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EFFECT OF MANGANESE IONS ON THE STATE OF CHLOROPHYLL - PROTEIN COMPLEXES OF PHOTOSYSTEM II AT LOW pH

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Abstract. In the presented article the effect of manganese ions on the reactions of millisecond delayed fluorescence of 8 day old seedlings of wheat (Triticum aestivum) incubated for 6 - 72 hours at pH 4.5 was studied. We also studied the effect of manganese on the components of delayed fluorescence and the formation of chlorophyll-protein complexes in etiolated seedlings during greening for 6, 12, 24, 48 hours at pH 4.5. Electrophoresis densitograms of chlorophyll-protein complexes indicate that the accumulation of manganese ions in the cell causes a decrease in the CPa and LHC_2 pigment complex and a decrease in their oligomeric and dimeric forms. The apoproteins of these complexes remained unchanged.

Keywords: *Photosystem II, Chlorophyll- Protein complexes, manganese toxicity.*

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Abbreviations CPa – Reaction Cneter of PSII, LHC – Light Harvesting Complex, LHC1 – olygomer, LHC2 – dimer, LHC3 – monomer, PSII – Photosystem II, Q_A – secondary acceptor in PSII, CP - monomer of Reaction Center in Phototsysten I, CPIa – oligomer of Reaction Center of PSI.

1. Introduction

High concentrations of metals in soils are a major problem worldwide as invironmental hazards increase, especially in acidic soils, when solubility of metals such as aluminum and manganese are increasing under such conditions (Kitao *et al*., 1997). The main effect of metal ions is their toxic effect on the photosynthetic apparatus of plants (Sigfridsson *et al*., 2004). The studies of the response of the photosynthetic apparatus to aluminum stress in two sorghum varieties, a decrease in the content of chlorophyll and decrease in the intensity of photosynthesis was reported (Peixoto *et al*., 2002). Myasaka et al. (1997) investigated the effect of aluminum and calcium ions on photosynthesis and chlorophyll fluorescence in taro and suggested that high levels of calcium help to stabilize the thylakoid membrane structure from destruction by aluminum. Similar studies on the assembly of light-harvesting complexes and the effect of pH in the presence of magnesium ions showed a strong concentration dependence on fluorescence quenching (Schaller *et al*., 2014).

The best known function of manganese ions in green plants is its participation in the photosynthetic oxygen evolution. PSII contains a mangano protein that catalyzes the

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initial steps of oxygen evolution. Manganese deficiency impairs the first step of electron transfer into the electron transport chain and has a negative effect on subsequent reactions such as photophosphorylation and carbon dioxide reduction. But manganese becomes toxic with excess and acidification of the soil at the subcellular level, in contrast to the stresses of other metals. Excess manganese simultaneously hits multiple targets in the cell. Depending on the plant species, excess manganese can be stored in cell walls, Golgi vesicles, and chloroplast thylakoids (Lidon & Reixeria, 2000). The main part of manganese ions enters the cytoplasm and binds to the other side of the thylakoid membranes of chloroplasts, affecting photosynthesis. A decrease in photosynthetic activity and an increase in polyphenol oxidase activity are the most sensitive indicators of manganese toxicity, preceding a decrease in chlorophyll and the appearance of visible symptoms (Küpper & Kroneck, 2005; Nable *et al*., 1997). A decrease in photosynthesis with an excess of manganese in the leaves is proposed as one of the mechanisms that determine the toxicity of manganese. An excess of manganese in leaves affects the activity of the CO_2 reduction cycle, but not the potential efficiency of photochemistry, which leads to an increase in the degree of Q_A reduction and thermal energy dissipation, as well as a decrease in the stationary state PSII quantum yield (Kitao et al, 1997). In presented work, we studied the toxic effect of the Mn^{2+} salt on the development of reactions responsible for the delayed chlorophyll fluorescence in the millisecond time range in 8-day-old wheat (*Triticum aestivum*) seedlings grown under low pH conditions, and the effect of Mn^{2+} on the formation of the components of the millisecond delayed fluorescence and chlorophyll-protein complexes (CP) in the process of greening.

