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The genetic potential of spring wheat resistance to cadmium and zinc

Abstract: The problem of obtaining safe foodstuff has remained relevant for a very long time. Long period of soil self-purification and the expensiveness of its artificial treatment forced mankind to look for new ways to solve problems related to soil pollution with heavy metals. The most perspective direction in this field is the identification of plant facilities, characterized by minimal accumulation of heavy metals. The purpose of our research is to identify wheat germplasm, resistant to heavy metals (zinc, cadmium) primarily in Eastern Kazakhstan, and discover donors for selection for metal resistance and perspective wheat forms, intended to implement into production. The research subject is various genotypes of spring wheat from the collection of Eastern Kazakhstan Agricultural Scientific Research Institute. The experiments were held in condition of modeling environmental pollution on 10-day-old sprouts of different wheat genotypes, grown on nutrient mixture, containing CaSO, 0.1mm and Cd ions at a concentration of 40 mg/L (as CdSO₄ salt) or Zn at a concentration of 400 mg/L (as salt ZnSO₄). Since a direct negative impact of heavy metals on plants is displayed in inhibiting of their growth and development (root and sprout growth weakening), indicators of wheat genotypes growth processes were examined during the experiment and Wilkins coefficient was defined. The study revealed sensitive and metal resistant samples. Studies of membrane permeability for electrolytes in terms of environmental pollution by cadmium and zinc, have led to the conclusion that plant resistance can generally be caused by cell membranes' resistance to the action of the stressor. Membrane permeability for electrolytes in the most resistant wheat variety Altai changed less comparing to a more sensitive variety of spring wheat Erythrospermum-606 during the action of both cadmium and zinc. This fact proves that the overall resistance of plants may be due to stability of cell membrane to the action of the stressor.

Key words: heavy metals, wheat, growth parameters, membrane permeability, accumulation of metals, sensitive and resistant genotypes.

Introduction

Plants, grown in contaminated soils, exhibit significant interspecies differences in responses to pollution [1]. It is known that the ability of plants to absorb, accumulate, and use chemical elements genetically determined [2].

On the basis of material obtained by screening the gene pool of wheat and by use of genetic models, Gamzikova O.I. with colleagues developed the idea of the ability to manage the edaphic stability properties by breeding method [3].

The available literature data showed that a significant positive correlation between the concentration of heavy metals in the grain and genotypes, indicating the possibility of breeding varieties with a low potential for accumulation of heavy metals [4].

Kazakhstan is characterized by the presence of mining and metallurgical industries, highly polluting by heavy metals, polluting the agricultural land in particular. Using and creating varieties that are tolerant to pollutants, especially heavy metals, is a component of environmentally clean technologies [1].

At the first stage of this process it is necessary to investigate the gene pool of cultivated and wild plants and segregate sustainable forms of plants and breeding donors, accumulating the minimum number of ecotoxicants.

Therefore, the purpose of work was the investigation of the different genotypes of winter wheat to identify the forms that are resistant to heavy metals such as zinc, cadmium, which are priority pollutants in the East-Kazakhstan region and identification of donors for breeding.

Materials and methods

Studies were carried out on various genotypes of spring wheat from the collection of Eastern Kazakhstan Agricultural Scientific Research Institute.

The following spring wheat genotypes were examined during the experiment: Altai, Ulbinka-25, Zaulbinka, Lyazzat, Glubochanka, Nargiz, Omskaya-18, Samal, Erythrospermum-606, Kutulukskaya.

Plant growth was held in terms of modeling environmental pollution on 10-day-old sprouts of different wheat genotypes, grown on nutrient mixture, which contains $CaSO_4$ 0.1mm and Cd ions at a concentration of 40 mg/L (CdSO₄ salt) or Zn at a concentration of 400 mg/L (ZnSO₄ salt). The plants were grown for 10 days in water culture with t-22°C during the day and 18°C at night, with a 14-hour photoperiod, light intensity – 5 thousand lx, humidity – 65%.

