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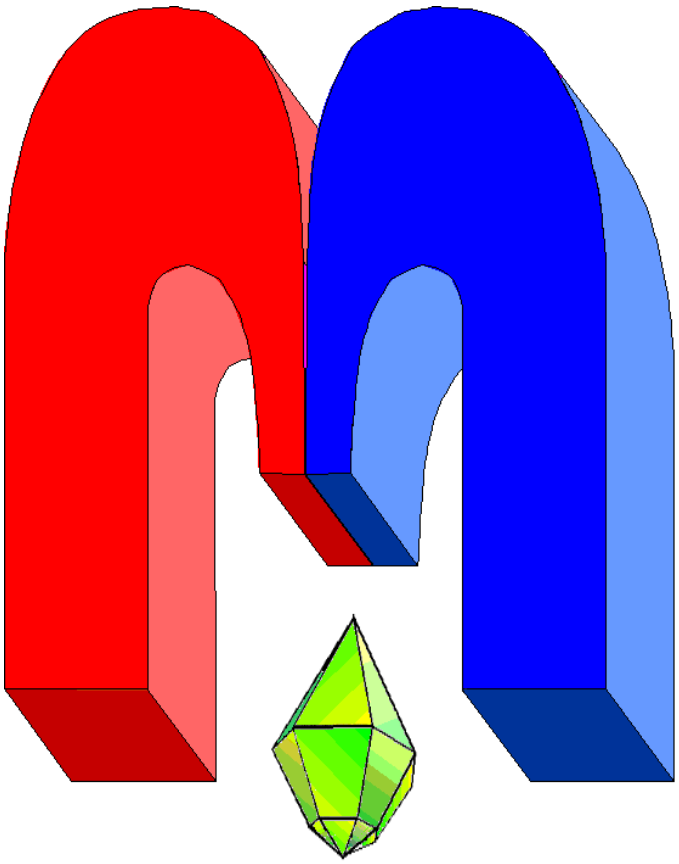

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* In Kazan University the Electron Paramagnetic Resonance (EPR) was discovered by Zavoisky E.K. in 1944.

The accumulation and toxic effect of manganese during the growth and development of *Solanum tuberosum* L.

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The migration and accumulation of manganese ions during the growing season of *Solanum tuberosum* L. potatoes have been studied. The complex form and content of the metal in potatoes cultivated under factor-static conditions are determined using elemental analysis and electron paramagnetic resonance (EPR) technique. The toxic effect of the metal manifests itself in biometric indicators with an increase of manganese salt content in the nutrient medium, which is accompanied by growth of the EPR signal and the accumulation of metal in plant organs. In addition, the EPR spectra show a narrow signal characteristic of stable semiquinone radicals.

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1. Introduction

It is known that trace elements, including manganese, play an important role in biochemical processes in plants [1,2]. Besides, the deficiency and excess of manganese can cause serious diseases of agricultural plants and reduce their yields [3]. Such elements as manganese are included into a separate group of micro fertilizers, which are required for plants in very small amounts (less than 1 kg/ha) to stimulate their growth [4] that makes a search for new fertilizers an urgent challenge [5,6]. Therefore, the comprehensive study of metabolism and the development of approaches to optimal nutrition of *Solanum tuberosum* L. potatoes, one of the most important agricultural crops not only in Russia, but also in the world, is of great interest to biochemists and plant physiologists.

Manganese is a cofactor of many plant cell enzymes involved in the redox reactions of photosynthesis, production of vitamins *C*, *B*, *E* and ascorbic acid [1,3]. In addition, manganese promotes to increase the sugar content and their elimination from the leaves, accelerates plant growth and seed maturation. Deficiency of manganese reduces the synthesis of organic substances and decreases the content of chlorophyll. The point chlorosis of the leaves is also a characteristic feature manganese deficiency. At the same time, an excess of the metal (its accumulation in plant) exerts negative effects on both the development of the plant itself and human health, when the plant is consumed.

In the present work, the qualitative and quantitative effect of manganese during the growing season of agricultural crops (potatoes) has been studied. Also, new data on the mechanism of manganese ions migration are revealed, the general toxicological influence of the metal on plant development is determined, and the optimal conditions for enrichment of plants with manganese are found using *Solanum tuberosum* L. as an example. It is especially important to investigate the development of potatoes on saline soils and on private farms in the vicinity of

industrial enterprises, as well as to evaluate possibilities of the plant for accumulation of the metal. We have chosen potatoes as an object of research, since it is sensitive to the content of manganese, like some other crops such as sugar beets, fodder beets, table beets, oats and apple trees. In this line, it should be emphasized that EPR spectroscopy is one of the most powerful instrument for the investigation of paramagnetic particles in plant organs. EPR spectroscopy has long established itself as a convenient and effective method for working with magnetic ions including manganese ones [7–9], the evaluation of radical activity in various living systems, the determination of role of metals in biochemical processes of plants, the study of metals migration and their accumulation in plants.