2. Materials and methods

Leaves of 8-day wheat seedlings (*Triticum aestivum*) incubated in the presence of Mn^{2+} salt ($MnCl_2 \cdot 2H_2O$) at a concentration of 100 mg/l for 2, 6, 24, 48 and 72 hours, as well as leaves and thylakoid membranes of etiolated wheat seedlings, turning green for 6, 12, 24, 48 hours (irradiation intensity 250 $\mu W/cm^2$) in the same nutrient solution with pH 4.5. Thylakoid membranes were obtained according to the standard method in 50 mM phosphate buffer (pH 7.8) at a ratio of sodium dodecyl sulfate (SDS) to chlorophyll equal to 2.5. Chlorophyll-protein complexes were isolated by SDS-PAGE electrophoresis with a concentration of 0.1% SDS in an electrode buffer with some modifications followed by staining with Coomasie blue as described previously (Kurbanova, 1987). To identify chlorophyll-protein complexes, a set of LKB markers (Sweden) was used. The obtained electrophoregrams were processed with the Origin Pro9 software package.

The kinetics of delayed fluorescence was measured using a phosphoroscope (Rubin *et al*., 1984) with a measurement of the time between excitation and emission equal to 1.25 ms.

3. Results and discussion

In this work, we studied the effect of manganese ions at pH 4.5 on the formation of chlorophyll-protein complexes. As can be seen in Fig. 1, the chlorophyll-containing profile (A) and the profile of chloroplasts stained with Coomasie blue (B) isolated from

wheat seedlings during greening (from 6 to 48 hours) showed that in the absence of manganese ions (control) CPIa (110 kDa), oligomeric form LHC (94 kDa), monomeric form RC PSI - CP (68 kDa), dimeric form LHC2 (47 kDa), PSII reaction center - CRa (43-45 kDa).) and the monomeric form of LHC (23 kDa) and their apoproteins.

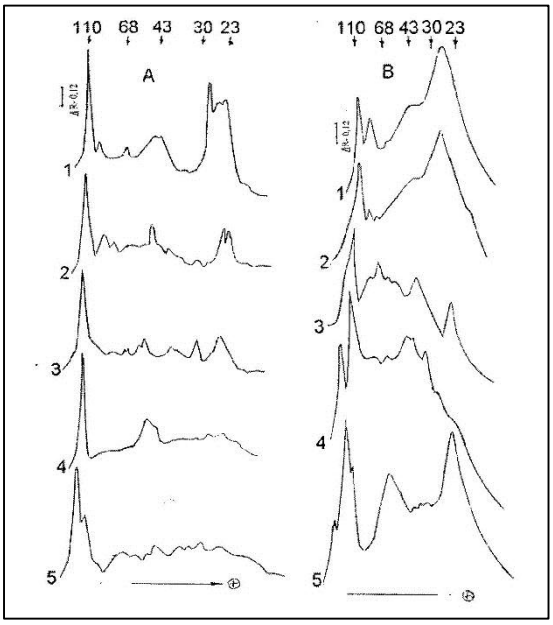

Fig. 1. Densitometric profile of chlorophyll-protein complexes of etiolated wheat seedlings turning green under normal conditions: (A) - 1 - control, 2 - 6 hours, 3 - 12 hours, 4 - 24 hours, 5 - 48 hours (B) – painted with Coomasie blue

The treatment of seedlings with Mn ions caused a significant degree of degradation of LHC (23 kDa) and CPa (43–45 kDa) with respect to CPIa, LHCI, and CP of chlorophyll-containing subunits (Fig. 2A).

Coomasie blue staining (Fig. 2) did not reveal apoprotein degradation in the 23 kDa and 43-45 kDa regions. In addition, under conditions where the effect of Mn^{2+} ions is manifested, profiles containing chlorophyll and stained with Coomasie blue consistently show an increase in the concentration of complexes with higher molecular weights (110, 94, 68 kDa).

The functional state of wheat seedlings under the action of manganese ions was assessed by the delayed fluorescence of chlorophyll ***a***, which, as is known, arises as a result of electron recombination during light-induced charge separation in the PSII reaction center (Wraight & Grofts, 1971). The fast phase of delayed fluorescence, which characterizes charge recombination, a change in the proton gradient, and the reduction of primary electron acceptors on the donor side of PSII, decreased after prolonged exposure to Mn ions during the accumulation of ions (Table 1). The slow component of delayed fluorescence, which characterizes the stability of the electron flow to the Q_A site, has been changed. The critical point of action of manganese ions is observed after 24 hours of incubation and lasts up to 72 hours.

The changes caused by the studied ions are clearly seen in the ratio of fast and slow components of delayed fluorescence, which reflects the interaction between the PSII RC, the electron transport chain, and the proton gradient. These processes are strongly disturbed when exposed to manganese ions (Table 1).

