CYTOGENETIC DAMAGE INDUCED BY MAGNESIUM IN WHEAT ROOT MERISTEMS

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Abstract: Like other metals, magnesium can be genotoxic for plants by generation of oxygen free radicals. This metal can induce mitotic alterations – chromosome breaks, achromatic lesions, chromosome aggregations, lagging chromosomes, micronuclei. Mitotic spindle modifications leading to poliploidy and aneuploidy were also evidenced. In this experiment, the effects of magnesium on wheat chromosome material were evaluated. Magnesium was administrated as magnesium sulphate ($MgSO_4.7H_2O$), for 3 hours, in four concentration variants - 1 mM, 25 mM, 50 mM şi 100 mM. The cell division was not significantly modified, comparatively to control, but the ana-telophase aberration frequency surpassed the control in all treated variants (especially in 1mM $MgSO_4.7H_2O$ (26.72%) and 100 mM $MgSO_4.7H_2O$ (22.44%). The most numerous abnormal metaphases were registered in 50 mM treated variant.

INTRODUCTION

Magnesium, like other metals such as cadmium, chromium, zinc, and manganese, may be genotoxic through generation of reactive oxygen species (ROS) (AMORIM et al., 2000). MgSO4 is found in nature as kieserite, MgSO4.H2O, which often accompanies the potassium salts. Some plant studies proved that magnesium sulphate induced mitotic abnormalities such as chromosome breakage, achromatic lesions, chromosome clumping, lagging chromosomes, micronuclei. Spindle abnormalities leading to the formation of polyploidy and aneuploidy were also observed in *Vicia faba* L. (ABRAHAM and RAJALAKSHMY, 1989). Also, in animal experiments, msignificantly more chromosomal abnormalities (terminal deletions, fragments, stickiness) than the situation encountered in respective controls (BELL et al., 1975). In this experiment, magnesium - considered as an essential anti-oxidant macromineral (AL-SHABANAH, 1998), was evaluated for its effects on genetic material of *Triticum aestivum*, in conditions of the seed treatment with 1, 25, 50, and 100 mM $MgSO_4.7H_2O$, for 3 hours.

MATERIAL AND METHOD

Wheat caryopses were maintained for three hours in solutions of 1 mM, 25 mM, 50 mM, and 100 mM $MgSO_4.7H_2O$. The amounts of magnesium in these solutions are: 0.0243 mg Mg/ml, for 1mM, 0.6076 mg Mg/ml, in 25 mM solution, 1.2152 mg Mg/ml, for 50 mM, respectively 2.4305 mg Mg/ml, for the most concentrated tested solution of magnesium sulphate. After treatment, the seeds were placed in Petri dishes, in dark, for germination. The rootlets were fixed in a mixture of absolute ethyl alcohol and glacial acetic acid, in a 3:1 ratio, and then stored in 70% ethyl alcohol, at refrigerator. A modified solution of carbol fuchsin was used for chromosome staining. Five preparations obtained by squash method/variant have been analyzed and 10 microscopic fields / slide were scored, in view of calculus of mitotic index and chromosomal aberrations.

RESULTS AND DISCUSSIONS

Mg^{2+} is the most abundant free divalent cation in the plant cytosol. The functions of Mg^{2+} in plants (as well as in other organisms) are mainly related to its capacity to interact with nucleophilic ligands. Mg^{2+} is essential for the function of many cellular enzymes (RNA polymerases, ATP-ases, protein kinases, phosphatases, glutathione synthase, and carboxylases) and for the aggregation of ribosomes, this metal playing an important role in reactions involved in replication, transcription, translation (Shaul, 2002). The magnesium ions maintain the tertiary structure of transfer RNA and assure the stability of double helicated DNA against thermal denaturation (Watanabe and Iso, 1984). The significance of Mg^{2+} homeostasis has been particularly established with regard to Mg^{2+} role in photosynthesis. It is known the fact that the

magnesium is the central atom of the chlorophyll molecule. At physiologically relevant concentrations, magnesium itself is not genotoxic, but is highly required to maintain genomic stability (Hartwig, 2001).

