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Review Article

Influence High Concentrations of Copper on Different Parts and Cell Compartments of Growing Wheat Seedling

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Abstract

This article sets out the material on the influence of the accumulation of copper accumulation in various parts of plants and cellular compartments of wheat seedlings. It was revealed that the addition of copper to the solution sharply lowered growth processes in plants. It was shown that the greatest accumulation of copper was observed in the roots, showing their protective barrier function. We studied various compartments of the cell. In 7 studied, copper was mainly accumulated in the cell membrane and in the cytoplasm. The results obtained contribute to the establishment of the basic laws of the distribution of copper in the cell, when its excess and increased understanding of the mechanisms of the action of heavy metals on plants.

Keywords: Wheat; Copper; Compartmentalization; Metal Distribution; Resistance

Introduction

It is known that when grown on polluted soils, heavy metals accumulate in the roots, they are less in the leaves, stem [1]. Due to their toxicity in plants, there is a special detoxification mechanism, including the formation of metal-protein complexes and the transfer of heavy metals (TM) to various organs and compartments. Proteins to which heavy metals bind are called thioneins [2]. As a result, TM become less toxic to cellular contents, and then deported to special compartments, in particular, vacuoles. If to date these proteins have been isolated and characterized, then the very distribution of heavy metals in the cell, the mechanism of their transportation to the storage compartments is poorly understood. In addition to metallothioneins, in response to metal stress, there is a stimulation of the synthesis of a number of enzymes that may also play a role in the binding of metals [3]. All these still poorly studied questions were studied by us at a model object - wheat of the Saratovskaya 29 variety. Copper was taken as a stressor, because copper stones have been studied much less than cadmium, zinc binding proteins. In addition, the mechanism of action of copper on plants has a kind of dual nature: at low concentrations it stimulates growth processes, and at high concentrations it inhibits. This explains the choice of toxic metal. Research on copper-binding proteins is also interesting in the sense that Kazakhstan has huge reserves of copper. There is also a lot of this metal in the waste of metallurgical plants in East Kazakhstan [4]. Knowledge of the mechanisms of binding this metal by native plants will allow to develop a system for more efficient selection of copper-accumulating and hardy species, as well as cleaning the soil from industrial emissions. No less interesting is the study of the molecular mechanisms of transport and distribution of heavy metals in cells.

Materials and Methods

To study the localization of copper in various compartments, wheat seeds of the Saratovskaya 29 variety, pre-treated for 25 minutes in 16% H_2O_2, were germinated on filter paper at 25°C. 4 daily wheat sprouts were transferred to a solution containing mineral salts and 4M $CaSO_4$, after three days $CuSO_4$ was added to the solution $CuSO_4 \cdot 5H_20$ at the rate of 150 mg per liter, where the plant was for another three days. In the control version, copper was not added. Experimental and control plants were dismembered into roots and an above-ground part before taking the attachment. The tissue attachment was homogenized in an environment of the following composition: tris-HC1, 0.05M, 0.5M sucrose, 0.1% albumin, pH 7.4. Ratio: Hitch: The medium was 1:4 and 1:5 respectively the above-ground part to the roots. The resulting homogenate was squeezed through kapron tissue, the precipitate was again washed with the same medium and designated as cell walls. The remaining fluid was centrifuged at 800 rpm for 20 minutes, the precipitate contained cell nuclei, and the supernatant was once again centrifuged at 2500 rpm for 15 minutes to deposit chloroplasts from above-ground organs. Supernatants obtained after deposition of chloroplasts and cell nuclei from the roots were centrifuged for 15 minutes at 14,000 rpm. Thus, mitochondria were obtained in the sediment, and the supernatant was designated as the cytoplasm of cells. Each of the obtained fractions: cell membrane, nuclei, chloroplasts, mitochondria, cytoplasm were subjected to wet ashing with concentrated perchloric acid. The copper content in the fractions was measured on the atomic absorption spectrophotometer-AAS-1.

Research Results and Discussion

Studies have shown that the addition of copper to the solution sharply inhibited the growth processes in wheat. As can be seen from the data of table 1, experimental plants were noticeably inferior to the control ones for the accumulation of biomass.

