HEAVY METAL IONS INFLUENCE ON CONIFER SEEDS GERMINATION AND MITOTIC DIVISION

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Abstract: The seed biostructure presents a differential response of both at the biochemical action of manganese ions - the inhibition or stimulation of specific enzymatic reactions - as at the osmotic characteristics of seeds tegument, depending on the studied plant species. The analysis of the cytological slides showed increased values of the mitotic index (MI) at dilution 10^{-3} $MnCl_2$ solution for larch (exposure time 48 hours) and 10^{-2} $MnCl_2$ solution for spruce (exposure time 21 days).

INTRODUCTION

It is well known the role of manganese in the cellular oxidative processes and also in the running of some enzymatic systems (Davidescu D. and all., 1988, Khan A. A., 1980, Roat-Malone, Rosette M., 2002), in connection with the iron one (Crichton, R., 2001). Thus, the bivalent manganese is part of the prokaryotic superoxide dismutase (SOD), enzyme which neutralize in the mitochondria the superoxide anions that induces numerous negative effects in the cells due to the formation of the hydrogen peroxide (Roat-Malone, Rosette M., 2002).

In the green plants, the photo system II uses another manganese enzyme that induces the water splitting and the production of molecular oxygen.

In a previous paper (Rîşca, I.M. şi colab., 2008) we studied the effect of manganese ions on the germination of wheat seeds, observing a number of specific changes during germination of wheat biostructure under the influence of Mn^{2+} ions. The present paper aims to study the effects that manganese has on seed germination of some forest species, especially of the spruce (*Picea abies*) and larch (*Larix decidua*) and the impact on the dynamics of mitotic division of the root apical meristems.

MATERIAL AND METHODS

Equipments. Germination was fulfilled in a growth chamber CONVIRON G30 whose parameters were set the following values: temperature 20^0C, humidity 90%, without lighting.

Biological material. Samples of spruce (*Picea abies*) and larch (*Larix decidua*) used were from UP 75A, respectively UP 45 P, 2009 harvest, 5.5 g/1000 seeds, respectively 5.0 g/1000 seeds. The following parameters of the germinated plants were determined: germination (FG), according to current standards (SR1634, 1999), length of hypocotyls (L_H) and rootlets (L_R).

Reagents. Reagents. $MnCl_2$ p.a. (Chimopar) and bi-distilled water were used.

Treatments. Seeds were treated with $MnCl_2$ solutions of seven concentrations: 1 m, 0.5 m, 0.1 m, 5 x 10^{-2} m, 10^{-2} m, 5 x 10^{-3} m and 10^{-3} m, against a distilled water witness. 50 seed, in three repetitions, were used, including for the witness, in Petri dishes on filter paper.

Four treatment regimes used, namely: the seeds were immersed in solutions of varying treatment periods (24 hours, 48 hours, 7 days and, respectively, for the entire duration of germination) and then, for the first three schemes treatment, the seeds were put to germinate in distilled water.

At 21 days the above mentioned parameters were determined, namely: the number of germinated seeds (FG) and the length and rootlets(L_R) and hypocotyls (L_H) at the germinated plants.

In order to perform microscopic squash-type preparations the rootlet meristem tips of spruce and larch were stained with Carr reagent (amended carbolic fuchsine). The samples were examined under the optical microscope at 10x and 40x objectives, and the cells in mitotic division were recorded. They were reported the total number of cells examined and the mitotic index, for both witness and treated samples.

RESULTS AND DISCUSSIONS

Experiments were conducted in order to determine the biological answer of the seeds of spruce and larch, respectively, under the influence of Mn^{2+} ions. The results are summarized in Tables 1 and 2 and Figures 1-5.

Table 1: values of the germination and hypocotyls and rootlets lengths of *Picea Abies* under the influence of the treatments with $MnCl_2$ solutions

Measured parameter (average values)	Concentration of Mn^{2+} / Immersion period	Witness	1 m	0,5 m	0,1 m	$5*10^{-2}$ m	10^{-2} m	$5*10^{-3}$ m	10^{-3} m
L_R (mm)	24 h	26,796	10,735	11,049	16,86	16,036	11,29	9,708	10,644
	48 h		2,471	5,81	7,839	10,12	19,487	17,025	12,98
	7 d		0,83	2,902	4,476	5,45	9,985	10,386	3,284
	21 d		0	0	1,505	5,955	27,31	27,804	29,884
L_H (mm)	24 h	28,706	16,238	21,154	25,032	25,252	21,237	19,466	18,213
	48 h		1,383	7,69	10,161	14,04	16,735	18,69	17,79
	7 z		0,933	3,025	8,154	9,569	21,877	17,818	6,337
	21 z		0	0	0,461	1,895	28,51	27,276	34,715
FG (%)	24 h	71,33	47,33	44	58,66	60	62,66	68	61
	48 h		18	48	39,33	61,33	52,66	65,33	56,66
	7 z		6,66	30	48	62,66	46,66	58	45,33
	21 z		0	0	13,33	39,33	80,66	78,66	64,66

