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EFFECT OF COPPER ON PHYSIOLOGICAL AND BIOCHEMICAL PECULIARITIES OF WHEAT (*TRITICUM AESTIVUM* L.) VARIETIES

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Abstract

The effect of different concentrations (0.25mM, 0.5mM) of Cu^{2+} on growth parameters were investigated in hydroponically grown five wheat (*Triticum aestivum* L.) varieties (Kazakhstanskaya rannaya, Kazakhstanskaya-3, Melturn, Kaiyr and Shagala). Sensitive and tolerant wheat varieties were taken for other physiological and biochemical analysis: RWC, proline and malondialdehyde (MDA) content, photosynthetic pigments and peroxydase and superoxide dismutase activity. Wheat varieties exposed to 0.5mM Cu^{2+} exhibited significant growth reduction. The results showed that growth, physiological and biochemical parameters were significantly reduced at 0.5 mM Cu^{2+} compared to untreated control plants. Wheat varieties tolerant and sensitive to Cu^{2+} were identified. MDA content in tolerant variety was less as compared to varieties sensitive to copper. In tolerant variety photosynthetic pigments (chlorophyll-*a*, chlorophyll-*b*, carotenoids) content was decreased to a less extent compared to sensitive varieties. The highest SOD and POD activity was observed at 0.5mM of copper.

Key words: Wheat, Heavy metal, Copper, Oxidative stress.

Introduction

Copper (Cu) is an essential trace element for plants. But when copper is absorbed in large quantities, it becomes toxic and causes inhibition of plant growth and development. IT has a negative impact on the physiological and biochemical processes, as well as the cause of various anomalies in the anatomical structure of the leaves and roots (Maksymiec, 1997; Hakmaoui *et al*., 2007; Gostin, 2009; Cvetanovska *et al*., 2010; Hussain, 2010; Gomes *et al*., 2011). As a result of disturbance of the physiological and biochemical processes, a reduction in the yield of crops is reported (Kabata-Pendias, 2001; Cheng 2003; Fuentes *et al*., 2007 Meng *et al*., 2007; Tekli *et al*., 2008; Haribabu & Sudha, 2011; Hatamzadeh *et al*., 2012. Azooz *et al*., 2012).

In plants like *Tanacetum vulgare* L. under copper stress the thickness of the lower and upper epidermis is changed. A decrease in leaf mesophyll cells, reduction in palisade parenchyma, as well as decrease in chlorophyll content in leaves have been reported (Mikovilovi & Dragosavac 2010).

Heavy metals, especially Cu causes an increase in the content of malondialdehyde - a product of lipid peroxidation. Malondialdehyde content indicates the level of lipid peroxidation, i.e. oxidative stress. Lipid peroxidation leads to damage of structure and functions of membranes, and can also cause permanent damage of cell functions (Reinheckel *et al*., 1988; Mathad & Pratima 2009; Pätsikkä *et al*., 2002). Copper participates in the Fenton reaction, and is capable of generating reactive oxygen species (ROS), and thus can cause oxidative stress. ROS cause oxidative modification of proteins and produce reactive aldehydes - products of lipid peroxidation, which leads to degeneration of membranes. Another possible object of reactive oxygen species - the oxidation of proteins. ROS cause oxidation of the amino acids (Mathad & Pratima, 2009). Copper causes a reduction in chlorophyll content of various plant species. The rice plants (*Oryza sativa* L.), which were grown in the medium, polluted by copper, the content of chlorophyll *a* and *b* and carotenoids was decreased with increasing concentration of metal (Lidon & Henriques, 1992).

Oxidative stress, caused by an excess of Cu ions, also destroys chlorophyll, which in turn leads to a decrease in carotenoid synthesis. Copper indirectly affects the synthesis of photosynthetic pigments by terpenoid biosynthetic pathways (Droppa, 1988).

Significant areas of soil and water near large industrial complexes in Kazakhstan are contaminated by copper ions; particularly in the area around the production and refining of copper, such as "BalkhashMed" and "ZhezkazganTsvetMed" (Panin, 2000; Tasekeev, 2004). Industrial wastes from copper mining companies in the Balkhash region contaminate soil and waters adjacent to the lake Balkhash, where wheat is a common crop (Aubekerov *et al*., 2014). In this regard, identification of wheat varieties that are resistant to Cu stress, and understanding the physiological and biochemical basis of resistance is one of the essential tasks in the area of food safety and to increase crop production. Selection of Cu tolerant wheat varieties is one of the most effective tools to improve productivity of Cu contaminated soils. The objective of the present work was to identify Cu-tolerant wheat varieties, and determine the physiological and biochemical features of selected varieties in response to Cu stress. Five commonly used wheat varieties in Kazakhstan were tested for their sensitivity/tolerance to Cu stress using phenotypic and biochemical parameters. These varieties were: Kazakhstanskaya-3, Kazakhstanskaya rannaya, Melturn, Kaiyr, and Shagala.