Variant	Dry weight mg/plant		Cu, mg/g dry. c-va		Cu, µg/plant	
	Roots	Above-ground h.	Roots	Above-ground h.	Roots	Above-ground h.
Control	2.40 ± 0,1	2.79 ± 0,1	1.31 ± 0,05	0.66 ± 0,02	3.14 ± 0,1	8.44 ± 0,1
Experience	1.74 ± 0,05	1.83 ± 0,1	4.29 ± 0,1	1.03 ± 0,05	7.46 ± 0,1	9.09 ± 0,1

Table 1: Resource requirements by component.
Accumulation of copper in various organs of wheat (in relative and absolute units).

Three days after the addition of copper to the solution, the weight of the roots in the experimental plants was less than that of the control plants by 28% and the above-ground organs by 31%. The relative content of copper in the organs of experimental plants has increased dramatically (in the roots by more than 3 times and in the above-ground organs by 56%). As can be seen, the greatest amount of copper in control and experimental plants was concentrated in the root system. This confirms the data of a number of authors that the roots occupy a dominant place in terms of metal accumulation [1-5].

The highest absolute copper content, taking into account biomass, accounted for above-ground organs, 73% versus 27% in the roots of control plants. In experimental plants, these differences were reduced by a significant increase in the concentration of copper in the root cells. So, if in the roots of experimental plants the absolute content of copper increased in comparison with the control more than twice, then in the above-ground organs it increased by only 8%. In general, the experimental plants contained 40% more copper than the control plants.

Analysis of data on the distribution of copper in the structural components of cells indicates that the metal mainly accumulates in the cell membrane and in the cytoplasm (Table 2). Moreover, in the cytoplasm of root cells, there are more metal ions than in the fraction of the cell wall, and in the above-ground organs, on the contrary, the greatest amount of copper is contained in the fraction of the cell walls. This applies to both control and experimental variants with the only difference that in the root cells of experimental plants, copper ions were distributed approximately equally between these compartments.

Compartments Cells	Control				Experience					
	Roots		Leaves		Roots			Leaves		
	mg	% of S	mg	% of S	mg	% of S	% of cont	mg	% of S	% of cont
Cell membrane	0.3	23	0.46	70	1.99	46	663	0.61	59	133
Kernel	0.11	8	0.03	5	0.27	6	245	0.07	7	233
Chloroplasts	-	-	-	-	-	-	-	0.03	3	-
Mitochondria	0.08	6	0.01	2	0.16	4	200	0.03	3	300
Cytoplasm	0.83	63	0.15	23	1.87	44	225	0.29	28	193
Total	1.31	100	0.66	100	4.29	100	327	1.03	100	156

Table 2: Resource requirements by component.

Effect of increased copper concentration on the distribution of copper in wheat cell compartments (in g per g of dry substances, NSR ≤ 005).

Similar patterns were obtained when analyzing the distribution of copper using another method. Here, copper was sequentially extracted from the leaves and roots of 0.05 M by an acetate buffer, then by the same +IM CSI buffer, then the remaining cell walls were dissolved in a solution of cellulase and pectinase, i.e. the soluble (1), ion-bound (P) and covalent-bound (W) fractions were sequentially extracted. However, unlike the previously used procedure, only covalently bound copper is determined in fraction III, which is localized on the cell wall and can be associated with it and ionic bonds (Table 3).

Organ	Exposure in copper solution (days)			Sum
	1	2	3	
3 days - root	0.233	0.018	0.07	0.319
letter	0.015	0.010	0.003	0.028
4 days - root	0.27	0.025	0.10	0.395
letter	0.021	0.012	0.064	0.097
5 days - root	0.33	0.031	0.128	0.489
letter	0.034	0.022	0.097	0.153
6 days - root	0.44	0.075	0.127	0.642
letter	0.019	0.029	0.110	0.158
7 days - root	0.43	0.076	0.127	0.631
letter	0.018	0.030	0.108	0.156

Table 3: Resource requirements by component.

Dynamics of copper accumulation by various organs of growing wheat seedlings Saratovskaya 29 (in mg/g). NSR ≤ 005.

Note: 1 is soluble, 2 is ion-bound, 3 is covalently bound fraction.

Conclusion

Thus, we have identified a different distribution of copper on the organs of plants. The greater accumulation of metal in the roots reflects their protective and distribution function in conditions of environmental pollution with heavy metal ions. The copper that entered the plants was mainly localized on the cell membrane of the above-ground organs and roots, which indicates its barrier ability with an excess of copper ions in the medium [5].

The data obtained contribute to the establishment of the basic patterns of copper distribution in the cell with its excess and expand the understanding of the mechanisms of action on plants of heavy metals.

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