THE CITOGENETIC EFFECTS OF TREATING MILLET *(PANICUM MILIACEUM L.)* WITH SALTS OF HEAVY METALS

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Key words: ferrous sulphate, copper sulphate, mitotic division, *Panicum miliaceum* L..
Abstract : This article presents the citogenetic effects that the copper sulphate and the ferrous sulphate have on the growth and development of the plant after being treated with such chemical substances. The application of this treatment determined significant alterations of the mitotic index and on the frequency of cells in various stages of division

INTRODUCTION

By the term “heavy metals” , we understand a series of elements with high density (over 5 kg/dm^3) with quite complex chemical properties, especially a high level of oxidation. A noticeable toxic action was was observed in the case of copper(d = 8,9), mangane (d = 7,43), lead(d = 11,34), mercury (d = 13,6) etc (Hătărăscu, 1982).

The biogeodynamic characteristics of each heavy metal has a very important ecologic significance within the framework of the relationships among the individual components of the ecosystems. The transfer of metals in the interior of trophic chains air – soil – plant – animal is not to be neglected. The heavy metals produce a series of profound changes in the metabolism of plants, most of the plants being sensitive to the effects of heavy metals: stomas’ opening is compromised, the photosynthesis diminishes considerably, the breathing process is disturbed, growth is slowed down, etc.

MATERIALS AND METHODS

As a biological material, we used seeds of millet (*Panicum miliaceum* L.) from the harvest 2003-2005. The seeds were placed to germinate in Petri dishes on filter paper soaked with distilled water. The germination took place 4 days later, in a proportion of 90%. From the Petri dishes with control-seeds, we harvested the roots that were 10-15mm long. From the rest of the Petri dishes, we harvested the seeds and replanted them in new Petri dishes in which the paper was soaked with copper acetate and ferrous sulphate, with concentrations of de 0,01%, 0,02% şi 0,05%, for 12, 24, 48 hours.

In the end, we got 27 samples for this species, plus the control-variant, on which no chemical substances had been applied. Once the roots obtained, we used the Squash method (Câmpeanu, 2002) to emphasise the chromosome aberrations. Also, we took digital camera pictures of various stages of division for all three concentrations and times.

RESULTS AND DISCUSSIONS

The mitotic index

In the fig. 1, we can notice a slight increase of the mitotic index compared to the control, for the concentration of 0,01%. This means that, for this acting time and concentration, the ferrous sulphate does not determine the decrease of the mitotic index. If the concentration of the chemical substance applied is raised, the mitotic index decreases for a concentration 0,02%. If in the cases of the minimal concentration, the ferrous sulphate did not lead to a decrease of the mitotic index, in the cases of medium and maximum concentrations, it decreased proportionally with their elevation.

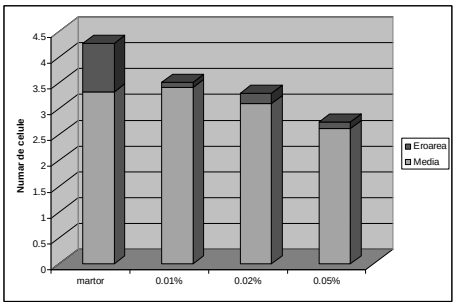

Fig. 1 The mitotic index for the millet, after being treated with ferrous sulphate for 12 hours

0,01%, 0,02%, 0,05% - concentration of the substance used;

In fig. 2, we can notice surprisigly significant increases of the values of the mitotic index for all three concentrations compared to those

of the controls. We can also notice a progressive change of this parameter as the concentration of the chemical agent gets higher. For a concentration of 0,05%, the value of the mitotic index is much higher compared to the controls and to the previous acting times.

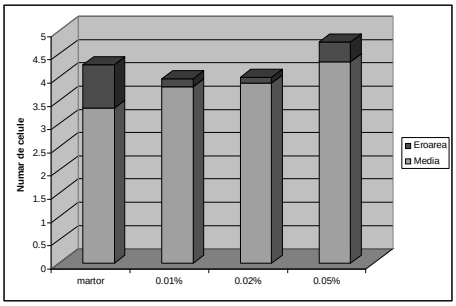

Fig. 2 The mitotic index for the millet, after being treated with ferrous sulphate for 24 hours

0,01%, 0,02%, 0,05% - concentration of the substance used;

The application of ferrous sulphate for 48 hours (fig. 3) on millet seeds determine an increase of the mitotic index for the first 2 concentrations (0,01 şi 0,02%), after which, for a new increase of the concentration, it drops below the value of the control. We can therefore conclude that prolonged exposure does not influence the decrease of this parameter.