The measurement of biometric indicators was carried out according to common methods. The plants were dissected to an over-ground part and roots. Roots and surface organs length were measured. In order to determine the plants' dry biomass, they were put in a drying cabinet and dried at t-105°C till constant weight, cooled down to room temperature and weighed.

The tolerance index or Wilkins coefficient were calculated using the formula: $I_t = I_{me} / I_c$, where I_{me} – growth of the roots on the solution of the tested metal, I_c – growth of the roots on the solution without metal [5].

The testing of contrasting by stability spring wheat genotypes for the permeability of cell membranes was conducted in laboratory conditions. To research the permeability of wheat cell membranes, identified during experiments on study of growth parameters wheat varieties contrasting by resistance were used, resistant – Altai and sensitive – Erythrospermum-606.

Membrane permeability determination for electrolytes was conducted according to Dexter conduct metric method [6]. Modified Dexter method was used to determine the release of electrolytes from plants leaves tissues, exposed to the stress effect.

A weighed sample of 500 mg leaves was immersed into 50 ml water with the known conductivity for 60 - 180 min. The exudate was drained out and electro conductivity was measured by conductivity meter at the end of the exposure.

After the counting the exudate was poured into the same cup with the sample and was boiled up and its electro conductivity was re-measured.

Calculation formula:% electrolytes elution = (a: b) x 100, where a - is the conductivity of dead tissues, b - is the conductivity of living specimens.

Results and their discussion

Our study of cadmium and zinc effect on growth parameters of wheat sprouts in the laboratory condi-

tions showed that the ions of both cadmium and zinc inhibit plant growth.

The research on wheat varieties from the Eastern Kazakhstan Agricultural Scientific Research Institute collection showed that by the growth of surface organs at a high concentration of cadmium, genotypes can be arranged as follows: Altai > Nargiz > Glubochanka > Ulbinka-25 > Samal > Omskaya-18 > Kutulukskaya > Zaulbinka > Lyazzat > Erythrospermum-606 (Figure 1).

Regarding zinc, the results are the following: by the growth of surface organs at a high zinc concentration, genotypes can be arranged as follows: Zaulbinka > Altai > Ulbinka-25 > Lyazzat > Glubochanka > Kutulukskaya > Nargiz > Omskaya-18 > Samal > Erythrospermum-606 (Figure 2).

Based on the received data, we see that in both cases, by the growth of surface sprouts, Altai is the wheat variety least exposed to effect of zinc and cadmium ions, while surface sprouts of variety Erythrospermum-606 acutely react on the presence of heavy metal ions.

Based on the received data, we see that in both cases, by the growth of surface sprouts, Altai is the wheat variety least exposed to effect of zinc and cadmium ions, while surface sprouts of variety Erythrospermum-606 acutely react on the presence of heavy metal ions. Samal, Omskaya-18, Kutulukskaya and Zaulbinka wheat varieties showed average resistance to the cadmium effect according to the growth parameters of surface organs. When introducing zinc to the cultivation medium, growth of surface organs of varieties Lyazzat, Glubochanka, Kutulukskaya, Nargiz, Omskaya-18 is inhibited at the average degree, comparing to other varieties, which is the indicator of average metal endurance. By the root growth, at introducing cadmium to the nutrition medium, genotypes can be arranged the following way: Nargiz > Ulbinka-25 > Altai > Glubochanka > Omskaya-18 > Zaulbinka > Lyazzat > Kutulukskaya > Erythrospermum-606 > Samal (Figure 3).

Altai, Ulbinka-25 and Nargiz have the most resistant to cadmium root system. Root growth of varieties Nargiz, Ulbinka-25 and Altai is inhibited in much less degree comparing to other genotypes at a high cadmium concentration in cultivation medium. Varieties Glubochanka, Omskaya-18, Zaulbinka and Lyazzat have an average root resistance level. Plant roots of varieties Erythrospermum-606 and Samal turned to be the most non-resistant to the adverse cadmium effect (Figure 3). When studying the impact of zinc on growth parameters of varieties from the Eastern Kazakhstan Agricultural Scientific Research Institute collection, it was shown that by the root growth at a high zinc concentration genotypes can be arranged as follows: Altai > Ulbinka-25 > Zaulbinka > Lyazzat > Glubochanka > Nargiz > Omskaya-18 > Samal > Erythrospermum-606 > Kutulukskaya (Figure 4).