Materials and Methods

Plant material, treatments: The five wheat varieties: Kazakhstanskaya-3, Kazakhstanskaya rannaya, Melturn, Kaiyr, Shagala were selected. Seeds were germinated in a growth chamber at 25ºC. Plants were grown in hydroponic conditions for 7 days in water, containing various concentrations of copper. Three treatments were defined as: no Cu added (control), 0.25mM$CuSO_4$ (low $CuSO_4$ concentration) and 0,5 mM $CuSO_4$ (high $CuSO_4$ concentration).

Copper tolerance index: Measurement of biometric parameters were made according to routine methods. Copper tolerance index (TI) was calculated as the quotient of the dry weight of plants grown in the presence of Cu and control conditions (Bálint *et al*., 2002) according to the formula:

$$TI\ (\%) = \frac{\text{Dry weight of Cu-treated plants}}{\text{Dry weight of control plants}} \times 100$$

Determination of photosynthetic pigments: It was performed as described by Shlyk (1971). Leaves 0.1 g were ground in 80% acetone, filter and bring up to 25 ml of a solution of acetone. Then the absorbance was measured at different wavelengths

Absorbance was measured with a spectrophotometer at wavelengths of 665, 649 and 440.5 nm. Calculation of the content of pigments was done by the following formula:

Chl_a (mg/L) = 11.63 x A_{665} – 2.39 x A_{649};
Chl_b (mg/L) = 20.11 x A_{649} – 5.18 x A_{665};
Chl_a+Chl_b (mg/L) = 6.45 x A_{665}+17.72 x A_{649},

where Chl – chlorophyll concentration, mg/L, A – Absorbance at a given wavelength.

Carotenoid content was determined by the formula:

Car (mg/L) = 4.695x$A_{440,5}$ – 0.268(Chl_a+Chl_{bB}),

where Car – carotenoids concentration, mg/L, Chl – chlorophyll concentration, mg/L; A – optical density at a given wavelength.

Then the pigment content per mg/g was recalculated:

$$A = ChlV/P \times 1000,$$

where A - pigment content, mg/g, Chl– chlorophyll concentration, mg /L; V - volume hoods, ml; P – plant sample weight, g.

Determination of relative water content (RWC): It was performed according to Schonfeld *et al.* (1988). It was measured wet weight of 20 disks of leaves. Turgid weight (TW) was determined after incubation of disks in distilled water for 16-18 hours. After incubation, the wheels quickly and carefully dried with a paper for determination of TW. The dry weight (DW) was measured after drying in oven at 70^{o}C for 72 hours.

RWC calculated using the formula:

$$RWC = [(FW - DW)/(TW - DW)] \times 100.$$

A quantitative method for the determination of proline content (Bates *et al*., 1973). The leaves of five seedlings were weighted and were poured by 5 ml of boiling distilled water. The tubes were placed in a water bath, heated to boiling, boiled for 30 min. and cooled. For control variant the tube containing 1 ml of distilled water, 1 ml of ninhydrin reagent and 1 ml of glacial acetic acid are placed in a water bath. For test variants instead distilled water it was taken 1 ml of vegetable extract. All tubes were heated for 1 hour and then cooled. Absorbance of solutions is measured spectrophotometrically at 522 nm. Proline concentration is determined using the previously prepared calibration curve. Then proline content in the samples is calculated using the formula:

A = n x V/P, where
a - content of proline
n - value of the calibration curve,
V - volume of dilution, ml
P - sample weight

Determination of lipid peroxidation: Lipid peroxidation was measured by a method based on the registered amount of malondialdehyde formed by reaction with 2-thiobarbituric acid (TBA) (Merzlyak *et al*., 1978).

Weigh roots (1 g) was grinded under cooling with 4 ml Tris-HCl buffer (pH 7.6), then filtered and the filtrate was centrifuged for 20 min at 8000 rpm. From the centrifugate was 2 ml of homogenate, 0.5 ml of distilled H_2O and 2.5 ml of 0.5% 2-thiobarbituric acid in 20% trichloroacetic acid (TCA). The samples was incubated for 30 min in a water bath and cooled. Then they were centrifuged for 20 min at 7000 rpm. Pellet carefully decanted into clean tubes and absorbance was measured by a spectrophotometer at 532 nm.Calculation of the content of malondialdehyde (MDA) was performed by the formula:

$$C = \frac{A \times V \times \gamma,}{\varepsilon}$$

where A - absorbance;
V - volume of the cuvette;
γ - dilution; ε - extinction coefficient 0.155 mM/cm^2.
The content of MDA was expressed in μM/g wet weight.

Determination of superoxide dismutase (SOD) activity: Leaves (approximately 0.1 g) were grinded in a mortar in an extraction mixture consisting of 50 mM phosphate buffer (pH 7.8), 1.0 mM EDTA, 0.05% Triton X-100, 2% polyvinilpolypirrolydon (PVPP) and 1 mM of ascorbic acid. The homogenate is centrifuged at 16,000 g for 15 min. The supernatant was used for determination of SOD activity.

