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ANATOMICAL PECULIARITIES IN WHEAT (*TRITICUM AESTIVUM* L.) VARIETIES UNDER COPPER STRESS

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Abstract

The effect of different concentrations (0.25 mM, 0.5 mM) of Cu^{2+} on anatomical parameters of leaves and roots was investigated in hydroponically grown five wheat (*Triticum aestivum* L.) varieties (Kazakhstanskaya rannaya, Kazakhstanskaya-3, Melturn, Kaiyr and Shagala). The results showed that wheat varieties exposed to 0.5 mM Cu^{2+} exhibited significant alterations in anatomical structure of leaves and roots. The thickness of the upper and lower epidermis, diameter of vascular bundles of leaves of almost all varieties showed a tendency to decrease under copper stress. Our experiments showed an activation of defense responses in the root anatomical structure like exodermis thickening in some varieties in the presence of copper in growth medium as compared to the control. This indicates that copper ions increase the thickness of exodermis, which reduce the absorption of toxic elements by root cells. Copper stress caused a decrease in the thickness of the lower and upper epidermis to varying degrees and reduction in the diameter of vascular bundles of wheat leaves. Copper stress caused a reduction in endodermis thickness thereby decreasing the diameter of the central cylinder of wheat roots.

Key words: Wheat, Copper, Anatomical structure, Exodermis, Endodermis, Vascular bundles, Central cylinder.

Introduction

Toxic man-made hazards are a major threat to humans. The development of modern technologies in industry and agriculture leads to an intense increase in the number of heavy metals in the environment, by times higher than background concentrations. Large areas of Kazakhstan are contaminated by heavy metals, like Zn, Cd, Pb, Cu, Co, especially around metallurgic plants in the eastern and southern Kazakhstan. Areas around copper mines and copper processing plants like BalkhashMed and JezkazganMed are contaminated with copper and other heavy metals.

In Balkash region, industrial wastes from copper mining enterprises pollute the soil and water areas adjacent to Lake Balkhash (Tasekeev, 2004; Panin, 2000). The pollution of the environment affects biodiversity in this region.

Copper is an essential element for plant growth as it is a part of most enzymes and other proteins. Cu is a key component ensuring functioning of a number of enzymes like cytochrome-c oxidase, ascorbate oxidase, and a number of nonenzymatic proteins. Cu is a part of plastocyanin, the component of the electron transport chain and photosynthesis. It plays an essential role in the life of plant organisms, i.e. strengthens oxidation processes and ensures formation of chlorophyll (Chernavskaya, 1989).

High concentration of microelements has a toxic effect on plants. They bind with sulfhydryl groups of proteins inhibiting the activity and causing structure degradation. Excess quantity of microelements causes toxicity symptoms and growth inhibition (Reinheckel *et al*., 1988; Chernavskaya, 1989). Copper ions (Cu^{2+}) bind with the cell wall polymers (histidine-rich glycoprotein) and can be reduced to Cu^{+} by apoplastic electron donors like ascorbate and superoxide ions, and Cu^{+} can then undergo the Fenton reaction (oxidation of the α-hydroxy acids hydroxyl group and α-glycols to a carbonyl group and formation of hydroxyl radicals in the presence of hydrogen peroxide) to the apoplastic hydrogen peroxide to generate hydroxyl radicals (*OH) (Stochs *et al*., 1995). The reaction proceeds by a free radical mechanism. This can cause non-enzymatic separation of cell wall polysaccharides that may loosen the cell wall (Chernavskaya, 1989).

Heavy metal ions have negative effect on anatomical structure of leaves and roots of plants (Kovačević *et al.*,1999, Shalini *et al.,* 1999, Khudsar *et al.,* 2001 and Papadakis *et al.,* 2004; Kasim, 2006; Cvetanovska *et al*., 2010; Mikovilovi & Dragosavac, 2010; Gomes *et al*., 2011), physiological and biochemical changes in plant cells (Maksymiec *et al*., 1997; Cheng, 2003; Fuentes *et al*., 2007; Hakmaoui *et al*., 2007; Meng *et al*., 2007; Tekli *et al*., 2008; Haribabu *et al*., 2011; Azooz *et al*., 2012; Hatamzadeh *et al*., 2012). Copper stress might inhibit photosynthesis through its direct effect on the photosynthetic apparatus (Pätsikkä *et al*., 2002; Mathad & H. Pratima *et al*., 2009; Azooz *et al*., 2012; Ren *et al*., 2015).

