INTERACTION BETWEEN CATION (NI^{2+}) AND ANIONS SULFATE AND CLORINE (SO_4^{2-} AND Cl^-) IN THE EXPRESSION OF PEROXIDASE ACTIVITY OF SUGAR BEET DEPENDING ON THE DOSE OF NICKEL AND TEMPORARY WATER STRESS

INTERACȚIUNEA DINTRE CATIONUL NICHEL (NI^{2+}) ŞI ANIONII SULFAT ŞI CLOR (SO_4^{2-} ŞI Cl^-) ÎN MANIFESTAREA ACTIVITĂȚII PEROXIDAZEI LA SFECLA DE ZAHĂR ÎN DEPENDENȚĂ DE DOZELE DE NICHEL ŞI STRESUL HIDRIC TEMPORAR

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Abstract. *The excess of Ni in the environment causing significant alterations in plant growth and development, carbohydrate metabolism, increased activity of peroxidase (POD) in leaves, roots and apoplast of these organs. Lower POD activity in leaves and apoplast under Ni environmental pollution of $SO4^{2-}$ anion compared to Cl^- anion, especially in water culture conditions, demonstrates the interdependence of the Ni cation and anion that accompanies it on the cation in solution nutrition: increased toxicity in the form of chloride Ni compared to sulfate. POD activity in leaves, roots and apoplast of these organs is in the close interdependence of changes in plant metabolism and are crucial in the expression level of tolerance of plants to increasing doses of nickel in the environment. This interdependence is manifested both in optimal soil moisture conditions (70% WSC), and temporary water stress (35% WSC).*
Key words: *Nickel, peroxidase, apoplast, sugar beets, water stress.*

Rezumat. *Excesul de Ni în mediu cauzează alterări semnificative în creşterea şi dezvoltarea plantelor, în metabolismul carbohidraților, majorează activitatea peroxidazei (POD) în frunze, rădăcini şi în apoplastul acestor organe. Activitatea mai scăzută a POD în frunze şi apoplast la poluarea mediului cu Ni şi anionul de $SO4^{2-}$ în comparație cu anionul de Cl^-, în special în condițiile culturii hidroponice, demonstrează de interdependență dintre cationul de Ni şi anionul ce-l însoțeşte pe cation în soluția nutritivă: majorarea toxicității Ni sub formă de clorură de Ni în comparație cu sulfatul de Ni. Activitatea POD în frunze, rădăcini şi apoplastul acestor organe este în strânsă interdependență de modificările în metabolismul plantelor şi sunt decisive în manifestarea gradului de toleranță a plantei la dozele crescânde de Ni în mediu. Această interdependență se manifestă atât în condiții optimale de umiditate a solului (70% CTAs), cât şi de stres hidric temporar (35% CTAs).*
Cuvinte cheie: nichel, peroxidaza, apoplast, sfecla de zahăr, stres hidric.

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INTRODUCTION

Nichel as ultramicroelement influences, like other micronutrients, on various physiological processes and foremost on the enzyme activity in the plants (Walsh, Orme-Johanson, 1987). The influence of Ni on the enzymes' activity can be direct as in the case of urease, where Ni is the direct component of the enzyme molecule (Brown et al., 1987) and, more often - indirectly, as a result of the ions imbalance in the plant due to deviations in the transport of other elements nutrients, like Zn, Fe, Cu and others. Nichel can influence indirect on the enzyme activity by interaction with SH groups of the enzymes, thus changing the conformation of protein molecule, which subsequently leads to inactivation of the certain enzyme activity (Kevresan et. al.1998).

The apoplast plays a significant role in various physiological processes including intercellular signaling, water and nutrients transport. So as it is formed assimilates' balanced background in the apoplast, this space is included in the general scheme of regulation of photosynthesis (Macronosov, 1983), nutrient transport (Bukhard, 2001). As a rule, first reaction, which shows the signs of deficiency or toxicity in nutrients resulting in intercellular space - apoplast, manifested both by the composition of the respective ions and the intensity of antioxidative reactions (Speer, Kaiser, 1991; Ana Lopez-Milan et al., 2000; Fecht Christoffers et al., 2003).

The aim of the current research was to reveal the influence of Ni on the peroxidase activity (POD) in leaves, roots and in the apoplast of these organs under insufficient, optimal supply and excess of Ni in the environment, determining the interaction between Ni and anion that accompanies it (Cl^- or $SO4^{2-}$), the expression of plant tolerance potential to environmental pollution with Ni and temporary water stress.