SOD activity was assayed by the method described by Beauchamp &Fridovich (1971), based on the inhibition of photochemical reduction of nitro blue tetrazolium (NBT) at 560 nm with the following modification:

The reaction mixture (1 ml) contains phosphate buffer (pH 7.8), EDTA, 13 mM methionine, 75 mM NBT, 16.7 μM riboflavin and 0.1 ml of enzymatic extract (protein content around 50 mg). Riboflavin was added last and the reaction initiated by placing the tubes under two fluorescent lamps of 9 watts. The reaction was stopped after 15 min by removing the tube from the light source. Maximum recovery of NBT and hence maximum light absorption test sample was compared with the control sample absorption of light and thus obtaining the percentage of inhibition. The amount of enzyme, required for 50% inhibition of photoreduction of NBT, was taken

as the unit of enzyme activity. Specific activity of SOD is expressed in units per mg of protein.

Determination of peroxidase (POD) activity: The cells were homogenized in a medium containing 0.05 M acetate buffer, pH 5.6. The ratio of the volume of homogenization medium and a plant sample was 10:1. The homogenate was centrifuged for 15 minutes at 14,000 rpm. The precipitate was discarded and the supernatant ("crude extract") was used to determine the activity of POD. The reaction mixture contained 0.05 M acetate buffer, 6.4 mM solution of o-dianisidine, 15 mM of hydrogen peroxide solution and the extract enzyme (10-50 mg protein/ml). After a quick mixing of the reagents the changes in optical density were followed. The initial speed o-dianisidine oxidation at room temperature at 460 nm was noted. The reaction rate was determined from the tangent of the angle of inclination of the initial straight portions of kinetic changes in optical density over time, according to the method of Lebedev (1977).

ANOVA with varieties and treatment as main effects for physiological and biochemical parameters was done. All values are expressed as the mean of three measurements for each treatment. Least significant difference (LSD) test was used to assess the differences between the mean values of control and Cu-treated plants; $p<0.05$ was considered statistically significant.

Results

Growth parameters: The effect of Cu (0.25 and 0.5 mM $CuSO_4$) on length and biomass of root and leaf organs of the five wheat varieties was studied. These parameters decreased as Cu^{2+} increased in all varieties. The analysis of variance for shoot and root length is presented in Table 1 and it showed significant effect ($p<0.01$) of both concentrations of Cu in both traits (Table 1). At high concentration of Cu^{2+}, length of roots of Kaiyr variety decreased significantly as compared to other varieties.

Application of the intense Cu stress (0.5 mM) showed a decrease in the length of roots and shoots in relation to control in all studied varieties: length of roots - Melturn (56%) > Kazakhstanskaya rannaya (55%) > Kazakhstanskaya-3 (53%) > Shagala (52%) > Kaiyr (42%); shoots - Melturn(53%) > Kazakhstanskaya rannaya (43%) > Kaiyr(36%) > Kazakhstanskaya-3 (32%) > Shagala (26%) (Table 1). The comparison of means between all varieties for the growth traits showed that high Cu^{2+} concentration had significant adverse effect on biomass of shoots of Kayir, Kazakhstanskaya-3, Shagala cvs. The shoots biomass of these varieties decreased by 64, 68, 74%, respectively.

Biomass accumulation by roots in Melturn and Kazakhstanskaya rannaya varieties was decreased in low extent as compared to other species. Roots biomass at 0.5 mM $CuSO_4$ was decreased in the following order: Kazakhstanskaya rannaya (53%) > Melturn (50%) > Kaiyr (45%) > Kazakhstanskaya-3 (30%) > Shagala (20%) ($p<0.01$) (Fig. 1). On Cu tolerance index (biomass accumulation by shoots as a % to control) varieties can be arranged as follows: Kazakhstanskaya rannaya (55%) > Melturn (53%) >Shagala (44%) > Kaiyr (30%) = Kazakhstanskaya-3 (30%) ($p<0.01$) (Fig. 2).

By growth parameters at high Cu concentration (0.5 mM). Kazakhstanskaya rannaya and Melturn were more tolerant compared with other varieties, Kaiyr and Kazakhstanskaya-3 were more sensitive to Cu.

Table 1. Effect of copper on growth parameters of wheat varieties. Differences within variety across treatments for shoots and roots are significant ($p<0.01$). For shoots LSD - 5.36; for roots LSD – 3.0 at $p<0.05$.

Treatments	Height of shoots**		Length of roots***	
	Sm	%	Sm	%
Kazakhstanskaya rannaya				
Control	18,68 ± 0,35a	100	8,94 ± 0,06	100
$CuSO_4$ - 0,25 mMol/L	8,78 ± 0,17b	47	6,15 ± 0,08	69
$CuSO_4$ - 0,5 mMol/L	7,95 ± 0,07c	43	4,9 ± 0,12	55
Melturn				
Control	15,18 ± 0,15 [a]	100	5,7 ± 0,05	100
$CuSO_4$ - 0,25 mMol/L	8,60 ± 0,16 [b]	57	4,0 ± 0,08	70
$CuSO_4$ - 0,5 mMol/L	8,00 ± 0,16 [c]	53	3,2 ± 0,09	56
Kaiyr				
Control	25,25 ± 0,13 [a]	100	9,11 ± 0,07	100
$CuSO_4$ - 0,25 mMol/L	12,20 ± 0,09 [b]	48	5,60 ± 0,09	62
$CuSO_4$ - 0,5 mMol/L	9,00 ± 0,086 [c]	36	3,85 ± 0,08	42
Kazakhstanskaya-3				
Control	15,85 ± 0,68 [a]	100	5,8 ± 0,17	100
$CuSO_4$ - 0,25 mMol/L	10,90 ± 0,13 [b]	69	4,5 ± 0,18	78
$CuSO_4$ - 0,5 mMol/L	5,00 ± 0,078 [c]	32	3,1 ± 0,15	53
Shagala				
Control	22,32 ± 0,23 [a]	100	8,25 ± 0,06	100
$CuSO_4$ - 0,25 mMol/L	12,51 ± 0,234 [b]	56	6,53 ± 0,068	79
$CuSO_4$ - 0,5 mMol/L	5,90 ± 0,023 [c]	26	4,33 ± 0,080	52