2. Experimental details and results

In this study, plants of the "Lukyanovskiy" variety were used in vitro. Potato plants were cultivated under factor-static conditions using the Murashige-Skoog nutrient medium [10], where manganese was introduced in the form of a salt of crystalline manganese sulfate $MnSO_4 \times 5H_2O$. The experiments on variation of the concentration were carried out starting from 24.1 g/l (0.1 mol/l) of manganese salt (mass content for the control) according to prescription in the Murashige-Skoog nutrient medium to a 20-fold excess over the control to study the toxic effects of manganese and its accumulation in plant organs in an environment with an increased metal content. The nutrient media, in which the salt content was by 2, 5, 10 and 20 times higher than the control, were employed. The plants were cultivated under factor-static conditions for 30 days, periodically measuring the length and counting the number of leaves. At the end of the experiment, the biomass of herb and roots were determined. Independent experiments were performed in triplicate; ten plants were grown for each concentration variant. For physicochemical experiments, three plants from each variant were used.

EPR spectra were recorded on a FT X-band Bruker ELEXSYS E-580 spectrometer (X-wave range 9.7 GHz). CW EPR spectra were recorded under the following conditions (in quartz ampoules, diameter of 3 mm): amplitude modulation 10 G, modulation frequency 100 kHz, time constant 0.02 s, conversion time 0.06 s, microwave power 0.6325 mW, field range 1000 G/centre field 3343 G, averaged scans 20, receiver gain 50 dB at room temperature.

The content of manganese was determined by atomic absorption analysis using electron microscope HITACHI TM 3000, detector SDD XFlash 430-H. Elemental analysis was performed on roots, stems, and leaves for each series of plants grown at different concentrations. The biological material was dried and then calcined at 350°C for 1 h in a LOIF LF-5/11-G1 muffle furnace, and the ash obtained was analyzed for metal content.

3. Result and Discussion

The experiments on variation of the concentration were carried out both without manganese sulfate in the nutrient medium and with up to 20-fold excess over the control to study the effects of deficiency an d accumulation of manganese in plant organs as well as its toxicity. It should be noted that manganese is considered to be an extremely immobile element. In plants, it only moves upward through the xylem to the leaves, and, reaching the latter, it is not transferred to other parts of the plant [1]. Indeed, the greatest accumulation of metal is observed in the green photosynthesizing part of the plant (Table 1).

Manganese ions are convenient objects for EPR monitoring since the ground state of high-spin complexes with d^5 configuration is an orbital singlet, and the EPR spectrum shows six lines of hyperfine components (HFS) from the manganese nucleus Mn^{2+} with spin 5/2 [11], see

Table 1. The accumulation of manganese (wt%) in plant organs.

Manganese sulfate content, mol/1	Roots	Stems	Leaves
0.1 (control)	0.11(2)	0.18(1)	0.16(4)
0.2	0.17(4)	0.21(4)	0.22(1)
0.5	0.54(1)	0.29(8)	0.20(8)
1.0	0.79(9)	0.88(28)	0.34(8)
2.0	0.97(8)	1.10(13)	0.74(15)

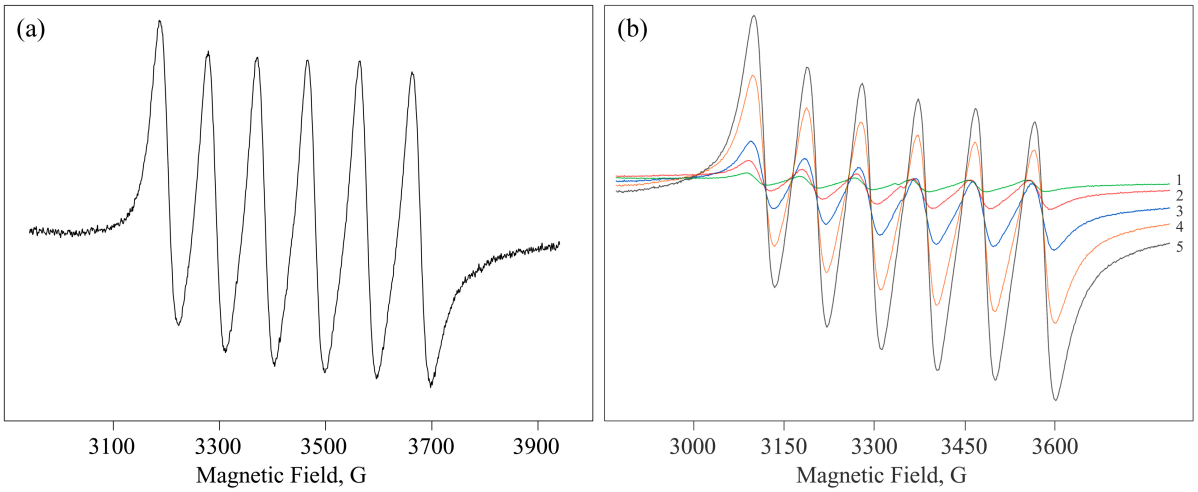

Figure 1. The typical (a) EPR spectrum of control for stem material and (b) EPR spectra for stem materials with the increase of manganese sulfate content in the nutrient medium: *1* - control, *2* - 0.2 mol/l, *3* - 0.5 mol/l, *4* - 1.0 mol/l, and *5* - 2.0 mol/l.