MATERIAL AND METHOD

The effect of Ni on the activity of POD, monosaccharides and sucrose content was studied in water and soil culture experiences. It was used Hoagland-Arnon nutrient solution. On the backdrop of nutrient solution (control) were administered increasing doses of Ni (as $NiCl_2$ and $NiSO_4$, with the equal amount of Ni in compared variants). Volume of the vessel - 250 ml. Variety of sugar beet - Baracuda. Experience scheme: Control - Hoagland-Arnon nutrient solution; Experimental variants with the administration of $NiCl_2$ and $NiSO_4$ - 0.05, 50, 100, 200, 300, 400 µM of Ni. In conditions of soil culture the effect of anions and Ni was studied in short-term experience in polyethylene pots, 300 g of soil. Soil - calcareous chernozem. Scheme of the experience: Control, 300 and 900 mg Ni / kg soil. Ni was used as $NiCl_2$ and $NiSO_4$. The POD activity was determined in leaves and roots at the stage of 8-10 leaves. The effect of Ni and chloride and sulphate anions under temporary water stress conditions (35% WSC) was studied at plant foliar treatment. Soil – calcareous chernozem. Foliar treatment was conducted in the intensive plant development phase and temporary water stress (35%WSC, 10 days) was created after 10 days of plants' salt treatment. Experience scheme: 70% WSC- Control, $NiSO_4$, $NiCl_2$; 35% WSC-Control, $NiSO_4$, $NiCl_2$. Extraction of apoplast assimilates (to determine POD activity) was performed by Riabuşchina and Brovcenco method (Brovcenco,Riabuskina, 1971), monosaccharides and sucrose content – by Bertran, POD activity - by Boiarchin (Ermacov, Arasimovici et. al., 1987).

RESULTS AND DISCUSSIONS

Nichel toxicity in plants is manifested by significant alterations in the distribution of macro– and microelements, changes in antioxidant enzyme activity, in the reduction of photosynthetic processes and in the assimilates translocation in plants (Rajni Shukia, Rageev Gopal, 2009; Kevresan et al.,1998; Toma et al., 2002; Pandolini et al., 2006; Lediko Josqak et al.,2008). Researches have shown that the application of the dose of 300 mg Ni / kg soil (table 1) significantly increases the activity of POD in leaves under application of Ni in chloride form. Peroxidase activity in roots under Ni sulphate application is virtually on the level of control variant while the application of nickel chloride significantly increased the enzyme activity. The visual symptoms of toxicity of the pollutant were also more pronounced in the application of nickel chloride salt.

Table 1

POD activity in leaves and roots (conventional units g. fresh weight / min) in sugar beet seedlings (v. Baracuda) depending on the ions Cl^- and SO_4^{2-} under soil pollution with Ni (300 mg element/ kg of soil).

Organ	Variant	POD activity
Leaves	Control	29,43
	$NiSO_4$	41,89
	$NiCl_2$	52,86
Roots	Control	88,53
	$NiSO_4$	86,86
	$NiCl_2$	138,83

There is of great interest the experimental data obtained in the experience under the conditions of water culture. Nichel dose of 0.05 µM (otherwise the microdose, where Ni has influences as essential micronutrient) acted differently on peroxidase activity in leaves, depending on the anion that accompanies it in nutrient solution. Ni sulphate significantly decreased the enzyme activity while Ni chloride - on the contrary, led to induction of enzyme activity. The effect of anions in the roots was less. Nichel dose of 50 µM significantly increased the enzyme activity in leaves regardless of the anion which shows the action of Ni that manifested as pollutant (table 2). Further increase the dose of Ni (100 and 200 µM) did not significantly affect the enzyme activity because on seedling leaves were observed visual symptoms of toxicity of the pollutant: inhibition, in fact, the accumulation of vegetative mass, the occurrence of chlorosis, partial drying of leaves and plants. Then, after 7-10 days from the Ni toxic dose administration (de facto) the majority of seedlings were dry in these variants and at the 400 µM dosage the seedlings were completely dry. In this case the peroxidase activity decrease is bound, perhaps, to, irreversible changes in plant metabolism.

Table 2

POD activity (conventional units g. fresh weight / min.) in leaves and roots of sugar beet seedlings under environmental pollution with nickel sulphate and chloride. Hoagland-Arnon nutrient solution. Phase of 6-8 leaves.