Means within columns for the shoots with the same letters are not significantly different. Means within columns for the roots are significantly different

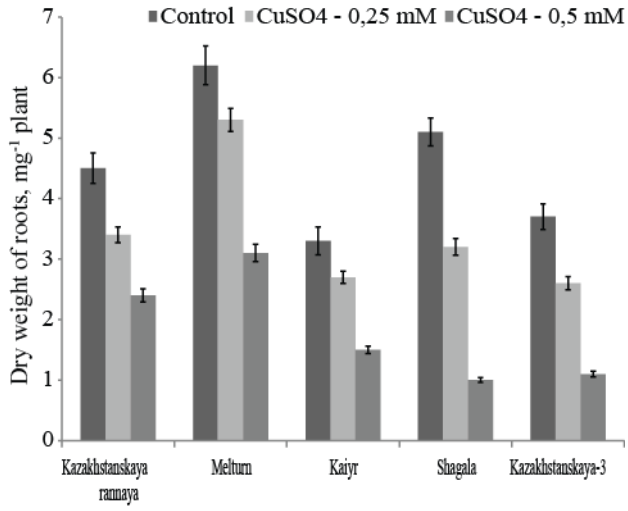

Fig. 1. Effect of copper (Cu^{2+}) on roots biomass of wheat varieties. Vertical bars represent ± SD of three replicates (*n*=3) LSD=1.08 at p<0.05.

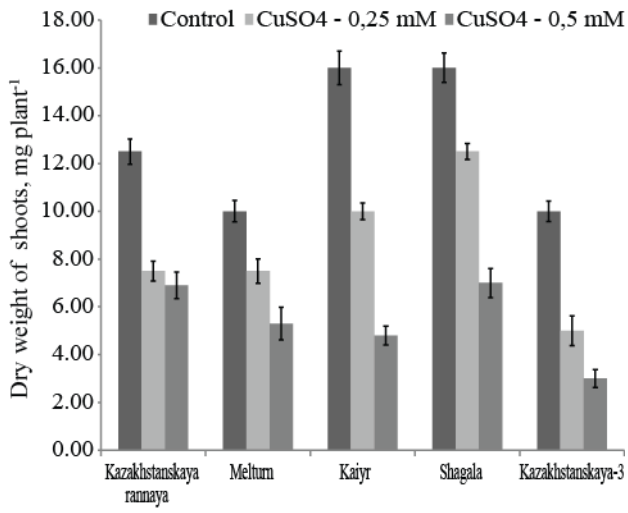

Fig. 2. Effect of copper (Cu^{2+}) on shoots biomass of wheat vatieties.Vertical bars represent ± SD of three replicates (*n*=3), LSD=3.19 at p<0.05

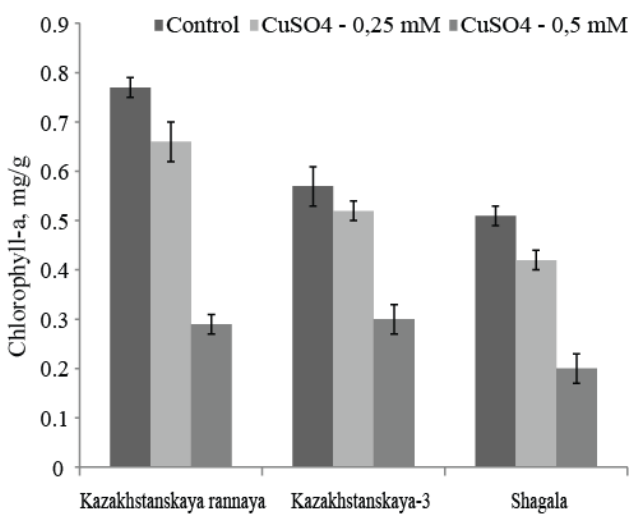

Fig. 3. Effect of copper on chlorophyll-*a* content in wheat leaves in the presence of copper in the medium. Vertical bars represent ± SD of three replicates (*n*=3), LSD=0.3 at p<0.05.