Concerning influence on wheat cell division (Table 1; Fig. 1), the magnesium treatments not determined significant differences of mitosis intensity comparatively to control. The mean values of mitotic index, expressed in %, ranged between 6.38±0.44 (1mM) and 7.93±0.41 (100 mM), the mean of control being 6.81±0.41. Very slight decreases of mitotic index appeared in 1mM, 25mM, and 50 mM $MgSO_4.7H_2O$ treated variants, while the maximum tested concentration (100 mM $MgSO_4.7H_2O$) exerted a stimulant effect (the number of dividing cells surpassed with 15% the control). It is visible a direct relation between the concentration increase of magnesium sulphate and behaviour of mitotic index. Some authors evidenced mitodepressive effect of magnesium sulphate.

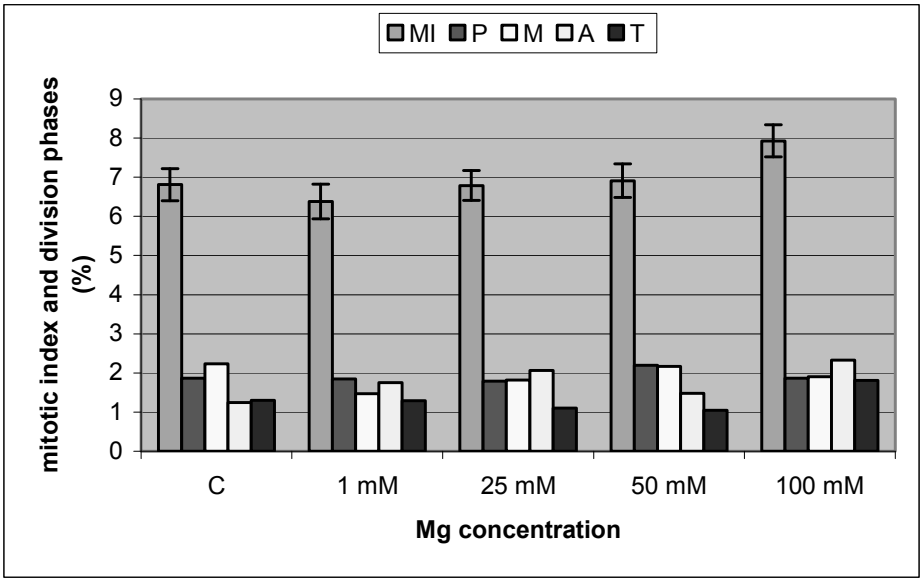

Fig. 1. The behaviour of mitotic index (MI) and preponderance (%) of mitotic phases (P=prophase; M=metaphase; A=anaphase; T=telophase), in wheat root meristems after sulphate magnesium treatment

In 100 mM $MgSO_4.7H_2O$ variant, the increase of mitotic index was realized by ana-telophase (A-T) number increase. In 1 mM $MgSO_4.7H_2O$ treated variant, with the lowest value of this cytogenetic parameter, a depression of metaphase percentage was registered.

Table 1. Variation of mitotic index and preponderance of each mitotic phase after sulphate magnesium treatment of wheat seeds

Variant	Total analyzed cells	Dividing cells		Mitotic index %	Prophases		Metaphases		Anaphases		Telophases	
		Nr.	x±SE		Total	x±SE	%	Total	x±SE	%	Total	x±SE	%
Control	3295	225	45±4.82	6.81±0.41	67	13.4±1.96	1.87	74	14.8±2.87	2.23	41	8.2±1.24	1.24	43	8.6±1.28	1.30
1 mM	3943	247	49.4±4.09	6.38±0.44	72	14.4±2.95	1.85	57	11.4±1.50	1.47	68	13.6±1.36	1.75	50	10±1.70	1.29
25 mM	2891	197	39.4±3.2	6.79±0.38	52	10.4±0.97	1.79	53	10.6±0.74	1.82	60	12±0.94	2.06	32	6.4±1.5	1.10
50 mM	3083	210	42±3.36	6.91±0.43	67	13.4±1.16	2.20	66	13.2±1.28	2.17	45	9±1.81	1.48	32	6.4±1.28	1.05
100mM	3024	245	51±4.15	7.93±0.41	58	11.6±2.20	1.87	59	11.8±1.90	1.90	72	14.4±0.52	2.33	56	11.2±1.15	1.81