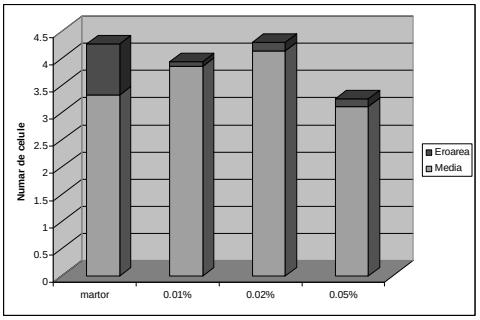

Fig. 3 The mitotic index for the millet, after being treated with ferrous sulphate for 48 hours

0,01%, 0,02%, 0,05% -concentration of the substance used;

The copper sulphate also determined some perturbations as far as the mitotic division is concerned, that are:

In the fig. 4, we can notice the decrease of the mitotic index compared to the control. For the first concentrations, the mitotic index grows, and for a higher concentraton, this parameter increases further. This shows us the influence of the copper sulphate on the mitotic index.

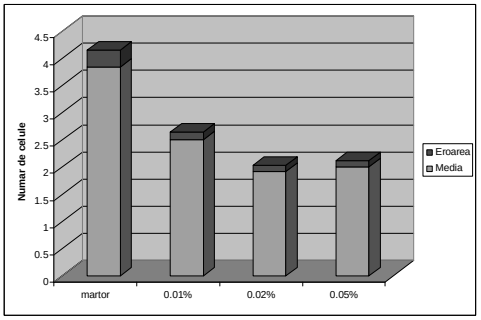

Fig. 4. The mitotic index for the millet, after being treated with copper sulphate for 12 hours

0,01%, 0,02%, 0,05% - concentration of the substance used;

In fig. 5, we can notice that, after 24 hours of treating the seeds with copper sulphate, there is a decrease of the mitotic index compared to the control. For the concentration of 0,01%, there is a decrease. For a higher concentration, the mitotic index increases again and, after increasing the concentration again, the mitotic index decreases further compared to the two concentrations used previously. So, a high concentration of copper sulphate determines a decrease of the drecrese of the mitotic index.

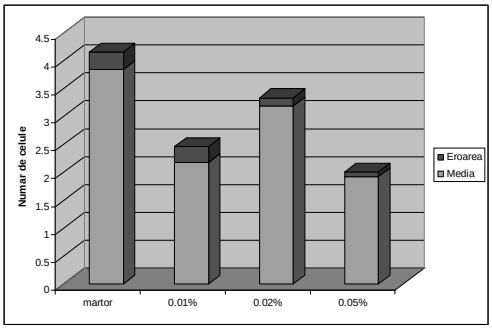

Fig. 5. The mitotic index for the millet, after being treated with copper sulphate for 24 hours

0,01%, 0,02%, 0,05% - concentration of the substances used;

The analysis of the radicular apex of the millet shows a series modifications as a result of using copper sulphate. In comparison to the control, for a 0,01% concentration, there is a decrease of the mitotic index. Once the concentration is raised, the value of this parameter drops, so we may speak of an influence of the chemical agent upon the cellular division. At a yet another increase of the copper sulphate, (0,05%), there is an inexplicable increase of the mitotic index compared to that of the control instead of a decrease, as we may have expected to a value much higher than that of the control (fig. 6). At any rate, this is the highest value of the mitotic index for all three acting times(12, 24, 48 hours)

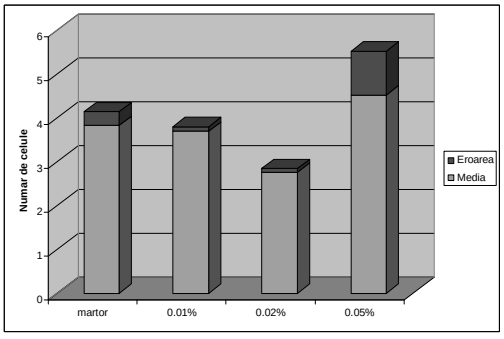

Fig. 6. The mitotic index for the millet, after being treated with copper sulphate for 48 hours