Figure 1a. The multiplet observed in the spectra of roots, stems, and leaves has the following spectral characteristics with a g-factor in the region of 2.004(2), constant (A) of 95(2) G, and linewidth (ΔH) of 38(5) G. The characteristic signal with HFS structure is typical for freely rotating manganese ions in octahedral oxide surroundings of the $Mn(H_2O)_6^{2+}$ complex and is very often detected in numerous biological plant materials [8].

The study of plant tissues show that intensity of the sextet grows (about 10-fold) with the increase of manganese sulfate content in the nutrient medium (Figure 1b), see Table 2. At the same time, no significant changes in the line width and shape are observed with increasing salt concentration (by 0.1–2.0 mol/L). The concentration of Mn^{2+} ions in plant organs is insufficient for significant line broadening due to dipole-dipole interactions, and line shape is explained by inhomogeneous broadening [12].

The experiments have revealed that the varying of manganese sulfate concentration in the nutrient medium dramatically affects the plant growth and biometric parameters (Figure 2). The analysis of the plant growth intensity in various media shows that after 10 days of incubation, this parameter does not noticeably differ in the plants. On the 30th day of incubation, visual examination of the plants demonstrates that in the control group, the color of the potatoes becomes rich green, the leaves are large, there are no withered leaves, and the root system is well developed. The plants with a 2-fold and 5-fold increased concentration of manganese sulfate have a light green color, there are already yellowed and wilted leaves, an elongation

Table 2. EPR characteristics of plant materials depending on the manganese sulfate content in medium.

Salt content, mol/1	Plant materials	g-factor	ΔH, G	A, G
0.1 (control)	Roots	2.0031(2)	33.3(5)	94.7(2)
	Stems	2.0041(4)	32.6(5)	94.9(2)
	Leaves	2.0031(3)	33.2(2)	95.0(5)
0.2	Roots	2.0050(4)	32.7(4)	94.6(3)
	Stems	2.0058(2)	34.2(2)	94.8(3)
	Leaves	2.0053(2)	33.7(4)	95.2(6)
0.5	Roots	2.0041(3)	34.4(1)	94.9(2)
	Stems	2.0052(6)	33.9(5)	95.1(2)
	Leaves	2.0042(3)	33.7(2)	95.8(1)
1.0	Roots	2.0036(5)	35.3(1)	93.8(8)
	Stems	2.0053(5)	34.3(7)	94.5(6)
	Leaves	2.0048(4)	37.4(4)	95.0(5)
2.0	Roots	2.0054(2)	36.5(3)	94.9(2)
	Stems	2.0055(3)	35.4(2)	94.9(2)
	Leaves	2.0055(2)	36.7(4)	94.4(5)