According to the literature data heavy metals decrease thickness of the lower and upper epidermis, mesophyll cells of the leaves, in the palisade parenchyma, and a decrease in chlorophyll (Mikovilovi & Dragosavac, 2010). Copper causes a decrease in root diameter, diameter of central cylinder, thickness of cortex. However, copper in the concentration of 150 μmol/L showed a significant increase of metaxylem elements (Gowayed *et al*., 2006). Heavy metals inhibit root elongation due to metal interference with cell division which may include chromosomal aberrations and violation of mitosis and cell elongation (Pasternak *et al*., 2005; Radha *et al*., 2010; Aery & Sarkar, 2012), a decrease in the elasticity of cell walls of the root (Sieghardt 1984; Barceló *et al*., 1986).

The root growth may be inhibited due to a decrease in cell division and increase in cell wall thickness, and due to change in the activity and contents of phytohormones like auxin (Schilcher *et al*., 2005; Sharma & Dietz, 2006; Seregin & Kozhevnikova, 2008; Soudeh & Zarinkamar, 2012). Copper and cadmium induce reduction in the cell size and reduce the root diameter (Kasim, 2006). The reduction in the diameter of metaxylem vessels influence on their translocation capacity (Poschenrieder & Barceló, 1999).

A study of the anatomical parameters under copper stress is one of the essential tasks because the changes in anatomical parameters can be the premise, or the cause of changes in physiological processes. The present investigation was, therefore, undertaken to study the effect of copper ions on anatomical structure of leaves and roots of wide spread wheat varietiescultivated in Kazkahstan.

Materials and Methods

The five wheat varieties, i.e., Kazakhstanskaya-3, Kazakhstanskaya rannaya, Melturn, Kaiyr, and Shagala were the research objects. The plants were grown in hydroponic conditions for 7 days, in solutions of various copper concentration. Three treatments were defined as: no Cu added (control), 0,25μM$CuSO_4$ (low $CuSO_4$ concentration) and 0,25 μM $CuSO_4$ (high $CuSO_4$ concentration).

Anatomical analysis: The anatomical structure of plants was studied using standard methods (Barykina, 2004). Morphometric studies were done with MOV-1-15 ocular micrometer (with x9 lens and x10.7 zoom).

Statistical analysis: The data was analysed statistically using the two-way ANOVA with species and treatments as main effects for shoot and root anatomical parameters. LSD was calculated using the following equation: $LSD_{0.05} = t_{0.05}\ \sqrt{(2 \cdot MSError/n)}$ was used to differentiate the means. All values were expressed as the mean of three measurements for each treatment. Values represent means ± standard error (SE).

Results

Anatomical structure of leaf blades in the presence of copper in growth medium. Upper and lower epidermis: The lower and upper epidermis thickness decreased almost in all studied wheat cultivars in the presence of Cu^{2+} in growth medium, except Kazakhstanskaya-3 and Melturn in some variances (Figs. 1, 2).

The upper epidermis thickness in Kazakhstanskaya-3 and Melturn varieties at low copper concentration (0.25 mM $CuSO_4$) exceeded the control by 6% and 2%, respectively (Fig. 1).

The upper epidermis thickness in Melturn variety was at the control level at low copper concentration and decreased as long as the copper stress intensified. The Shagala variety showed the highest level of the upper epidermis (by 22%) (Fig. 1) at low copper concentration. The results of this study showed that Kazakhstanskaya rannaya cv had the highest level of the upper epidermis width decrease (by 32%) under intensive copper stress of 0,5mM$CuSO_4$.

At 0.5 mM $CuSO_4$ the thickness of upper epidermis in wheat varieties decreased in the following order: Melturn (85%) > Kaiyr (80%) > Kazakhstanskaya-3 (75%) > Shagala (70%) > Kazakhstanskaya rannaya (68%) ($p<0.05$) (Fig. 1).