Varieties Altai, Ulbinka-25 and Zaulbinka have the most zinc resistant root system. Lyazzat, Glubo-

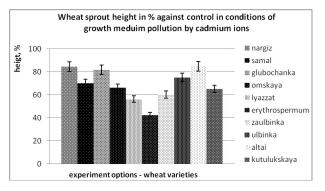


Figure 1 – Influence of cadmium ions presence in growth medium on sprout growth of different wheat genotypes.

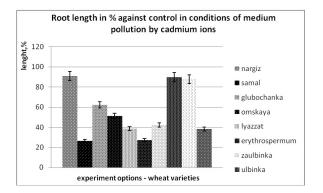


Figure 3 – Influence of cadmium ions presence in growth medium on root growth of different wheat genotypes

Varieties Nargiz, Ulbinka-25 and Altai have the highest, Glubochanka, Omskaya-18, Zaulbinka, Lyazzat Zaulbinka and Kutulukskaya have an average, and Erythrospermum-606 and Samal have the lowest Wilkins coefficient or index of tolerance at a high concentration of cadmium (Table 1).

Based on the results of the study of root growth in polluted by cadmium ions medium and on index of tolerance varieties Nargiz, Ulbinka-25 and Altai can be identified as genotypes with the most resistant towards the adverse effect of cadmium root system. chanka and Nargiz have an average roots resistance level. Altai, Ulbinka-25 and Zaulbinka varieties' root growth is inhibited less comparing to other genotypes that were introduced to zinc growth medium.

Varieties Omskaya-18, Samal and Erythrospermum-606 are less resistant to the adverse effect of zinc.

The most non-resistant to the adverse effect of zinc are plant roots of variety Kutulukskaya.

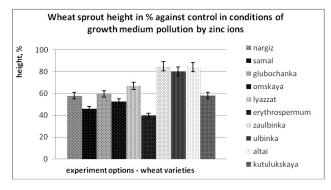


Figure 2 – Influence of zinc ions presence in growth medium on sprout growth of different wheat genotypes

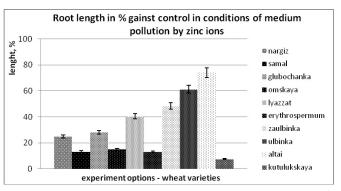


Figure 4 – Influence of zinc ions presence in growth medium on root growth of different wheat genotypes.

Altai variety has the highest, varieties Ulbinka-25 and Zaulbinka have an average, and Kutulukskaya and Erythrospermum-606 varieties have the lowest Wilkins coefficient at a high zinc concentration (Table 2). Based on the results of the study of root growth in polluted by zinc ions medium and on index of tolerance genotypes Altai and Ulbinka-25 can be identified as the most resistant toward the adverse effect of zinc root system.

The regularities of accumulation and distribution of Zn and Cd on the bodies of spring wheat depending on the genotypic differences of samples in a model experiment conditions have been studied. Investigation of zinc content in aerial parts and roots of seedling of spring wheat of different genotypes showed that the smallest accumulation of zinc is in winter wheat aerial organs, when its content in the medium is 400 mg/l, observed in seedlings of varieties Samal and Lazzat. The highest zinc content in the aboveground organs observed in plants of variety Ulbinka.