0,01%, 0,02%, 0,05% - concentration of the substance used;

The frequency of cells with chromosomial aberrations

If we consider the values obtained after 12 hours of treating the samples with ferrous sulphate, we notice that , compared to the control, the frequency of the aberrant ana-telophases increases progressively very much, proportionally with the increase of the concentrations of the mutagen agent. If for a cconcentration of 0,01% we recorded a value approximately 5 times higher than the control’s, for the maximum concentration of 0,05%, their frequency reached an even higher value (fig. 7). Thus, the increase of the concentration of the chemical agent is directlz

proportional with the increase of the aberrant ana-telophases. The number of chromosomial aberrations compared to the controls also increases, such as:double bridges, retarded or expulsed chromosomes. This proves the perturbing effect of the ferrous sulphate on the millet seeds.

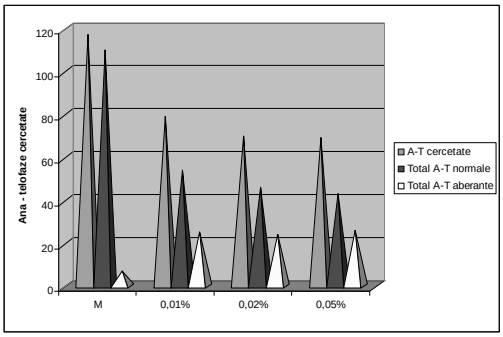

Fig. 7. The frequecy of the ana-telophases with aberrations in millet, after being treated with ferrous sulphate for 12 hours.

M–control; 0,01%, 0,02%, 0,05% - concentration of the substance used;

By applying ferrous sulphate on millet seeds for 12 hours, we can see that there appears an increase of the frequencies of the aberrant ana-telophases. For the first two concentrations, the values are relatively constant compared to the previous acting time (12 hours), but once the concentration gets to its highest, the frequency is approximately 6.42 higher than the control.((fig. 8).

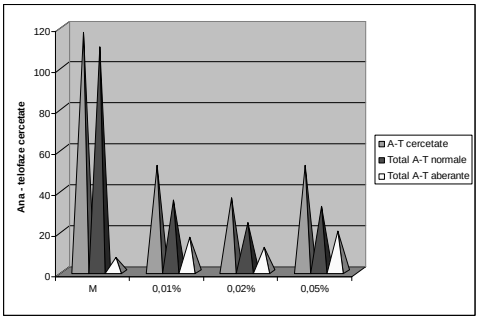

Fig. 8 The frequecy of the ana-telophases with aberrations in millet, after being treated with ferrous sulphate for 24 hours.

M–control; 0,01%, 0,02%, 0,05% - concentration of the substance used;

The frequency of the aberrations in the ana-telophases of the mitosis, present in the cells in the radicular apex of the millet increases for the minimal concentration of the chemical substance (0,01%), compared to the control, (fig. 9). When the concentration of the ferrous sulphate is higher (0,02%), there is a decrease of the frequency, so there is a slightly perturbing effect for this concentration, and, for the concentration of 0,05%, the frequency of the aberrant ana-telophases drops significantly, most aberrations consisting in double bridges, expulsed chromosomes. No fragments, retarded or inelar chromosomes were observed for this acting time. (48 hours).

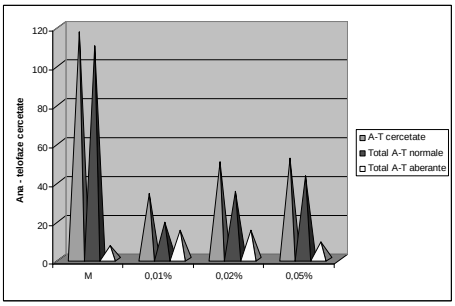

Fig. 9. The frequecy of the ana-telophases with aberrations in millet, after being treated with ferrous sulphate for 48 hours.