Lower epidermis: At low copper concentration the thickness of lower epidermis of the leaves of the Kazakhstanskaya-3 variety exceeded the control to a great extent – by 30%. In Kazakhstanskaya rannaya this parameter was at the control level (100%to control). The greatest decrease of the lower epidermis thickness at 0,25 mM$CuSO_4$ was observed in Melturn cv (decreased by31%), the lowest – in the Shagala variety (by 2%).

The leaf blade of Kazakhstanskaya rannaya and Melturn varieties presented a smaller thickness of lower epidermis at 0.5 mM $CuSO_4$ compared to control (69 and 67% to control) (Fig. 2). At 0.5 mM $CuSO_4$ the thickness of lower epidermis of wheat varieties decreased in the following order (% to control): Kazakhstanskaya-3 (103%) > Kaiyr (87%) > Shagala (79%) >Kazakhstanskaya rannaya (69%) > Melturn (67%) ($p<0,05$) (Fig. 2).

Thus, the least change of upper epidermis thickness occurred in the Melturn variety (85% to control), the highest – in Kazakhstanskaya rannaya (68% to control). The most reduced thickness of lower epidermis was observed in Kazakhstanskaya rannaya and Melturn cvs (69% and 67% to control, respectively), the least - in Kazakhstanskaya-3 (103% to control).

The results showed the decline in the thickness of both upper and lower epidermis of wheat leaf in almost all studied varieties, except the Kazakhstanskaya-3 variety.

Diameter of vascular bundles: The diameter of vascular bundles of leaves of almost all varieties showed a tendency to decrease at all Cu^{2+} concentrations. The diameter of vascular bundles decreased significantly almost in all studied varieties, except the Kayir variety (decreased by 3% in copper treated plants). Greatest changes in diameter of vascular bundles occurred in Melturn (64% to control). The diameter of vascular bundles decreased in equal level at both copper concentration almost in all varieties. Higher concentration of Cu^{2+} in the Melturn variety made this parameter to reduce more (by 82 and 64% to control at 0.25 and 0.5 mM Cu SO_4, respectively).

The diameter of vascular bundles at 0.5 mM Cu SO_4 decreased in the following order (% to control): Kaiyr (97%) > Kazakhstanskaya-3 (87%) > Shagala (85%) > Kazakhstanskaya rannaya (77%) > Melturn (64%) (Fig. 3).

Anatomical structure of wheat roots in the presence of copper in growth medium

The exodermis and endodermis thickness: Copper ions decreased the thickness of exodermis and endodermis of wheat roots. At 0.25 mM $CuSO_4$ the smallest decline of the thickness of exodermis was observed in the Kaiyr variety (by 4%), the most reduction - in the Melturn variety (by 16%). At a high concentration of copper the thickness of exodermis reduced in Kazakhstanskaya rannaya and Kaiyr varieties to a low extent (by 9%) compared to other varieties, and to a significant extent –in the Shagala variety (by 30%) (Fig. 4).

