	2.05±0.03*	132.3
TL19						
Control	0.63±0.017	100.0	1.25±0.015	100.0	1.88±0.03	100.0
10^{-6}	0.63±0.017	100.0	1.00±0.025***	80.0	1.63±0.04	66.7
10^{-5}	1.00±0.06***	158.7	1.30±0.021	104.0	2.30±0.03	122.3
10^{-4}	0.64±0.05	101.6	1.64±0.006 ***	131.2	2.28±0.01	121.3

Table 1. Free phenols content in roots, shoots and whole plants of two wheat lines (LTL1 and TL19) differing in tolerance to increased Cu^{2+} concentrations. LSD at * - $P<0.05$, **-$P<0.01$, ***-$P<0.001$.

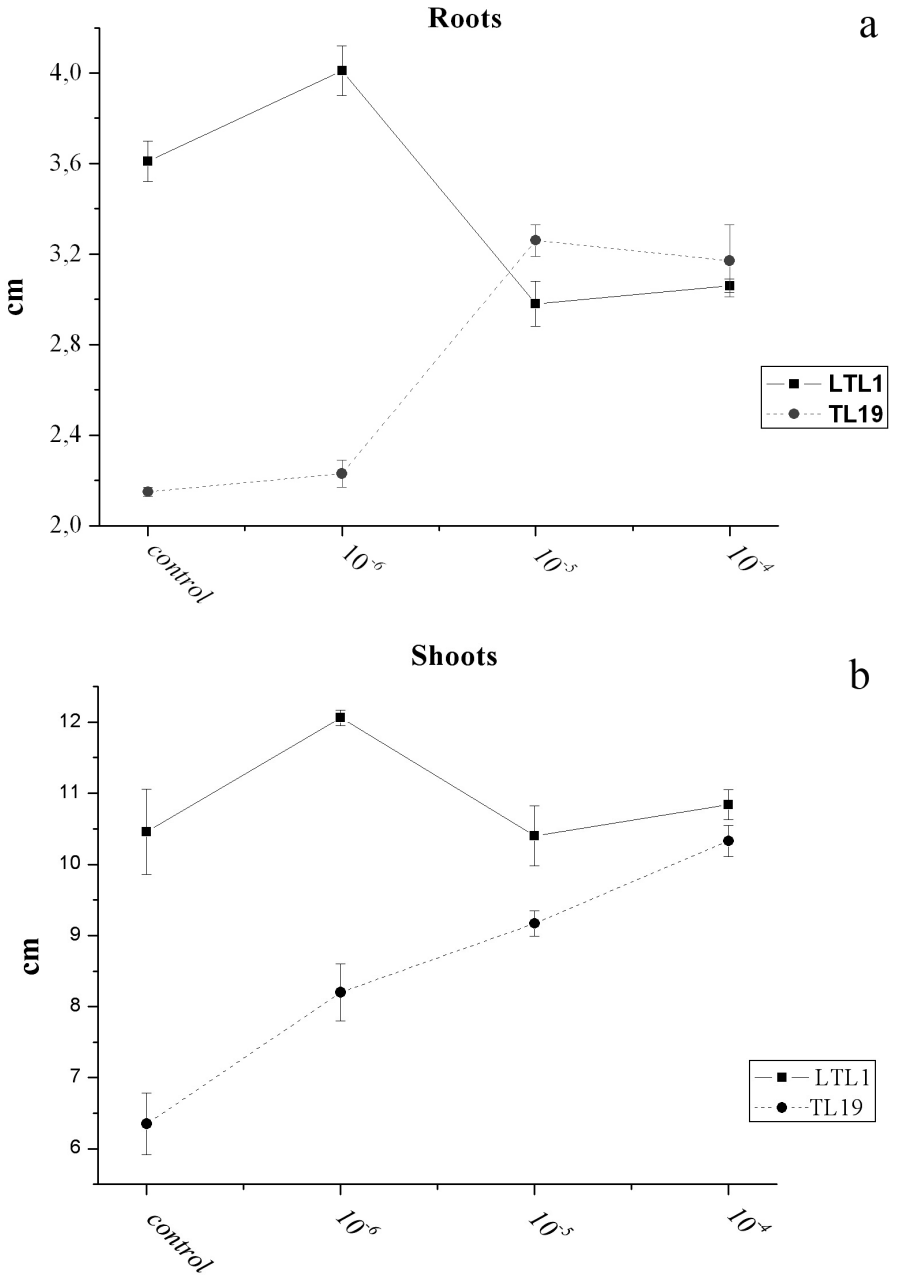

Fig. 1. Lengths of roots (a) and shoots (b) in plants of two wheat lines (LTL1 and TL19) grown at different Cu^{2+} concentrations (10^{-4}, 10^{-5} and 10^{-6} M). Values represent the means of three different experiments ± SD.

growth in the control, as well (Fig. 1). The most pronounced differences between the two lines were observed at a concentration of 10^{-5}M Cu^{2+} which was inhibitory for LTL1 and stimulating for TL19. These results indicated that TL19 line was more tolerant to Cu^{2+} toxicity.

The tolerance to Cu^{2+} excess is determined also by plant ability to reduce the translocation of Cu^{2+} in the above-ground parts which delays reaching the toxicity threshold level (De Vos et al., 1991; Dietz et al., 1999). Our results showing lack of inhibitory effect of Cu^{2+} treatment on the height of the young plants of the tolerant line TLT19 (Fig. 1) confirmed the defense role of this mechanism against heavy metal toxicity. It has been reported that heavy metal-tolerant ecotypes show delayed growth when compared with the sensitive ecotypes (Macnair, 1993). Our results showed lower values for the growth parameters in the tolerant line (Fig. 1). On the other hand, the content of soluble phenols was higher when compared with LTL1 line, especially in the roots (by 50%) (Table 1). These results were expected as phenols are included in the inhibitory plant complex and a reduction in their content is accompanied by morphogenetic processes whereas their increase correlates with a reduction in growth (Kefeli, 1997).

A lot of data have been reported showing that accumulation of phenols is species-, seasonal- and organ-specific. Our data were in accordance with the data reported by Zagoskina et al. (2005) showing low phenol levels in the roots compared with the above-ground parts in *Triticum aestivum* L. Furthermore, the differences observed between the controls of the two wheat lines suggest the existence of interspecies specificity in the accumulation of phenols (Table 1). Our results showing variations in the levels of free phenols due to Cu^{2+} treatment confirmed the role of these compounds in the plant defense system under stress conditions. Similar data for increased content of some phenolic compounds after Cu^{2+} treatment were reported also for *Raphanus sativus* (Sgherri and Navari-Izzo, 2003). The highest phenol content in the roots of both wheat lines was measured in plants grown at 10^{-5}M of $CuSO_4$ whereas in the above-ground parts the most effective concentration was 10^{-4}M (Table 1).

The changes in the content of free phenols at increasing Cu^{2+} concentrations showed the same trend in both lines studied (LTL1 and TL19). Furthermore, the increase measured in the roots, being more

sensitive to Cu^{2+} excess compared with the above-ground parts, was similar in both wheat lines. The major difference was the ability of TL19 line, showing lower reduction in the growth, to maintain higher phenol levels at all Cu^{2+} concentrations applied. Based on the well known functions of free phenols as growth regulators and antioxidants, the tolerance to Cu^{2+} toxicity observed in TL19 line could be at least partly accounted for by the higher capacity for accumulation of free phenolic compounds.

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