Table 2. Frequency and types of ana-telophase (A-T) chromosome aberrations and of metaphase abnormalities in wheat root meristems, after sulphate magnesium treatment

Variant	A-T	Aberrant A-T		Simple ana-telophase aberrations (%)			Complexe aberrations (%)	Total metaphases	Abnormal metaphases		Fragmented metaphases (%)	C-metaphases (%)	M with expulsed chromosomes (%)
		% from total A-T	% from dividing cells	bridges	expulsed chromosomes	lagging chromosomes	multipolar ana-telophases			% from total metaphases	% from dividing cells		
Control	84	27.38	10.22	5.77	0.00	2.22	0.44	1.77	74	12.16	4.00	1.33	0.44	2.22
1 mM	118	55.93	26.72	14.57	0.40	4.85	0.80	5.66	57	15.78	3.64	1.21	0.00	2.42
25 mM	92	40.21	18.78	10.15	0.00	5.07	0.00	3.55	53	7.54	2.03	0.00	0.50	1.52
50 mM	77	44.15	16.19	10.47	0.00	1.90	0.47	3.33	66	18.18	5.71	0.00	0.00	5.71
100mM	128	42.96	22.44	11.01	0.40	4.48	0.40	5.71	59	15.25	3.67	0.40	0.00	3.26

The frequency of ana-telophases with chromosome aberrations, expressed in % from total dividing cells, was higher in all experimental variants, comparatively to control (Table 2, Fig. 2). The most numerous aberrations were encountered in 1mM $MgSO_4.7H_2O$ (26.72%) and in 100 mM $MgSO_4.7H_2O$ (22.44%). In literature, some of published data confirm that the lower Mg concentrations led to increased noxious effects (STILWELL and CORUM, 1982). Both in control and in the four magnesium treated variants, the simple chromosome aberrations with the highest incidence were the bridges (Fig. 5), followed by lagging chromosomes (Fig. 3, 6) and multipolar ana-telophases. A high number of complex chromosome aberrations (bridges + expulsed chromosomes, multipolar ana-telophases + bridges, multipolar ana-telophases + bridges + expulsed chromosomes etc.) (Figures 3, 4) were registered in ana-telophases of variants treated with 1mM and 100 mM $MgSO_4.7H_2O$.

The chromosome bridges - connections containing chromosome material remained between the two nuclei – often cause tetraploidizations. The undivided chromosome parts appear to impede the final separation of the two daughter cells. However, not every chromosome bridge leads to a tetraploid cell. Observations over long periods of time showed that many of the partially divided cells with chromosome bridges later completed cell division, although the process was slower compared to cells without chromosome bridges. Concerning the lagging chromosomes at anaphase, they represent a potential source of aneuploidy. After cytokinesis occurs, a lagging chromosome may give rise to a monosomic daughter cell and a trisomic one.