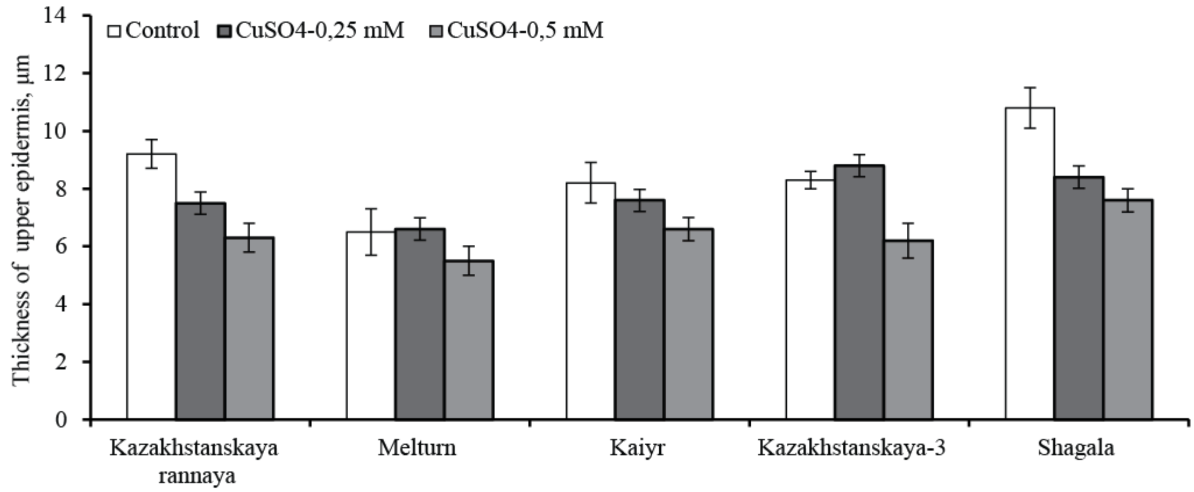

Fig. 1. Effect of copper on thickness of upper epidermis of wheat leaves.
Vertical bars represent ± SD of three replicates (*n*=3), LSD=0.86 at $p < 0.05$

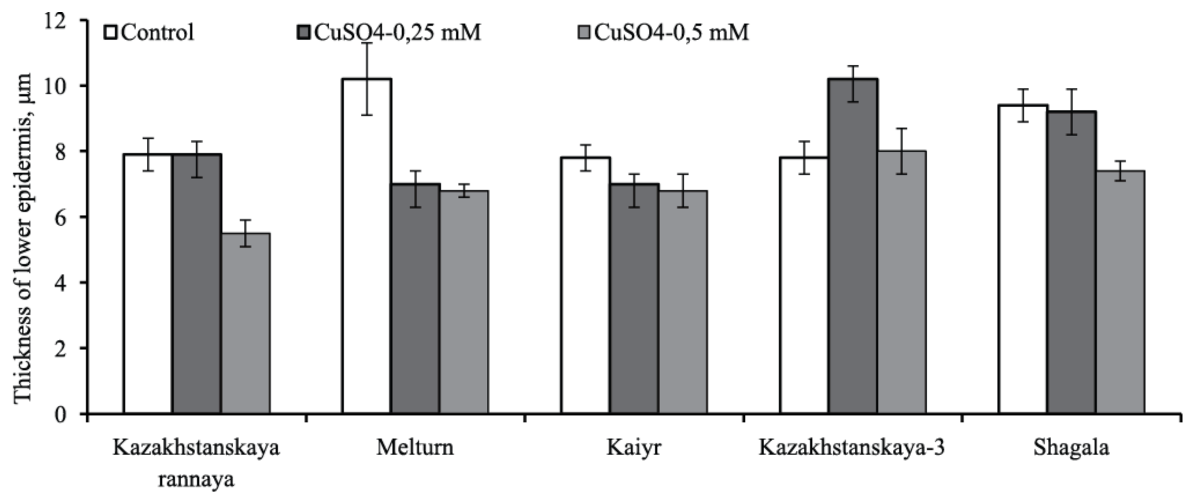

Fig. 2. Effect of copper on thickness of lower epidermis of wheat leaves.
Vertical bars represent ± SD of three replicates (*n*=3), LSD=4.4 at $p < 0.05$