M–control; 0,01%, 0,02%, 0,05% - concentration of the substance used;

The copper sulphate determines major perturbances as far as the number and types of chromosomial aberrations are concerned:

If we look at fig. 10, we can see an increase of the aberrant ana-telophases duet o the increase of the concentrations of the chemical substance.. For a lowest concentration, the percentage increases compared to the control 3.31 times. There is also an increase of cells with chromosomial aberrations. There is also a growth of the number of chromosomial aberrations such as double bridges, retarded and expulsded chromosomes. At medium concentration of the copper sulphate, the frequency of the aberrant ana-telophases is 5 times higher than in the control. As the concentration of the chemical agent gets to 0,05%, the ratio of the aberrant ana.telophases is 8,59 times higher than the control's. This proves the effect of the copper sulphate on the millet seeds, effect that determined the increase of the frequency of the ana-telophases as well as the number of the aberrations for these concentrations. Indeed, the copper sulphate determines the appearance of a quite high numbr of aberrations during division.

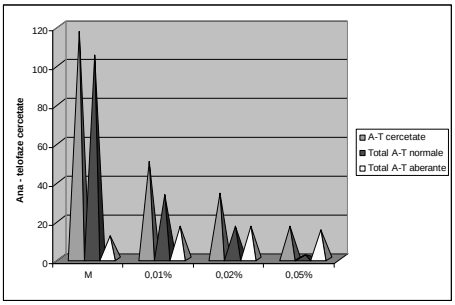

Fig. 10. The frequecy of the ana-telophases with aberrations in millet, after being treated with copper sulphate for 12 hours.

M–control; 0,01%, 0,02%, 0,05% - concentration of the substance used;

For this particular acting time (12h), we can notice an increase of the normal ana-telophases compared to the previous time and a slight decrease of the aberrant ones.If after 12 hours of treatment at highest concentration, the frequency of the aberrant ana-telophases 8,59 times higher than the control's, in this case, for highest concentration, there was only a 4,03-time increase. So, the treating time does not influence the increase of the aberrant ana-telophases. (fig.11).

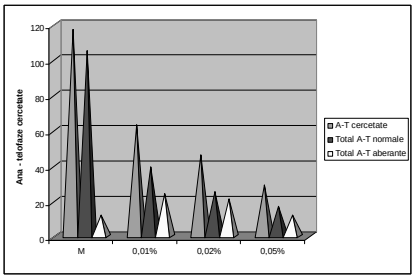

Fig. 11. The frequecy of the ana-telophases with aberrations in millet, after being treated with copper sulphate for 24 hours.

M–control; 0,01%, 0,02%, 0,05% - concentration of the substance used;

For a concentration of 0,01%, the frequency of the ana-telophases remains relatively constant (fig.12) as well as in the cases of the other times (12, 24 hours), after which it drops for medium concentration (0,02%) and, at concentrations of 0,05%, there appeared no cell with aberrations

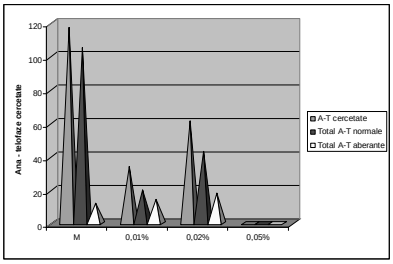

Fig.12. The frequecy of the ana-telophases with aberrations in millet, after being treated with copper sulphate for 48 hours.

M–control; 0,01%, 0,02%, 0,05% - concentration of the substance used;

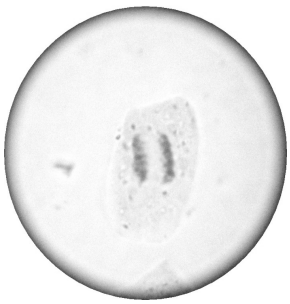

Foto 1. Telophase (0.01% ferrous

CONCLUSIONS

In the case of treating millet seeds with ferrous sulphate, there could be observed an slightly inhibiting effect of this salt. This substance determined minor modifications as far as the mitotic index, aberrant ana-telophases are concerned, but no high sensitivity could be observed.

The maximum percentage of the chromosomial aberrations are concerned was registered for the maximum concentration of chemical substance (0,05%).

The copper sulphate determined major alterations: it determined the decrease of the mitotic index, directly proportionally with the increase of the concentrations of the chemical substance,led to the decrease of cells in division, so of the cell division in general,. So, if for the first 2 concentrations, (0,01 and 0,02%), there ware no major modifications, in the case of maximum concentration, this substance proved its highly inhibiting effect upon the millet seeds genetic material.

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