CITOGENETICS EFFECTS INDUCED BY THE ADMINISTRATION OF SUBSTANCES WITH REDUCTION POTENTIAL AT *LARIX DECIDUA* MILL. SSP. *CARPATICA*, *PICEA ABIES* (L.) KARST. AND *THUJA ORIENTALIS* L.

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Abstract: The paper present the influence of ascorbic acid, riboflavin, citric acid and sodium bisulphit upon the mitotic division of *Larix decidua* Mill ssp. *carpatica, Picea abies* (L.) Karst and *Thuja orientalis* L. The treatment was applied in one variant, germinated seeds in ascorbic acid, riboflavin, citric acid and sodium bisulphit in 3 concentrations.

INTRODUCTION

Food additives are substances added intentionally to foodstuffs to perform certain tehnological functions, for exemple colour, to sweeten or to preserve. They have been used by mankind for centuries. Most food additives are considered safe. But, some are known to be carcinogenic or toxic. Hyperactivity in children, allergies, asthma, and migraines are often asociated with adverse reactions to food additives. For all this affirmations we considered apropos our studies in this direction.

In this approach we propose to make citogenetics studies at *Larix decidua* Mill. ssp. *carpatica*, *Picea abies* (L) Karst and *Thuja orientalis* L.

In this experiment we observed the stimulator or inhibitor effect of ascorbic acid, riboflavin, citric acid and sodium bisulfit to the mitotic division, and estimated the instalment of the abberrations appearance (Bedeleanu, 1985; Lehninger, 1987; Neamţu, 1981).

In this direction, in literature we found citogenetical researches on *Secale cereale* which was treated with tartrazine (E102), carmoisine (E122), patent blue (E131) and acid green 50 (E142), (Zaharia, Pavel, 2003).

MATERIALS AND METHODS

The biological material used in this experiment was represented by seeds of *Larix decidua* Mill ssp. *carpatica, Picea abies* (L.) Karst. from the forest Departament of Piatra Neamţ (2005) and *Thuja orientalis* L. from the Bottanical Garden of Iaşi.

The seeds were put to germination in lab conditions. When the roots reached 5 - 10 mm in length, they were treated each of four substances with reduction potential, and then were immersed in distillate water (three time for 10 minutes) for washing.

Substance: ascorbic acid (vitamin C, E 300), riboflavin (vitamin B_2, lactoflavin, E 101), citric acid (E 330), sodium bisulphit (E 222): 0.1 %, 0.25 %, 0.5%;

Action variants:

A - germinated seeds in ascorbic acid, riboflavin, citric acid and sodium bisulfit: 0.1 %, 0.25 %, 0.5 %;

Except this variants, there also used a control plot and in this case no treatment were applied to the radicular meristems.

For the cytogenetic investigations, the roots were fixed in 3:1 fixing solution for 24 hours , then hydrolised with HCl (50 %) for 6 minutes and coloured with coloring Carr.

The radicular meristems was displeyd using squash technique (Cîmpeanu et al., 2002).

The microscopical examination was carries out using the optic microscop Novex K-Range.

The microphotographics were made with digital camera Canon.

RESULTS AND DISCUTIONS

1. Proportion of cells in mitotic division at *Larix decidua* Mill. ssp. *carpatica* (Dom.), *Picea abies* (L.) Karst, and *Thuja orientalis* L.

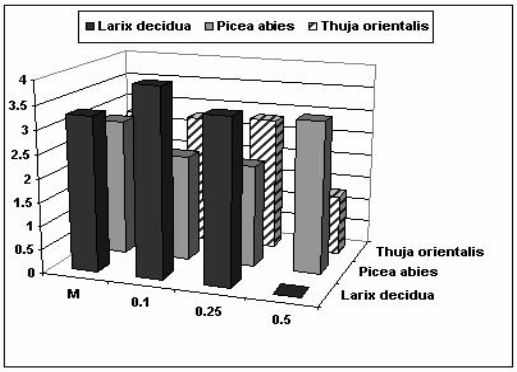

Fig.1. Dynamics of mitotic index after the treatment with ascorbic acid

We observed that the mitotic index increase at 0.1 % and 0.25 % at *Larix decidua* and *Thuja orientalis* and decrease at 0.5 % concentration. The ascorbic acid at high concentration affect cells division at 0.5 % (*Larix decidua*) and to *Picea abies* stimulate it (Fig. 1).

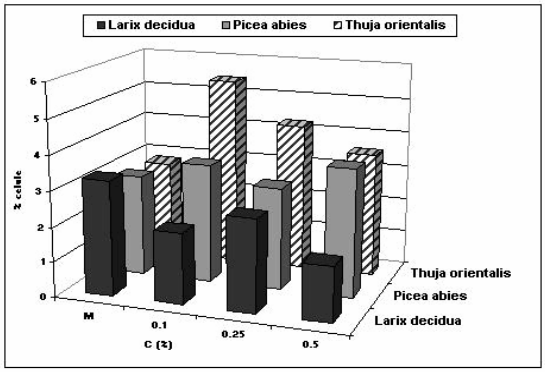

Fig. 2. Dynamic of mitotic index after the treatment with citric acid

The citric acid stimulate the mitotic division at *Picea abies* and *Thuja orientalis* and inhibit to *Larix decidua* comparative the etalon (Fig.2).