Photosynthetic pigments content: Three wheat varieties differing in tolerance to Cu by growth parameters were subjected to further physiological and biochemical analyses. The effect of Cu^{2+}on chlorophyll content in leaves depended on the concentration of Cu. Chlorophyll content was decreased in all varieties. At 0.25 mM $CuSO_4$ the chlorophyll content decreased in Kazakhstanskaya rannaya, Kazakhstanskaya-3, and Shagala varieties by 9, 14 and 18%, respectively (Fig. 3) ($p<0.01$). Application of 0.5 mM $CuSO_4$ reduced of chlorophyll-*a* content significantly in comparison with control plants. Content of chlorophyll-*a* in Kazakhstanskaya rannaya and Shagala was decreased to the same extent - by about 60 % of control, respectively. In Kazakhstanskaya-3, chlorophyll content was decreased the least (by – by. 47% in: Kazakhstanskaya rannaya (53%), and > Shagala (39%) > Kazakhstanskaya-3 (38%) ($p<0.05$).

The content of chlorophyll-*b* at 0.5 mM $CuSO_4$ in different wheat varieties decreased in the following order: Kazakhstanskaya rannaya (54%) > Kazakhstanskaya-3 (39%) > Shagala (38%) ($p<0.05$) (Fig. 4). At low concentrations of Cu^{2+}, the content of carotenoids was increased by more than 20% in Kazakhstanskaya rannaya (Fig. 5); however, in Kazakhstanskaya-3 and Shagala, the same concentration of Cu carotenoids caused a reduction by >30%. At high concentration of Cu (0.5 mM) carotenoid content were lower in all varieties by almost 70%. According to the content of carotenoids at 0.5 mM $CuSO_4$ the varieties ranged as follows: Kazakhstanskaya rannaya (31%) > Shagala (29%) > Kazakhstanskaya -3 (24%) ($p<0.05$) (Fig. 5).

The relative water (RWC) and proline content in leaves of wheat: By lowering relative water content at the concentration of 0.5 mM $CuSO_4$ wheat varieties showed the following results (% of control): Kazakhstanskaya rannnaya (90) > Shagala (86) > Kazakhstanskaya-3 (81) (Fig. 6). RWC value of Kazakhstanskaya-3 variety is reduced to the greatest extent, and this variety among others is less tolerant to the Cu effect on growth parameters. To cope the water deficit generated due to Cu^{2+} stress plant need to accumulate osmolites like proline.

By proline content under the effect of 0.5 mM $CuSO_4$ wheat varieties are arranged in the following order (% to control): Shagala (809%) > Kazakhstanskaya rannaya (693%) > Kazakhstanskaya-3 (478%) (Fig. 7). In Shagala and Kazakhstanskaya rannaya varieties the proline content was increased to the greatest extent relatively to control.

Malondialdehyde content: At 0.25 mM $CuSO_4$ the level of malondialdehyde in Kazakhstanskaya rannaya variety was increased to a lesser extent as compared to other varieties (by 11% compared to control) (Fig. 8). In Shagala and Kazakhstanskaya-3 varieties malondialdehyde content at 0.25 mM $CuSO_4$ increased by 18 and 14%, respectively. At a high concentration of Cu (0.5 mM $CuSO_4$) the level of malondialdehyde in Shagala and Kazakhstanskaya-3 varieties was increased by 25 and 19%, respectively, the least increasing of malondialdehyde content was observed in Kazakhstanskaya rannaya variety – by 14%. By increasing the degree of lipid peroxidation at 0.5 mM $CuSO_4$, varieties can be arranged as follows: Shagala (125%) > Kazakhstanskaya-3 (119) > Kazakhstanskaya rannaya (114%) ($p<0.05$).

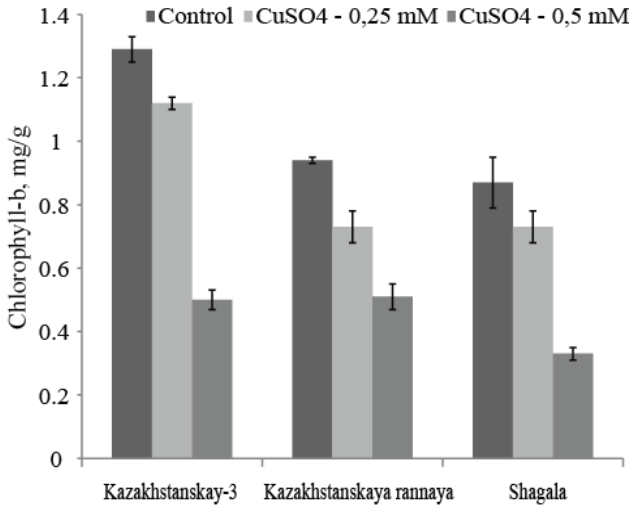

Fig. 4. – Chlorophyll-*b* content in leaves in the presence of copper in the medium. Vertical bars represent ± SD of three replicates (*n*=3), LSD=0.19 at p<0.05.