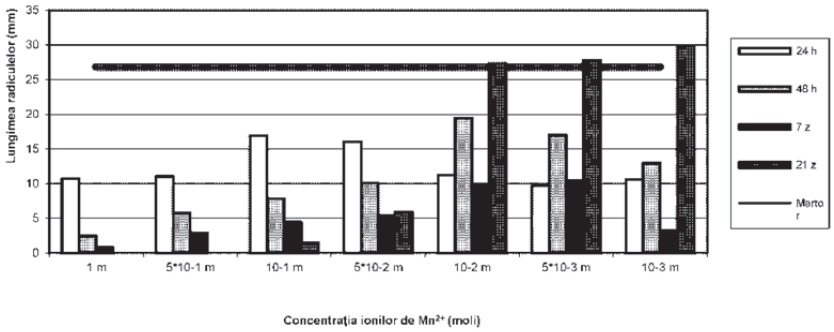

Fig. 1: influence of manganese ions against spruces of *Picea Abies*

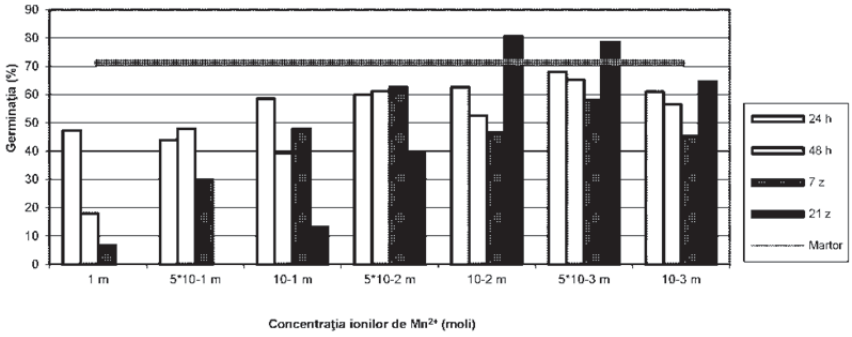

Fig. 2: influence of manganese ions against the seeds germination of *Picea Abies*

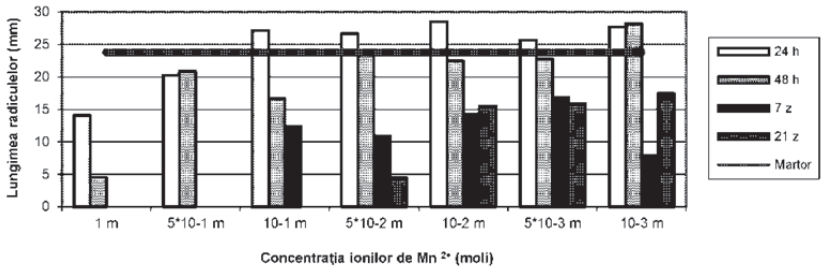

Fig. 3: influence of manganese ions against the rootlets of *Larix decidua*

Table 2: values of the germination and hypocotyls and rootlets lengths of *Larix decidua* under the influence of the treatments with $MnCl_2$ solutions

Measured parameter (average values)	Concentration of Mn^{2+} / Immersion period	witness	1 m	0,5 m	0,1 m	$5*10^{-2}$ m	10^{-2} m	$5*10^{-3}$ m	10^{-3} m
L_R (mm)	24 h	23,777	14,079	20,256	27,107	26,665	28,477	25,649	27,687
	48 h		4,566	20,839	16,662	24,171	22,442	22,76	28,155
	7 z		0	0	12,394	10,919	14,323	16,846	7,944
	21 z		0	0	0	4,53	15,518	15,89	17,433
L_H (mm)	24 h	24,972	5,095	20,003	27,319	22,567	24,463	24,72	29,937
	48 h		5,733	20,679	25,639	28,875	27,812	33,573	27,005
	7 z		0	0	16,344	14,567	24,872	21,063	22,348
	21 z		0	0	0	6,666	25,259	24,031	25,996
FG (%)	24 h	16,66	6	14	22,66	21,33	13,33	14,33	16
	48 h		9	16,66	19,33	15,33	24,67	15,33	18,66
	7 z		0	0	10	13,33	18,33	15,33	13,33
	21 z		0	0	0	6	13,33	12,67	16