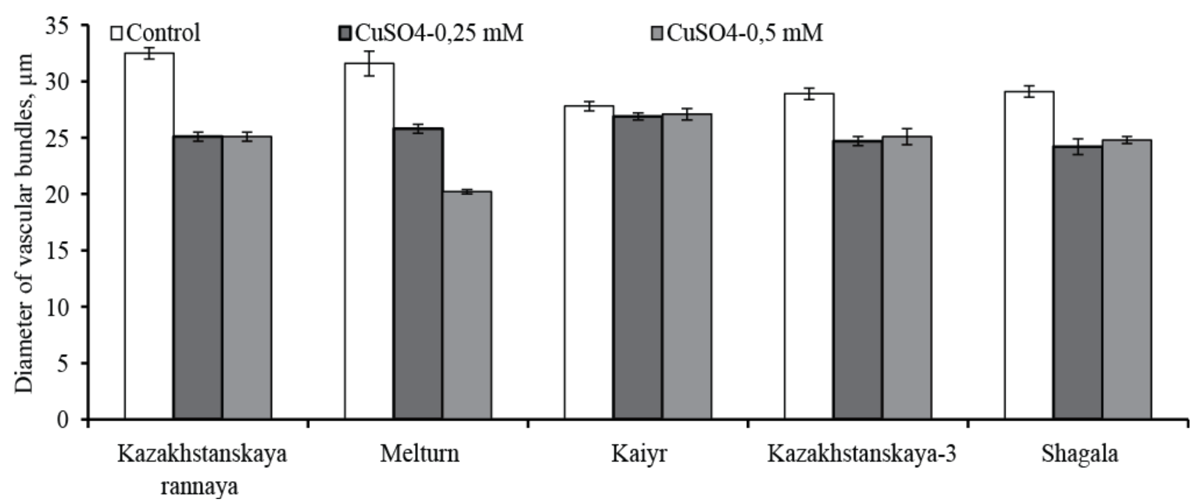

Fig. 3. Effect of copper on thickness of diameter of vascular bandles of wheat leaves.
Vertical bars represent ± SD of three replicates (*n*=3), LSD=5.0 at $p < 0.05$

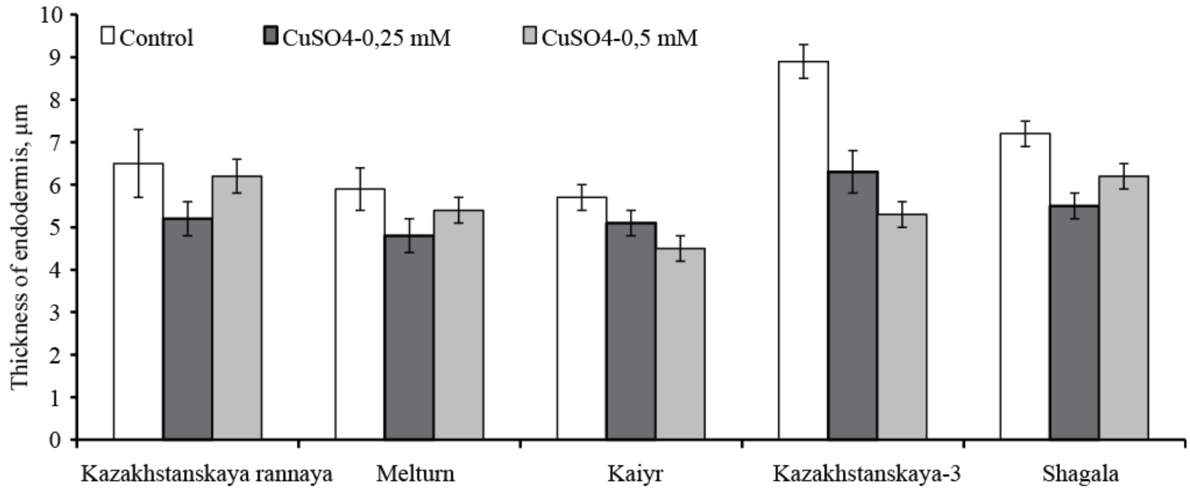

Fig. 4. Effect of copper on thickness of endodermis of wheat roots.
Vertical bars represent ± SD of three replicates (*n*=3), LSD=1.5 at $p<0.05$