Variant	$NiSO_4$		$NiCl_2$	
	Leaves	Roots	Leaves	Roots
Control	47,995	64,065	47,995	64,085
Ni 0,05μM	35,296	64,009	74,105	59,343
Ni 50μM	96,956	55,227	81,963	44,362
Ni 100μM	62,527	49,861	87,759	35,003
Ni 200μM	60,727	46,295	10,453	38,762

In the conditions of temporary water stress (table 3) POD activity in leaves increases significantly and this indicates to the decisive role of the enzyme in the detoxification of free radicals and, ultimately, in reducing the negative effect of drought on plant physiological status. On the other hand, plant foliar treatment with both salts, leads to reducing of POD activity in this organ. It is possible to conclude the positive role of micronutrient in these conditions on physiological processes in plants (non-specific nature), probably by free radical content decrease in leaves.

It was found the most significant effect of Ni sulphate on the activity of POD (after the 3^{th} and 10^{th} days of water stress) compared with nickel chloride. The same regularities in enzyme activity occur, in principle, and in leaf apoplast: more significant decrease of enzyme activity from the application of nickel sulphate in comparison with nickel chloride (the background of increased enzyme activity in leaf apoplast - control variant). More pronounced decrease in apoplast POD activity under the influence of sulfate ions in water stress conditions demonstrates the decisive role of sulphate (except of Ni cation) to include more decisively in plant metabolism and is reflected directly on reducing the negative effect of drought on plants.

Table3

POD activity (conventional units g. fresh weight / min) in leaves and in leaf sugar beet apoplast depending on foliar treatment of plants with solutions of nickel sulphate and chloride and temporary water stress, 10 days, 35% WSC.

Variant	After 3-th days of water stress				After 10-th days of water stress			
	Leaves		Apoplast		Leaves		Apoplast	
	70% WSC	35% WSC	70% WSC	35% WSC	70% WSC	35% WSC	70% WSC	35% WSC
Control	36,52	51,10	1,37	1,41	69,10	79,84	1,38	1,69
$NiSO_4$	38,51	39,38	0,87	0,99	58,40	56,26	1,59	1,14
$Ni Cl_2$	45,55	36,63	1,48	1,17	83,57	65,11	1,94	1,62

The temporary water stress conditions have not been established an strict regularities in the distribution of monosaccharides in the leaves in dependence of

Cl^- and SO_4^{2-} ions: it was observed only the tendency to increase the content of monosaccharides under sulphate application, and maintaining on the same level, or there were an insignificant decrease of their content under the plant foliar treatment solution of 0.01% nickel chloride. Decrease of the monosaccharides' content in the application of Cl^- - ions is due to increasing transport of these substances to the roots because sucrose content increases more substantially under water stress (with 0.9%, in control - 14.4%, tab. 4).

Table 4

Weight of roots of one plant depending on plant foliar treatment with solutions of nickel sulphate and chloride and temporary water stress

	Variant	Root weight, kg	%	Content of sugar,%	Increase, %
70%WSC	Control	0,67 ± 0,06	100	14,4	0
	$NiSO_4$	0,71± 0,02	105,9	14,8	0,4
	$NiCl_2$	0,66± 0,08	98,5	14,4	0
35%WSC	Control	0,54± 0,05	100	14,4	0
	$NiSO_4$	0,56 ± 0,08	103,1	14,9	0,5
	$NiCl_2$	0,52 ± 0,01	96,8	15,3	0,9

The plant foliar treatment with Ni sulfate contributed to the increase of root mass in both the optimal soil moisture conditions (with 5.9%, in the control – 0.67 kg) and temporary water stress (with 3.1 %, in control -0.54 kg). Under Ni chloride treatment the weight of roots was on the level of control but with higher sugar content in them (with 0.9%, in control - 14.4%).

CONCLUSIONS

1. The excess of Ni in the environment causing significant alterations in plant growth and development, carbohydrate metabolism, increased antioxidant enzyme activity of POD in leaves, roots and in the apoplast of these organs.

2. Lower POD activity in leaves and apoplast under Ni environmental pollution and the SO_4^{2-} anion compared to Cl^- anion, especially in water culture conditions, demonstrates the interdependence of the Ni cation and anion to accompanies it in nutrient solution: increase toxicity of Ni chloride as compared with nickel sulfate.

3. POD activity in leaves, roots and apoplast of these organs is in the close interdependence of changes in plant metabolism (carbohydrates) and are decisive in the manifestation of the degree of tolerance of plants to increasing doses (pollutants) of Ni in the environment. This interdependence is manifested both in optimal soil moisture conditions (70% WSC) and temporary water stress (35% WSC).

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