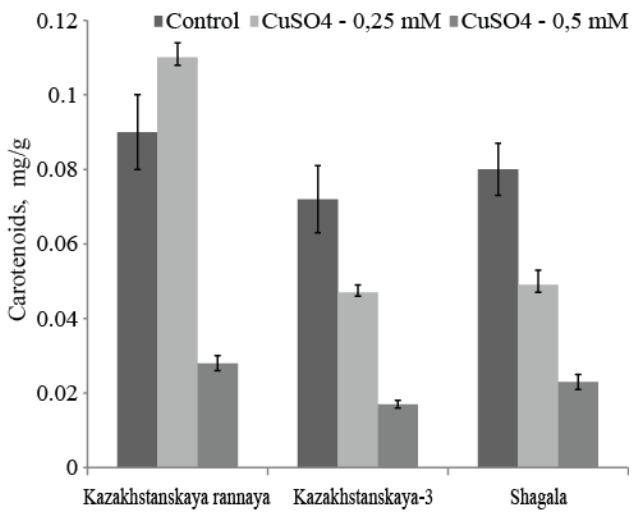

Fig. 5. Effect of Cu^{2+} on carotenoids content in wheat leaves. Vertical bars represent ± SD of three replicates (*n*=3), LSD=0.028 at p<0.05.

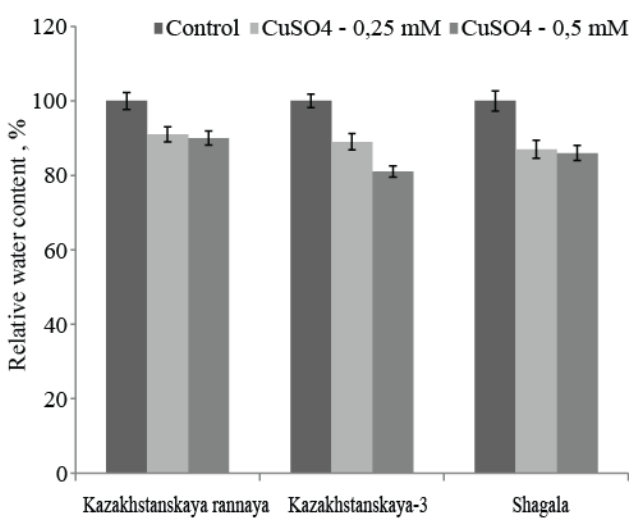

Fig. 6. Effect of copper (Cu^{2+}) on relative water content in whet leaves. Vertical bars represent ± SD of three replicates (*n*=3), LSD=4.29 at p<0.05.

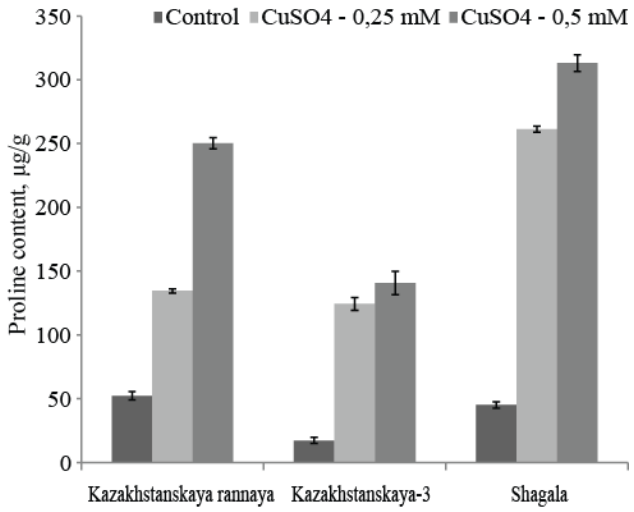

Fig. 7. Effect of copper (Cu^{2+}) on proline content in wheat leaves. Vertical bars represent ± SD of three replicates (*n*=3), LSD=7.54 at p<0.05.

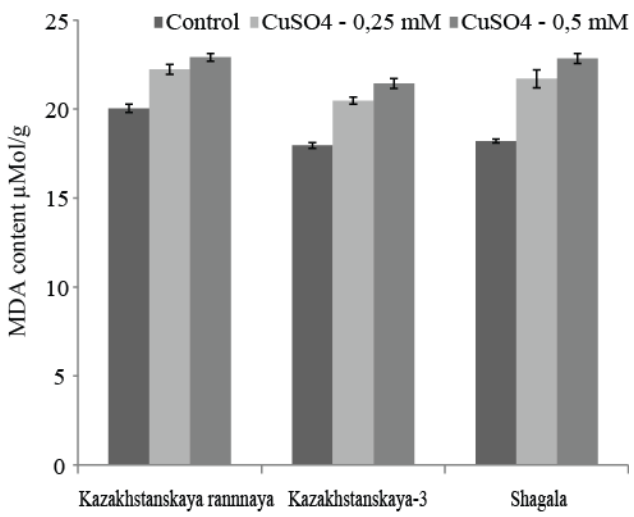

Fig. 8. Effect of copper on malondialdehyde content in wheat leaves. Vertical bars represent ± SD of three replicates (*n*=3), LSD=0.79 at p<0.05.