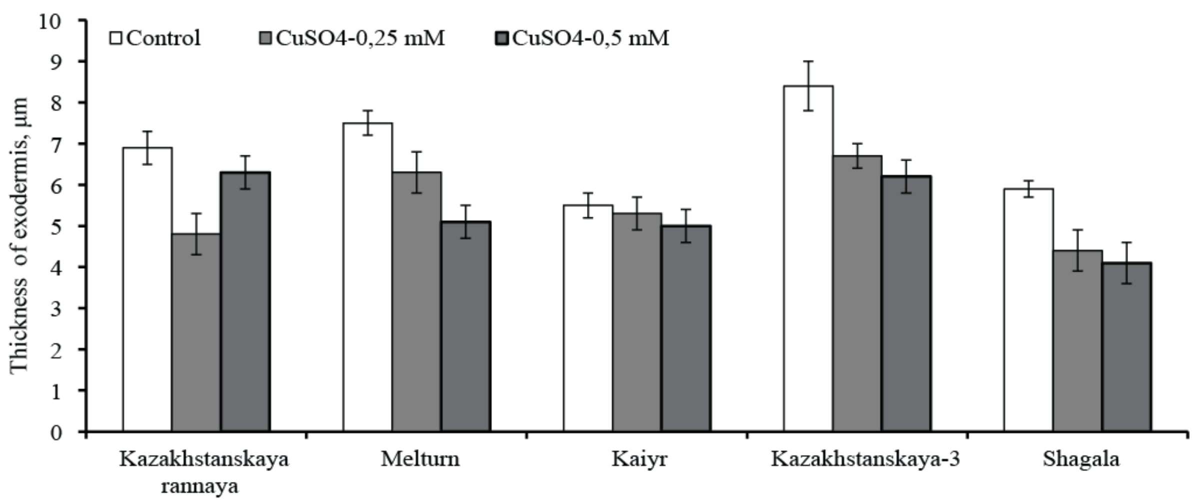

Fig. 5. Effect of copper on thickness of exodermis of wheat roots.
Vertical bars represent ± SD of three replicates (*n*=3), LSD=0.72 at $p<0.05$

By the exodermis thickness under the influence of 0.5 mM $CuSO_4$, the varieties arranged as follows (% to control): Kazakhstanskaya rannaya (91%) = Kaiyr (91%) > Melturn (84%) >Kazakhstanskaya-3 (74%) > Shagala (69%) ($p<0.05$).

At 0.25 mM $CuSO_4$, the minimum decrease of the endodermis thickness was observed in the Kayir variety – by 11%, the highest decrease – in Kazakhstanskaya-3 variety (by 24%).

With 0.5 mM $CuSO_4$ concentration, the most significant thinning of endodermis was observed in Melturn and Kazakhstanskaya rannaya. For the sensitive to copper Kazakhstanskaya-3 variety this indicator decreased to a large extent – by 40% (Fig. 4). In this case the reduction degree in anatomical parameters correlated with the degree of the roots of resistant cultivars.

By endodermis thickness change under the influence of 0,5 mM $CuSO_4$ the varieties were ranged as follows (% to control): Kazakhstanskaya rannaya (95%) > Melturn (92%) > Shagala (86%) > Kaiyr (79%) > Kazakhstanskaya-3 (60%) ($p<0.05$) (Fig. 5).

Endodermis/endodermis ratio: The absolute values of the exodermis/endodermis thickness ratio at 0.5 mM $CuSO_4$ are as follows: Melturn (1.17) > Kazakhstanskaya-3 (1.16) > Kaiyr (1.11) > Kazakhstanskaya rannaya (1.01) > Shagala (0.66). The differences between varieties and treatments were not significant ($p>0.05$). In relation to control the means of the exodermis/endodermis thickness ratio have a tendency to increase in some varieties, but the differences between varieties and treatments were not significant ($p>0.05$). This value decreased in the following order (% to control): Kazakhstanskaya -3 (124%) > Kaiyr (115 %) > Kazakhstanskaya rannaya (96%) = Melturn (96) > Shagala (80%) (Fig. 6).

In Kazakhstanskaya-3 and Kaiyr varieties, the exodermis and endodermis ratio exceeded the control: at low concentration (0.25 mM) of copper - by 15 and 8%, respectively, and at 0.5 mM copper - by 14 and 15% (in Kazakhstanskaya-3 and Kaiyr varieties, respectively).