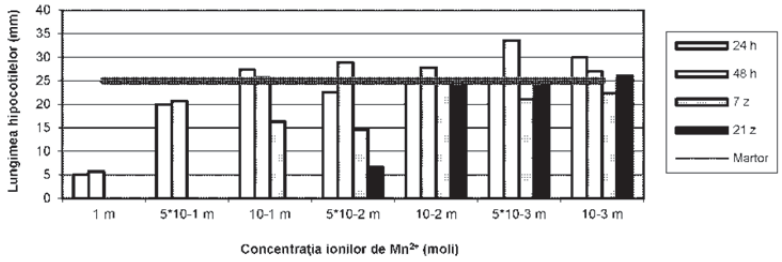

Fig. 4: influence of manganese ions against spruces of *Larix decidua*

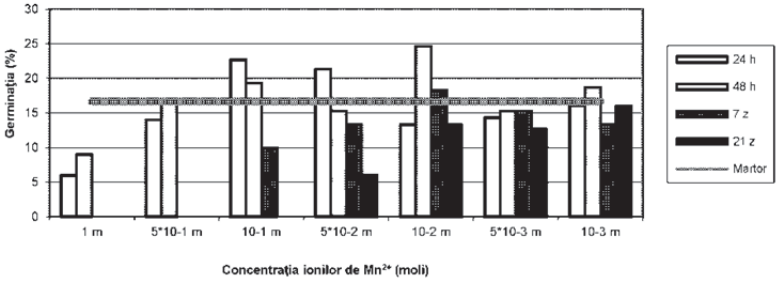

Fig. 5: influence of manganese ions against the seeds germination of *Larix decidua*

Table 3. Mitotic index in larch

Nr. of cells	Division cells					Mitotic index
Blank		P	M	A	T	
1350	170	130	17	10	13	0,13
Sample						
1280	210	166	21	16	7	0,18

Table 4. Mitotic index in spruce

Nr. of cells	Division cells					Mitotic index
Blank		P	M	A	T	
1350	155	127	12	6	10	0,11
Sample						
1280	189	156	16	11	6	0,14

A first finding is that at high concentrations manganese has - without exception - inhibitory effects on seed germination, the more pronounced effects as the seed to manganese exposure is longer. Individual tolerances vary, larch showing - for the short time of treatment - a

greater tolerance to high concentrations of manganese ions (10^{-1} m), unlike spruce shows stimulant symptoms at higher dilutions 10^{-2} m, and only long-stroke treatment. On the other hand, the effects of manganese occurs – depending on treatment periods and dilution - differentiated for spruce and larch. Thus, if the spruce is stimulated by high dilution and long immersion times, the larch has the reverse effect.

Spruce has an increased sensitivity to phytotoxic effects of manganese, but - paradoxically - maintained the entire period in dilute solutions of Mn^{2+} - it has significantly growth, especially in terms of length rootlets and, to a lesser extent, the hypocotyls (Fig. 1 and 2).

Larch is stimulated by dilution from 10^{-1} m, but only for short treatment times (Figures 3-5); the stimulation is manifested both in terms of germination and the size and hypocotyls and rootlets. For long exposure periods the larch resists at concentrations smaller than 10^{-2} m, with the exception of roots that have a pronounced inhibition (Fig. 4).

CONCLUSIONS

The above data lead us to the idea of a differential response of seed biostructure both at the biochemical action of manganese ions - the inhibition or stimulation of specific enzymatic reactions - as at the osmotic characteristics of seeds tegument, depending on the studied plant species.

Thus, the larch tegument is more permissive to the diffusion of manganese ions, inducing the rapidly activating of the enzyme systems (exposure times of 24 and 48 hours) but prolonging exposure to manganese causes an evident toxic accumulation. High concentrations of manganese have brutal effects, unbalancing irreversible the seed biostructure. At dilutions greater than $5x10^{-3}$ m changes are practically insignificant compared with witness.

For spruce seeds, their tegument presents an increased resistance against the diffusion of manganese ions, so that at short diffusion times significant results are not achieved. On the other hand, as already stated, spruce seeds are more sensitive so that, once "pierced" the osmotic defense line of the tegument, the seeds biostructure can maintain homeostasis parameters much closer limits, reflected by a more pronounced inhibition for all the measured parameters (Fig. 1-2) the most affected seemed to be root systems of spruce (Fig. 1).

The analysis of the cytological slides showed increased values of the mitotic index (MI) at dilution 10^{-3} for larch (exposure time 48 hours) and 10^{-2} for spruce (exposure time 21 days). In these conditions were registered 0,18 (0,13 blank) MI in larch mitotic division and 0,14 (0,13 blank) MI in spruce mitotic division (tab. 3 and 4).

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