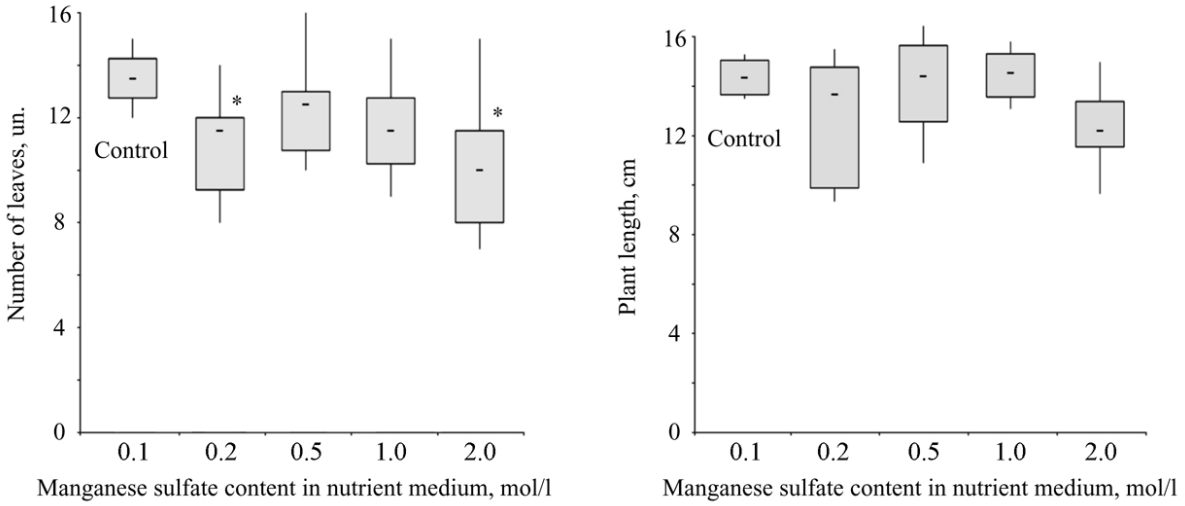

Figure 2. The biometric parameters depending on manganese sulfate concentration in nutrient medium. The asterisks in the graphs mark valid differences according to the Mann-Whitney test.

of internodes is observed, and the root system is developed well. For the plants with a 10-fold increase in the manganese salt content, half of the plants have a light green color, the leaves of the lower layer have necrosis and extensive yellowing, and the root system is poorly developed. The observed necrosis indicates the presence of a strong stress in plants, apparently caused by a change in the cultivation environment. An increase in concentration by 20 times reduces the growth of plants, which, due to the toxic effect of manganese, have a pale green color of leaves and stems, tissue necrosis is observed in the lower part of the stem, there are a large number of yellowed and wilted leaves, and the root system is poorly developed. Biomass of the aboveground part of plants is by 1.5 times lower than that of control plants.

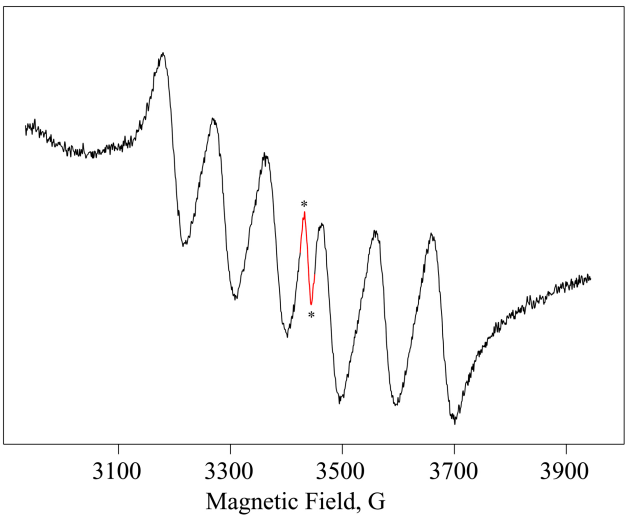

Figure 3. The EPR spectrum recodered for leaves material (control). The additional line is indicated by the asterisks symbols.

Note that in the green part of the plant, in stems and leaves, even sometimes in roots, additional singlet line with g-factor equal 2.0040(2) and line width (ΔH) of 8(1) G can be seen in the EPR spectra (Figure 3). The registered signal in a narrow range of the magnetic field remains unchanged and does not change the intensity. Therefore, such signals can be attributed to long-lived radicals. The parameters of singlet are characteristic of stable semiquinone radicals [8,13], and are found in many plant materials. And the level of semiquinone radicals often can be an indicator of plant resistance to stress, in such cases as damage to the cell structure, during water loss and other processes.

The Thus, the toxic effect of manganese accumulation in the *Solanum tuberosum* L. culture is manifested by an increasing scale of dysfunctions in the plant organism. For instance, the number of yellowed leaves reaches 13-15%, when the concentration of manganese sulfate in the nutrient medium increases by 2–10 times. The maximum yellowing of leaves (42%) and their necrosis are detected, when manganese content augments by 20 times, the development of the root system being also decreased.

4. Summary

It has been shown that the maximum intensity of *Solanum tuberosum* L. growth is observed in the control group and in a series with a manganese concentration in the nutrient medium of 0.2 mol/L. The increase of manganese content in the medium up to 2.0 mol/l slows down the growth of plants, and leads to necrosis of stems, yellowing and drying of potato leaves. The EPR spectra show a typical sextet from Mn^{2+}, which is characteristic of hydrated manganese complexes in plant tissues. The EPR spectra also contain a weak narrow singlet assigned to stable semiquinone radicals. However, without additional comparative experiments, it is difficult to evaluate the stress resistance of the studied potatoes. An excess of manganese in the nutrient medium is accompanied by its accumulation in the plant organs, especially in the photosynthesizing part of the plant. The high concentration of manganese salt in the nutrient medium has a pronounced toxic effect on potatoes, which manifests itself in yellowing and wilting of leaves, as well as in the appearance of necrosis on the stems. This effect may be caused by disordered assimilation of other elements by the plant, as well as by their transition into toxic compounds for plants. In addition, an excess of manganese reduces the intensity of photosynthesis due to a decrease of chlorophyll content in plant tissues, which is accompanied by a drop of the plant biomass.

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