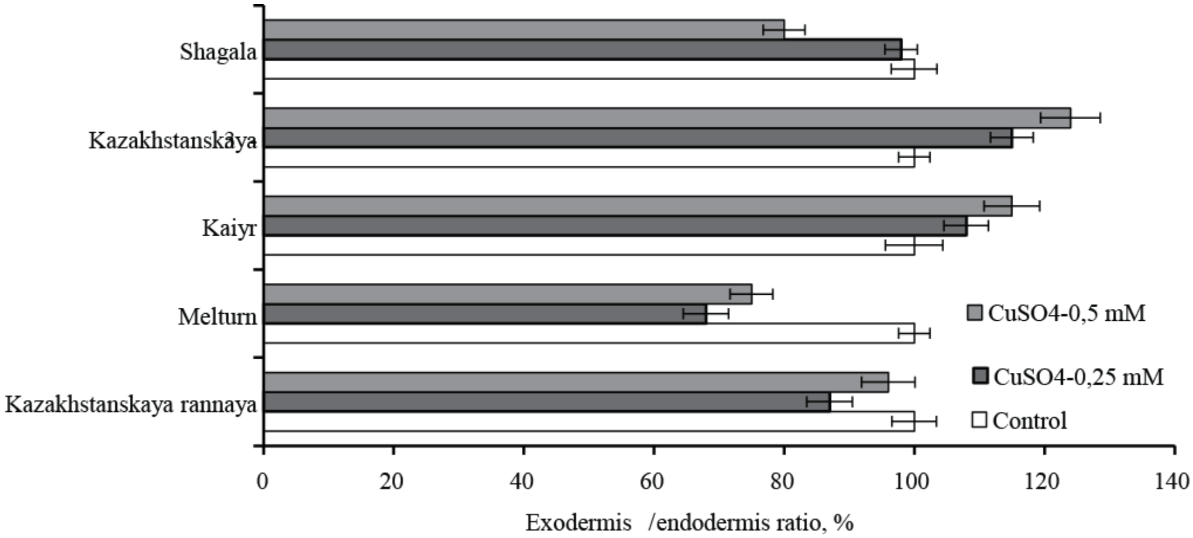

Fig. 6. Effect of copper on exodermis/endodermis ratio of wheat roots.
Vertical bars represent ± SD of three replicates (*n*=3), $p > 0.05$

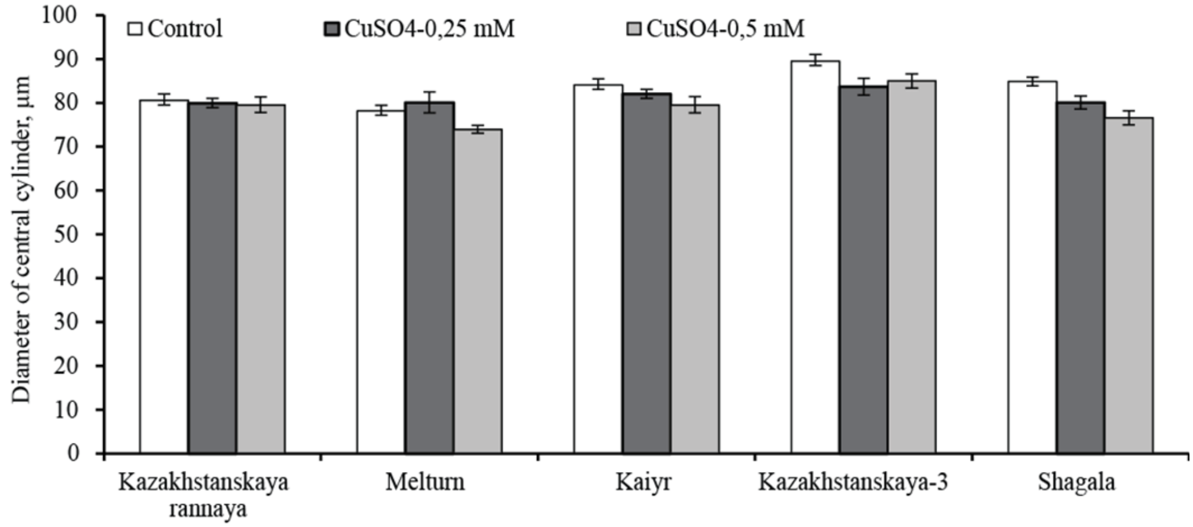

Fig. 7. Effect of copper on diameter of central cylinder of wheat roots.
Vertical bars represent ± SD of three replicates (*n*=3), LSD=4.6 at $p<0.05$

Diameter of central cylinder: The diameter of the central cylinder in wheat varieties decreased to a low extent in both copper concentrations. At low concentration of copper in Melturn variety this parameter slightly increased (by 2%), the greatest decrease was observed in Kazakhstanskaya-3 cv (by 7%).

The greatest decrease of the central cylinder at high Cu concentration was observed in Melturn (by 12%) cv. In Kazakhstanskaya rannaya and Kayir varieties this parameter remains on the control level (99%, 98% to control, respectively) (Fig. 7).

At high copper concentration (0.5 mM) greatest change in diameter of the central cylinder occurred in Shagala variety (10% below control), and the lowest - in Kazakhstanskaya rannaya and Kazakhstanskaya-3 varieties (1 and 4% lower than the control, respectively). The diameter of the central cylinder of different varieties at 0.5 mM $CuSO_4$ ranged as follows (% to control): Kazakhstanskaya rannaya (99%) > Kazakhstanskaya-3 (95%) = Kaiyr (95%) = Melturn (95%) > Shagala (90%).

