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 (MgSO₄.7H₂O), for 3 hours, in four concentration variants - 1 mM, 25 mM, 50 mM and 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 MgSO₄.7H₂O (26.72%) and 100 mM MgSO₄.7H₂O (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, significantly 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 MgSO₄.7H₂O, 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 MgSO₄.7H₂O. 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

Mg²⁺ is the most abundant free divalent cation in the plant cytosol. The functions of Mg²⁺ in plants (as well as in other organisms) are mainly related to its capacity to interact with nucleophilic ligands. Mg²⁺ 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 Mg²⁺ homeostasis has been particularly established with regard to Mg²⁺ 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 MgSO₄·7H₂O treated variants, while the maximum tested concentration (100 mM MgSO₄·7H₂O) 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](image)

In 100 mM MgSO₄·7H₂O variant, the increase of mitotic index was realized by anaphase (A-T) number increase. In 1 mM MgSO₄·7H₂O treated variant, with the lowest value of this cytogenetic parameter, a depression of metaphase percentage was registered.
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## 1. Vizualizarea de mișcări și repartizarea de celulară în plamă de bulb la A. magnifica

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**Table 1:** Vizualizarea de mișcări și repartizarea de cellulară în plamă de bulb la A. magnifica.
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\(_2\)O (26.72%) and in 100 mM MgSO\(_4\).7H\(_2\)O (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 + expelled chromosomes, multipolar ana-telophases + bridges, multipolar ana-telophases + bridges + expelled chromosomes etc.) (Figures 3, 4) were registered in ana-telophases of variants treated with 1mM and 100 mM MgSO\(_4\).7H\(_2\)O.

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.

![Graph showing the frequency and types of ana-telophase (A-T) chromosome aberrations (in %) in wheat root meristems, after sulphate magnesium treatment](image-url)
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 expelled chromosomes – potential source for aneuploidy (Fig. 7).

![Fig. 3. Anaphase with lagging chromosomes and expelled chromosomes – 50 mM](image1)

![Fig. 4. Multipolar anaphase with bridges and expelled chromosomes – 100 mM](image2)

![Fig 5. Anaphase with bridges -100 mM](image3)

![Fig. 6. Telophase with laggard – 100 mM](image4)

**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 expelled chromosomes (Fig. 7, 8).

Fig. 8. Metaphase with expelled 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 MgSO4.7H2O treated variants. The frequency of ana-telophase chromosome aberrations surpassed that of control in 1mM MgSO4.7H2O (26.72%) and 100 mM MgSO4.7H2O (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

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