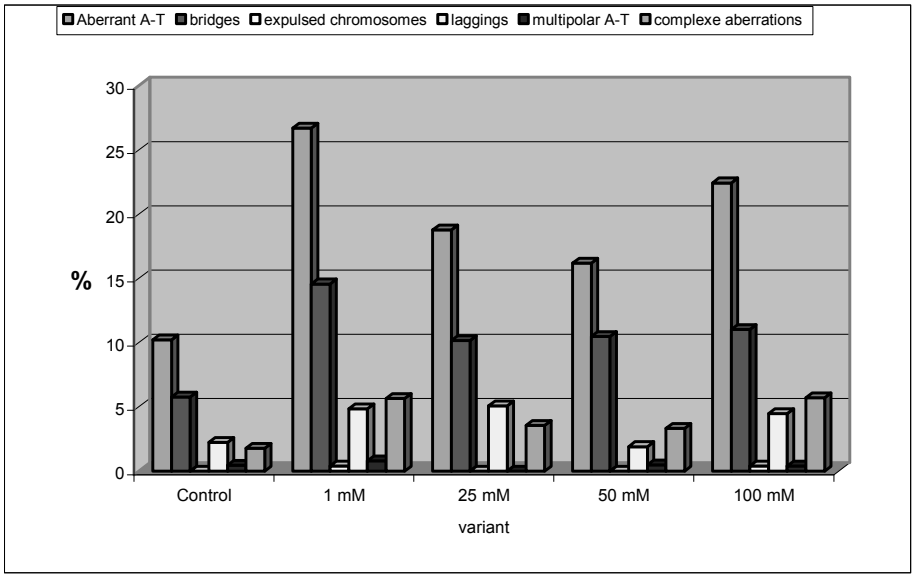

Fig. 2. Frequency and types of ana-telophase (A-T) chromosome aberrations (in %) in wheat root meristems, after sulphate magnesium treatment

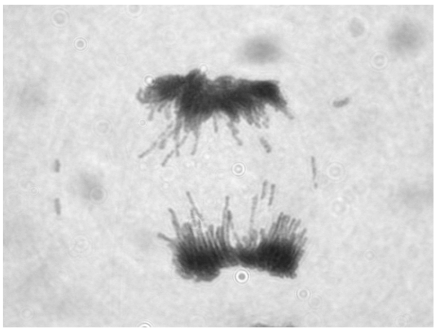

Fig. 3. Anaphase with lagging chromosomes and expulsed chromosomes – 50 mM

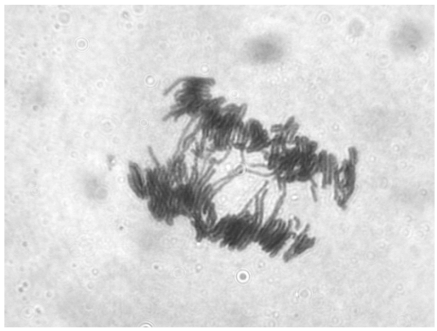

Fig. 4. Multipolar anaphase with bridges and expulsed chromosomes – 100 mM

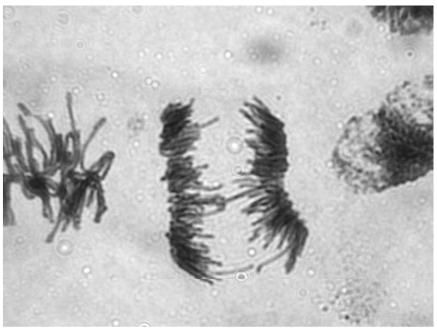

Fig 5. Anaphase with bridges -100 mM

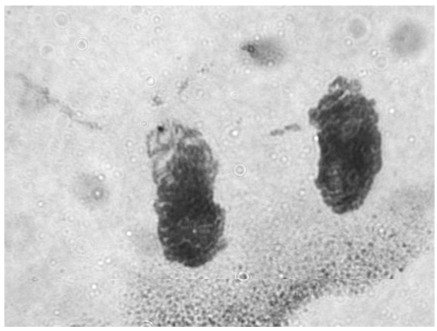

Fig. 6. Telophase with laggard – 100 mM

The metaphases were also influenced by magnesium treatments. The most important number of abnormal metaphases (5.71%) was registered in 50 mM variant. In this case, they consisted only in expulsed chromosomes – potential source for aneuploidy (Fig. 7).