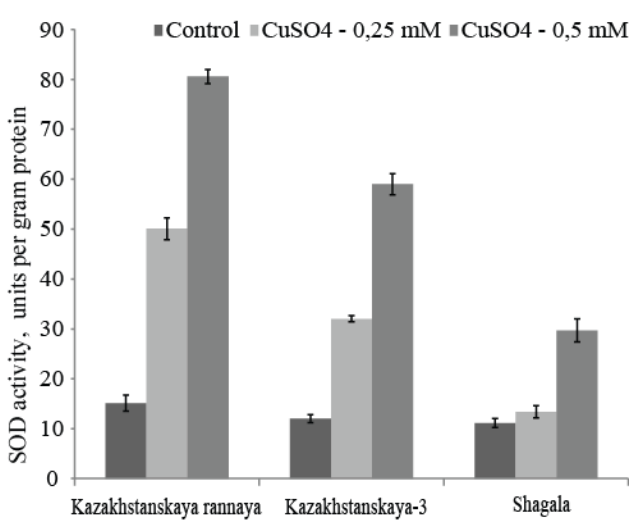

Fig. 9. Effect of copper (Cu^{2+}) on SOD activity in wheat leaves. Vertical bars represent ± SD of three replicates (*n*=3), LSD=19.9 at p<0.05.

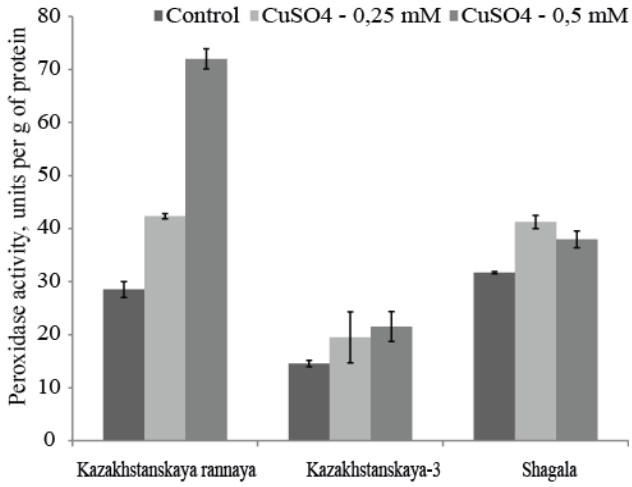

Fig. 10. Effect of copper (Cu^{2+}) on POD activity in wheat leaves. Vertical bars represent ± SD of three replicates (*n*=3), LSD=18.9 at p<0.05.

Superoxide dismutase and peroxidase activity of wheat leaves: SOD activity significantly increased under Cu stress. In the resistant variety Kazakhstanskaya rannaya the enzyme activity was increased more than 5 times. By the increase of the SOD activity wheat varieties at 0.5 mM $CuSO_4$ can be arranged in the following order (% to control): - Kazakhstanskaya rannaya (534) > Kazakhstanskaya-3 (266) > Shagala (120) ((Fig. 9).

By POD activity increase under Cu stress (0.5 mM) wheat varieties can be arranged in the next row (% to control): Kazakhstanskaya rannaya (253) > Kazakhstanskaya-3 (148) > Shagala (119) (Fig. 10).

Discussion

The data presented here demonstrate that the growth parameters like length of the roots and shoots, and the shoot biomass accumulation of wheat was significantly affected by Cu. These results are consistent with the previous reports (Kabata-Pendias, 2001; Chen *et al*., 2004; Meng *et al*., 2007).

Similar results were obtained by Wang & Zhou (2005) when they studied the effect of chlorimuron-ethyl, copper and cadmium on wheat growth. They concluded that wheat sensitivity to toxicity of these pollutants were as follows: root elongation> elongation of above-ground organs > germination rate. It has been found that various concentrations of copper are toxic for wheat growth and germination percentage, plumule length, root length, lateral root number, fresh weight, dry weight and relative water content was also decreased with increasing concentration of copper (Singh *et al*., 2007).

Copper and other heavy metals cause inhibition of elongation growth, as well as reducing the accumulation of plant biomass, which is a consequence of the breach of the photosynthesis process. In our study, the chlorophyll content decreased as Cu concentration increased in the growth medium. Weak growth of wheat under copper stress Singh *et al*. (2007) explained by poor breakdown of starch by low amylase activity because in their experiments the amylase activity in seeds under the Cu stress decreased in comparison to control. Heavy metals have a negative impact on the synthesis of chlorophyll at the level of the synthesis of enzymes or reduce the absorption elements necessary for the synthesis of pigments. The chlorophyll content was reduced in wheat plants (*Triticum aestivum* L.), grown in the presence of copper ions in the soil (Lanaras *et al*,. 1996), in Citrus plants (Ilyas *et al*., 2015) and *Brassica oleraceae*, exposed to copper, cobalt and chromium (J. Chatterjee and C. Chatterjee 2000), in citrus and rice plants that were growing in a medium, polluted by copper (Lidon & Henriques, 1992).

Heavy metals have a negative influence on chlorophyll biosynthesis and inhibit the activity of protochlorophyll reductase and synthesis of aminolevulinic acid. The high ratio of chlorophyll a and chlorophyll b indicates changes in relation of FS2/FS1 under stress (Fry *et al*., 2002). Copper can substitute Mg in chlorophyll. Cu-porphyrine dissipate energy in the form of heat and strongly decrease the fluorescence efficiency (Skórzyńska-Polit *et al*., 2010).