Discussion

The study of anatomical peculiarities of leaves and roots of wheat under copper stress is very important because the changing of anatomical structure of plants parts are the is a prerequisites of plant response to stress. Reducing of vascular bundles diameter is directly related to decrease in the area xylem vessels, which as conductive elements are clearly responding to holding various elements by changing its diameter (Ortega *et al*., 2006). It is supposed that reduction in the number of conducting elements has been reported in literature as being an adaptive measure to secure water flow (Baas *et al*., 1983).

Reduction in size and number of conducting elements of the xylem in response to heavy metals has been reported by Sandalio *et al*. (2001). It should be noted that tolerant to copper wheat varieties of Melturn and Kazakhstanskaya rannaya showed the greatest reduction of the diameter thickness of vascular bundles at a high level of copper.

It should be noted that the thickness of exodermis in Kazakhstanskaya rannaya and Melturn varieties and the thickness of endodermis in Kazakhstanskaya rannaya, Shagala, and Melturn varieties at higher concentrations were greater than at lower ones. Perhaps, it indicates that activation of protective reactions with strengthening of stress is expressed in the thickening of exodermis and endodermis (Figs. 4, 5).

So, at a high concentration of copper in Kazakhstanskaya rannaya and Kaiyr varieties the least reduction (by 9%) of the exodermis thickness was observed compared with the other varieties, the most showing in the Shagala variety. Kazakhstanskaya rannaya and Kaiyr varieties showed greater stability compared with the Shagala variety.

The smallest decrease of endodermis thickness was observed in resistant varieties of Melturn and Kazakhstanskaya rannaya (5 and 8% in varieties, respectively). For the sensitive variety of Kazakhstanskaya-3 this indicator decreased the most (40%).

The exodermis to endodermis thickness is an important indicator of adaptive reactions to stress and plant resistance. Thickening of exodermis is an indicator of adaptive responses against stressors (Hose *et al*., 2001; Mikovilovi & Dragosavac, 2010; Gomes *et al*., 2011; Ceccoli *et al*., 2011). Exodermis of root cells protects against penetration of excess toxic agents from the environment into the root cells. Exodermis thickening showed the development of an anatomical adaptations to stress conditions.

The value of the ratio exodermis/endodermis relative to control was the highest in sensitive to copper on growth parameters Kazakhstanskaya-3 variety (124%) and middle tolerant Kaiyr variety (115%). It indicates that, in response to increased stress the thickness of the exodermis is increased compared with the control. Tolerant to copper on growth parameters Kazakhstanskaya rannaya and Melturn varieties had not any change on this parameter ($p>0.05$) relative to control. Shagala variety had the lowest mean of this ratio (0.66 and 80% to control).

Our experiments showed activation of defense responses in root anatomical structure as a thickening of exodermis in the presence of copper in growth medium compared to control in some varieties. This indicates that copper ions increase the thickness of exodermis, which reduces the absorption of toxic elements by root cells. Under stress conditions exodermis as a peripheral barrier against the penetration of unfavorable dissolved substances into the apoplast. Exodermis forms a barrier of variable resistance to the flow of water and nutrients inside root cells and conductive elements (Hose *et al*., 2001).

The decrease of diameter of the central cylinder is possibly a prerequisite of low growth parameters under the effect of toxic elements in the varieties. Vascular bundles diameter reduction is an indicator of water and minerals conductivity decrease (Ceccoli, 2011).

Thus, study of the anatomical features of the leaves and roots of wheat plants subjected to the copper effect showed that structure of the leaves and roots is undergoing significant changes. Copper ions in varying degrees reduced the thickness of the lower and upper epidermis, and vice versa increased in some varieties. The thickness of the vascular bundles changed variously, too. The diameter of the central cylinder also changed under stress: it decreased in some varieties, increased in others, and remained at the control level in third samples.

Conclusion

Copper stress caused a decrease in the thickness of lower and upper epidermis in varying degrees and the reducing of the diameter of the vascular bundles of wheat leaves. Copper stress caused a reduction of exodermis, endodermis thickness, the diameter of the central cylinder of wheat roots. In some varieties there was an increase in the exodermis/endodermis thickness ratio compared to control as an adaptive response.

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