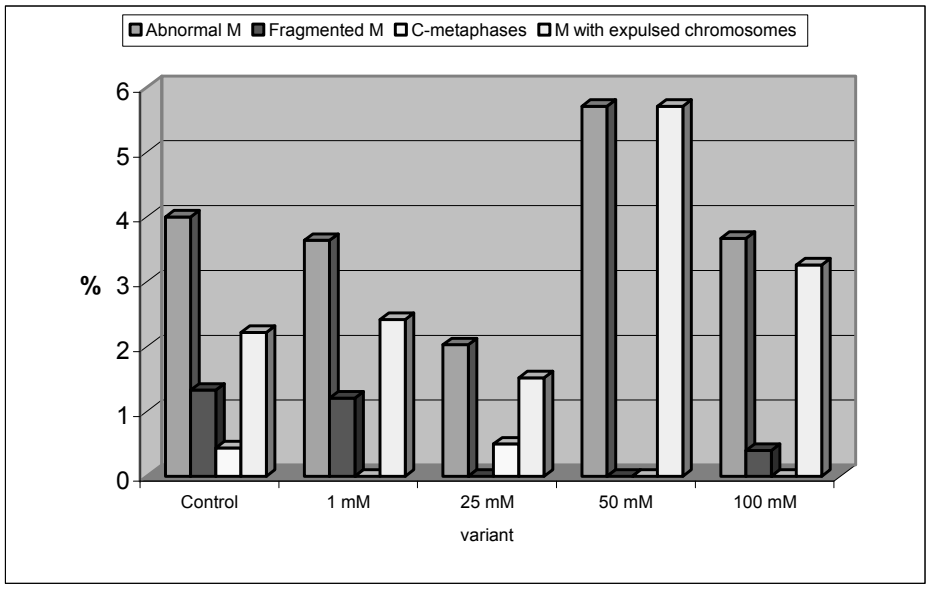

Fig. 7. The incidence (%) of abnormal metaphases (M) and of types of metaphase abnormalities, after sulphate magnesium treatment

The metaphase abnormalities were represented by fragmented metaphases, colchicine-like metaphases (C-metaphases) and metaphases with expulsed chromosomes (Fig. 7, 8).

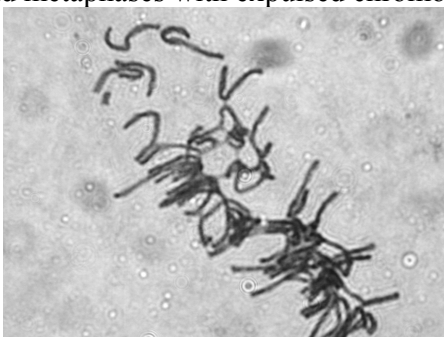

Fig. 8. Metaphase with expulsed chromosomes – 100 mM

Therefore, the results obtained in this experiment confirm the potential of magnesium to damage the genetic material when this metal is administrated at critical concentrations.

CONCLUSIONS

After the treatment of wheat caryopses with sulphate magnesium solutions of different concentrations, they were not evidenced significant differences between treated variants and control concerning the intensity of cell division; only in 100 mM sulphate magnesium treated variant, a stimulation of mitoses with 15% was observed. Very slight decreases of mitotic index appeared in 1mM, 25mM, and 50 mM $MgSO_4.7H_2O$ treated variants

The frequency of ana-telophase chromosome aberrations surpassed that of control in 1mM $MgSO_4.7H_2O$ (26.72%) and 100 mM $MgSO_4.7H_2O$ (22.44%) variants. The chromosome bridges were the most numerous.

The highest number of abnormal metaphases (5.71%) was encountered in 50 mM variant.

REFERENCES

Abraham, S., Rajalakshmy, B. N., 1989. *Cytologia*, 54, 559 - 563.
Amorim, M. I. M., Mergler, D., Bahia, M. O., Dubeau, H., Miranda, D., Lebel, J., Burbano, R. R. & Lucotte, M., 2000. *An. Acad. Bras. Ci.,* 72(4), 497 – 507.
Al-Shabanah O. A., 1998. C*hemotherapy,* 44(4), 272 – 283.
Bell, T. L., Branstrator, M., Roux, C. & Hurley, L. S., 1975. *Teratology*, 12(3), 221 – 226.
Hartwig, A., 2001. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, 475(1-2), 113 - 121
Shaul, O., 2002. *BioMetals,* 15, 309–323.
Stilwell, E. F., Corum, K. E., 1982. *Marine Biology*, 66, 227 – 230.
Watanabe, K., Iso, K., 1984. *Biochemistry,* 23(7), 1376 – 1383.

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