A study of the effect of manganese on the greening of seedlings during the formation of a photosynthetic membrane revealed a decrease in the ratio of the fast phase to the slow phase of delayed fluorescence (Table 2). After 48 hours, the degree of greening decreased by 2.5 times in the presence of $MnCl_2$ compared with the control.

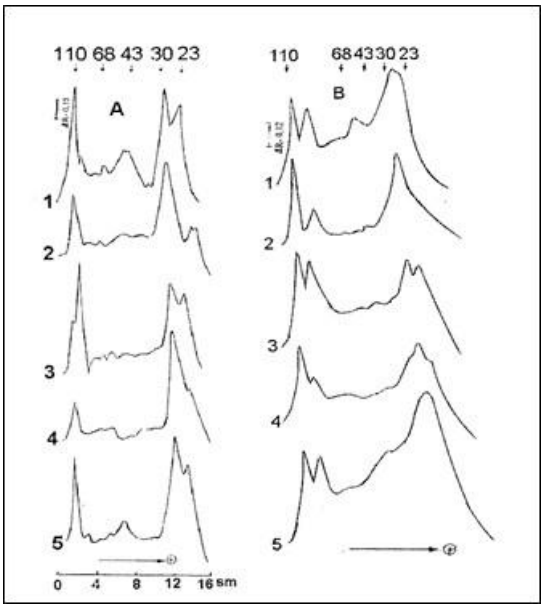

Fig. 2. Densitometric profile of chlorophyll-protein complexes of etiolated wheat seedlings turning green in the presence of MnCl2 (100 mg/l) at pH 4.5 (A) - 1 - control, 2 - 6 hours, 3 - 12 hours, 4 - 24 hours. , 5–48 h. (B) stained with Coomasie blue

Table 1. Effect of manganese ion incubation time on changes in the fast and slow components of delayed fluorescence in wheat seedlings at pH 4.5

	$MnCl_2$ (100 mg/l)		
Incubation time (hour)	fast	slow	fast/slow
Control	58.7	27.1	2.17
2	65.2	26.7	2.5
6	45.8	10.5	4.36
24	38.2	2.7	14.15
48	32.1	2.1	15.3
72	22.2	1.6	18.3

Table 2. The effect of manganese ions on the ratio of fast to slow components of delayed fluorescence in etiolated wheat seedlings at different stages of greening

Greening time (hours)	Control	$MnCl_2$ (100 mg/l)
	fast/slow	fast/slow
6	14.1	10.9
12	29.3	17.7
24	43.2	25.7
48	70.6	27.1

Resuming, we can conclude that the formation of the thylakoid membrane and their structure are very sensitive and subject to the influence of Mn^{2+} in acidic conditions of the nutrient medium. The nature of the kinetic curves of delayed

fluorescence during the formation of the thylakoid membrane indicates the deterioration in the biosynthesis of photochemical centers. Data in Table 2 indicate a decrease in the proton gradient and the rate of the electron transfer reaction in PSII, which was observed earlier by authors (Gaziyev *et al*., 2011). Changes in the photochemical function of the thylakoid membrane correlate with the obtained electrophoresis data. It is known that under stress the photosynthesis is impacts by different levels, including the synthesis of chlorophyll. The main changes under the action of manganese ions are observed within 6-12 hours of greening, when granules are formed and chlorophyll pools and the ratio of reaction centers are optimized. Under the action of manganese ions, the absence of the oligomer and dimer, as well as the monomer of light-harvesting complexes, is also observed. This can be explained by the fact that proteins that should interact with chlorophyll changed their configuration under the action of Mn ions, which led to the cessation of the biosynthesis of the light-harvesting complexes. The explanation of the observed effect fits into the concept of an electric double layer (EDL) proposed (Barber J., 1980) and described in detail (Kana Govindjee, 2016). EDL is affected by ion valence and interferes with the regulation of transition states, the interaction of protein complexes, and the transfer of excitation energy from Photosystem II to Photosystem I. These effects are reflected in changes in the intensity of chlorophyll ***a*** fluorescence, which is also a measure of photoprotective non-photochemical quenching of the excited state of chlorophyll ***a***. Thus, it can be assumed that manganese ions disrupt not only chlorophyll biosynthesis, but also prevent the formation of chlorophyll-protein complexes.

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