Wheat varieties	Nargiz	Samal	Glubo- chanka	Oms- kaya-18	Lyaz-zat	Eryth- rosper- mum-606	Zaul- binka	Ulbin- ka-25	Altai	Kutu luks- kaya
I _{me}	12.08	8.36	15.17	12.63	10.33	4.6	6.9	8.8	11.15	9.6
I _c	13.29	31.46	24.36	24.60	26.70	16.9	16.3	9.8	12.7	25
I	0.91	0.27	0.62	0.51	0.39	0.27	0.42	0.90	0.88	0.38

Table 1 - Wilkins coefficient or tolerance index of wheat sprout roots in polluted by cadmium ions cultivation environment

Table 2 - Wilkins coefficient or tolerance index of wheat sprout roots in polluted by zinc ions cultivation environment

Wheat varieties	Nargiz	Samal	Glubo- chan- ka	Oms- kaya-18	Lyaz-zat	Eryth- rosper- mum-606	Zaul- binka	Ulbin- ka-25	Altai	Kutu- luks- kaya
I _{me}	3.32	4.17	6.87	3.72	10.79	2.2	7.9	6	9.4	1.9
I _c	13.29	31.46	24.36	24.60	26.70	16.9	16.3	9.8	12.7	25
I	0.25	0.13	0.28	0.15	0.40	0.13	0.48	0.61	0.74	0.08

Study of zinc accumulation in plant roots, at its content in the medium of 400 mg/l, showed that its least amount is contained in the seedling roots of varieties Altai, Lyazzat, Glubochanka and Samal. The highest zinc content in the roots was observed for plant varieties Ulbinka 25.

According to the study of the effect of zinc on the growth parameters of 10-day-old wheat seedlings as well as according to the study of the accumulation of zinc in the roots and aerial parts of plants of different winter wheat genotypes, variety Altai can be identified as a genotype with the highest root resistance to zinc action and variety Lyazzat and Samal as a resistant to zinc translocation into above-ground organs.

Study of zinc accumulation in roots and aerial parts, as well as growth parameters of wheat seedlings of different genotypes allowed to reveal the most sensitive and resistant genotypes. The most resistant to zinc genotypes are varieties of spring wheat Altai, Lyazzat and Samal, and most sensitive – spring wheat variety Ulbinka 25.

Study of the content of cadmium in the aerial parts and roots of seedlings of different genotypes of spring wheat showed that at a dose of cadmium in the environment of 40 mg/L, the lowest number in its roots accumulate sprouts of spring wheat varieties

Kutulukskaya, Nargiz, 25 Ulbinka and Samal. The greatest amount of cadmium in the roots of plants accumulates variety of spring wheat Erythrospermum 606. The remaining varieties occupy an intermediate position between them. Thus, the smallest accumulation of cadmium is observed in the roots of spring wheat varieties Kutulukskaya, Nargiz, Ulbinka 25 and Samal.

Cadmium content in aboveground organs of studied seedlings of spring wheat genotypes showed that at a dose of cadmium in the environment of 40 mg/l, in above-ground organs least amount accumulate sprouts of spring wheat varieties Lyazzat, Omskaya 18 and Altai.

In these experimental conditions the greatest amount of cadmium in the aerial parts accumulate seedlings of spring wheat variety Erythrospermum 606.

The remaining varieties occupies an intermediate position between them. Thus, the greatest resistance to the inflow of cadmium in the aerial organs of winter wheat varieties showed Lyazzat, Omskaya 18 and Altai.

According to the study of the accumulation of cadmium in the roots and aerial parts of plants of different spring wheat genotypes can be identified varieties Kutulukskaya, Nargis, Ulbinka 25 and Samal as the genotypes with the greatest root resistance to cadmium and varieties Lyazzat, Omskaya 18 and Altai as the genotypes resistant to the translocation of cadmium in above-ground organs. The study of cadmium accumulation in the roots and aerial parts, as well as growth parameters of wheat seedlings of different varieties allowed to reveal the most sensitive and resistant genotypes. The most resistant to cadmium were spring wheat genotypes Nargiz, Ulbinka 25 and the Altai, and the most sensitive – variety of spring wheat Erythrospermum 606.