The manganese content in plants decreases in the presence of large amounts of copper in the growth medium. The reduction of manganese leads to a decrease in phytoene since manganese is required for the synthesis of phytoene. Iron deficiency, caused by an excess of copper in the growth medium leads to a reduction of protochlorophyll. According to other researchers heavy metals cause disruption of cell structure of the leaves, reducing intercellular space, structural changes in the thylakoids of chloroplasts (Stobart *et al*., 1985; Oncel *et al*., 2000; Yin *et al*., 2008), closing of stomata, thickening of the cell wall, disorganization of thylakoids and stroma that cause a reduction of chloroplast photosynthetic activity (Pätsikkä, 2002; Vassiliev *et al*., 2003). Thus, chlorophyll content and photosynthesis rate is an index of plant tolerance to stress.

In our research RWC was decreased under Cu stress. Relative water content (RWC) decreased under heavy metals stress as a proposed indicator of phytotoxicity in Indian and Chinese mustard, brake fern (Su *et al*., 2005; Brunet *et al*., 2008). Water deficiency caused the lowering of absorption ability of PSII antenna pigment in *Populus euphratica* seedlings; this lead to the decreasing of photosynthesis activity (Yang *et al*., 2015). Plants with decreased water content enhance the synthesis of osmolytes, particularly proline. In our study, proline content increased as RWC decreased. Proline is a non-essential amino acid and is synthesized in a plant whenever it is subjected to stress. Accumulation of proline in plants is a common response to abiotic stresses (Brunet *et al*., 2008). It is known that proline is a source of carbon and nitrogen for quick recovery under stress, and that it stabilizes cell membrane and some macromolecules, and it is a free radical scavenger (Jaleel & Azooz, 2009).

One of the fastest non-specific reactions of cell membranes caused by any stress is increased lipid peroxidation (LPO) membranes. Copper is a redox metal which generates reactive oxygen substances (ROS) (Fry *et al*., 2002). Copper ions Cu^{2+} complexes with polymers of the cell wall and can be reduced to Cu^{+} by apoplast electron donors as ascorbate and superoxide ions, Copper ions Cu^{+} then can be involved in Fenton reaction (Yin *et*

al., 2008; Skórzyńska-Polit *et al*., 2010) ROS have negative effect on lipids, proteins, carbohydrates, and nucleic acids (Brown *et al*,. 1964; Mittler, 2002; Khatun *et al*., 2008; Wang *et al*., 2009; Golshan *et al*., 2011). Measurement of malondialdehyde level was used as an index of lipid peroxidation in the presence of Cu ions in growth medium. In our study in sensitive wheat cultivar Cu plants the MDA content was increased to a greater extent as compared to other varieties.

Plant cells have the enzymatic mechanisms to remove or reduce the harmful effects of stress factors. Enzymatic antioxidants play a vital role in the neutralization of ROS, and protect cells from oxidative damage. The balance between ROS production and development of antioxidant defense mechanisms determines the survival of the organism under stress conditions (Jouili & Ferjani, 2003; Khatun *et al*., 2008; Golshan *et al*., 2011). Superoxide dismutase (SOD) is an important component of antioxidative defense systems and it is the first enzyme in the detoxifying process, which converts $\cdot O_2^-$ radicals to H_2O_2 and O_2 ¯ (Ozden *et al*., 2009). A simultaneous increase in SOD activity and the amount of the corresponding proteins under salt and heavy metals stress was observed. It indicates an increase in the synthesis of the enzyme under stress conditions has been reported? (De Vos *et al*., 1992; Pukacki, 2004; Baranenko, 2006).

Peroxidase is a multifunctional enzyme protective and adaptive system of plants to stress factors. Peroxidase (POD) is one of the first marker enzymes and is activated in response to stress (Andreeva, 1988). Peripheral (cell membrane, plasmalemma) localization of peroxidase explains the rapid change in its activity. High POD activity would enable the plants to scavenge H_2O_2 in cells, thus maintaining cellular membrane integrity and protecting against the oxidative stress induced by excess Cu (Mazhoudi *et al*., 1997). In our study the activity of antioxidant enzymes in more tolerant wheat varieties was higher as compared to sensitive varieties. Consequently, the antioxidant enzymes activity can be a sensitive indicator of the plants state under stress.

Conclusion

Wheat varieties were screened for resistance to the Cu effect on the growth and physiological/biochemical parameters at the seedling stage. It was identified that Kazakhstanskaya rannaya and Melturn varieties were more tolerant to Cu compared to other varieties, middle tolerant - Shagala cv, sensitive varieties - Kaiyr, Kazakhstanskaya-3. Cu decreased photosynthetic pigments (chlorophyll-*a* and chlorophyll-*b*, carotenoids) content. There is a positive relationship between the level of reduction of photosynthetic pigments content and tolerant index of wheat varieties. Lipid peroxidation in wheat varieties increased in response to Cu stress. Wheat varieties sensitive to Cu stress showed greatest increase of lipid peroxidation compared to tolerant wheat varieties. SOD and POD activity increased in all wheat varieties under Cu stress. Wheat variety tolerant to Cu showed greatest increase of antioxidant enzymes activity. The decrease of these physiological and biochemical parameters affects yield and quality of wheat grain growing on Cu contaminated areas.

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