During the research testing of spring wheat contrasting by resistance genotypes we tested on permeability of cell membranes. Membrane permeability change is one of the symptoms of plants reaction to the external influence. It is known, that the exit of electrolytes is the membrane permeability function, which in its turn describes the damage extent [7]. Therefore, genotypes resistant to adverse environmental factors must have different physicochemical membrane characteristics.

Study on the cadmium ions influence on cell membrane permeability of surface organs of resistant Altai and sensitive Erythrospermum-606 wheat genotypes, showed that the permeability of cell membranes for electrolytes increase at a high cadmium (40 mg /l) concentration (Table 3).

The leaf tissues cell membranes permeability of winter wheat variety Altai increased for 23% under the cadmium influence regarding control.

Leaf cell membrane permeability of spring wheat variety Erythrospermum-606 increased for 75% under the cadmium influence (Table 3). It should be mentioned that cell membrane permeability for electrolytes of more resistant wheat variety Altai's changed less comparing to more sensitive genotype Erythrospermum-606.

Table 3 - Influence of cadmium ions on the electrolyte output from leaf tissue of 10-day-old wheat seedlings

Variants	% output of electrolytes	% to control				
Variety Altai						
Control	2.52±	100				
Cd 40 mg/l	3.17±	123				
	Variety Erythrospermum					
Control	3.15±	100				
Cd 40 mg/l	5.52±	175				

Study on the influence of zinc ions on the cell membrane permeability of surface organs of resistant Altai and sensitive Erythrospermum-606 wheat genotypes, showed that cell membrane permeability for electrolytes increases at a high concentration of zinc (400 mg/L) (Table 4). Cell membranes permeability of leaf tissues of spring wheat variety Altai increased for 13% under the zinc influence regarding control.

Leaf cell membrane permeability of winter wheat variety Erythrospermum-606 increased by 45% under the zinc influence relatively to control (Table 4).

Table 4 - Influence of zinc ions on electrolytes output from leaf tissue of 10-day old wheat seedlings

Variants	% output of electrolytes	% to control					
Variety Altai							
Control	2.52±	100					
Zn 400 mg/L	2.84±	113					
Variety Erythrospermum							
Control	3.15±	100					
Zn 400 mg/L	4.63±	145					

It should be mentioned that cell membrane permeability for electrolytes of resistant wheat variety Altai changed less comparing to more sensitive Erythrospermum-606, both under the cadmium and under zinc effect. The reason for strong inhibition of spring wheat Erythrospermum's-606 growth processes, as well as the relative stability of Altai varieties to the heavy metals toxic effect can be cell membrane's different ability to resist the stress, particularly the strong effect of heavy metals.

Membrane permeability change under stressful conditions proves structural restructure of membranes, which defines plants' potentially possible mechanisms to resist adverse environmental factors [8]. As a result of change of cell membrane permeability under influence of various factors, the intensive output of electrolytes from cells is happening. Intensity of secretion of electrolytes depends on varietal features.

Conclusion

As a result of a comprehensive study of zinc and cadmium effect on the growth of surface organs and sprout roots of different wheat genotypes, resistant and sensitive by growth parameters winter wheat genotypes were identified in the laboratory conditions.

According to the study, as the effect of zinc on the growth parameters of 10-day-old wheat seedlings and the accumulation of zinc in the roots and aerial parts of plants of winter wheat different genotypes can be identified variety Altai as a genotype with the highest root resistance to zinc action and variety Lyazzat and Samal as a resistant to zinc translocation into above-ground organs.

According to the study the accumulation of cadmium in the roots and aerial parts of plants of different spring wheat genotypes can be identified varieties Kutulukskaya, Nargis, Ulbinka 25 and Samal as the genotypes with the greatest root resistance to cadmium and varieties Lyazzat, Omskaya 18 and Altai as the genotypes resistant to the translocation of cadmium in above-ground organs.

Membrane permeability of more resistant wheat variety was changing in less degree comparing to more sensitive wheat genotype, both under the cadmium and the zinc effect. This fact indicates that the overall resistance of plants may be due to their cell membrane resistance towards the stressor effect.

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