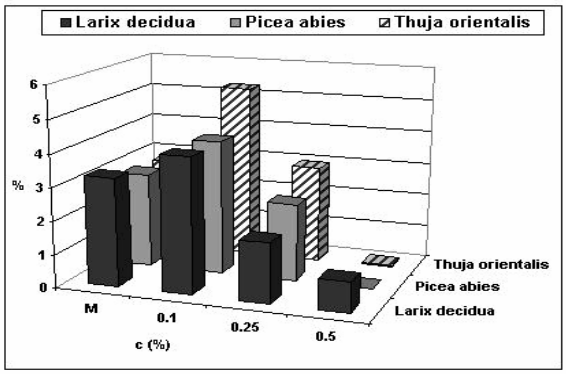

Fig. 3. Dynamic of mitotic index after the treatment with sodium bisulphit

Concentrations of 0.1 % and 0.25 % sodium bisulphit stimulate the cells division at all the three species, and the 0.5 % concentration has an inhibitor effect (Fig. 3).

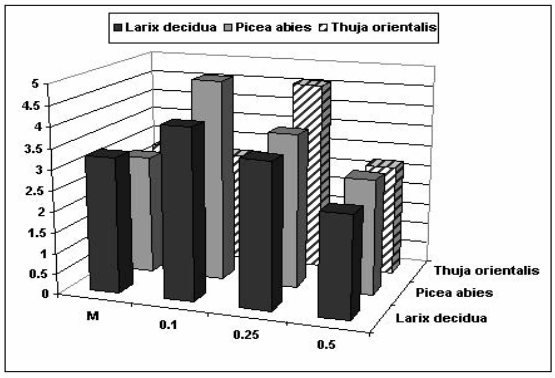

Fig.4. Dynamic of mitotic index after the treatment with riboflavin

We observed that the mitotic index increase (comparativ with the control) at concentration 0.1% and 0.25%, than decrease at 0.5 % concentration. This values are observed at all of three species and four substances with a few variations. In the same time we could noticed that the numerous phases are prophases and telophases.

At the treatment with riboflavin, the frequency of cells in mitotic division are biggest at 0.1 % and 0.25 %, than decrease at 0.5 % concentration, at all the three species (Fig. 4).

2. The dynamics of aberrations at at *Larix decidua* Mill. ssp. *carpatica* (Dom.), *Picea abies* (L.) Karst. and *Thuja orientalis* L.

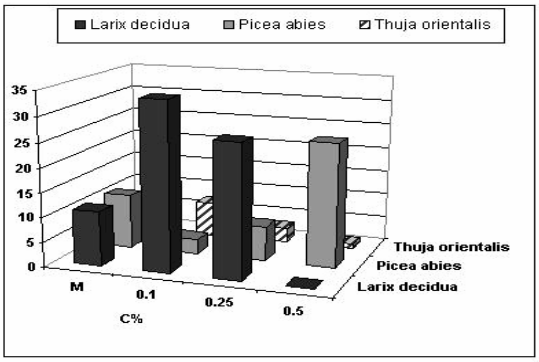

Fig.5. Aberrations frequency induced by the treatment with ascorbic acid

At *Larix decidua* we observed an increase of the aberrations frequency at 0.1 % and 0.25 % concentration and at *Picea abies* at 0.5 %. *Thuja orientalis* seems to be more resistant comparative the other two species, after the treatment (Fig. 5).

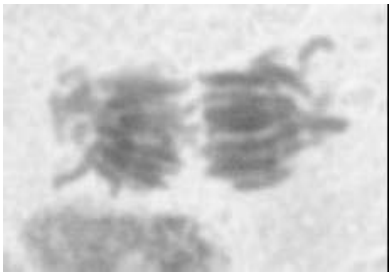

Fig.6. *Thuja orientalis*, 0.25 %
A-T with expelled chromatic material

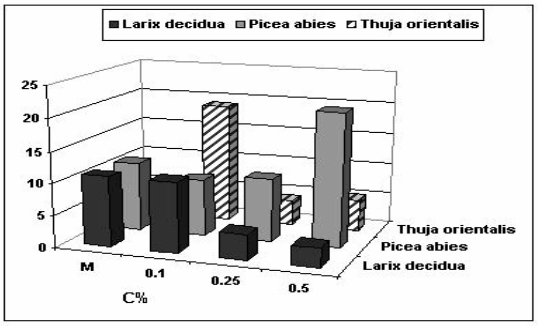

Fig.7. Aberrations frequency induced by the treatment with citric acid

The citric acid induce an increase of aberrations frequency at 0.5 % (*Picea abies*) and 0.1 % (*Thuja orientalis*) (Fig.7).

Fig.8.*Larix decidua*, 0.1 % A-T with bridge and retardatary chromosomes

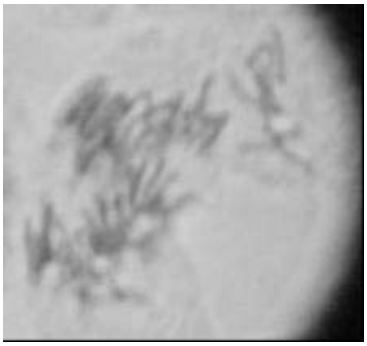

Fig.9. *Picea abies*, 0.25 % A-T with expelled chromatic material

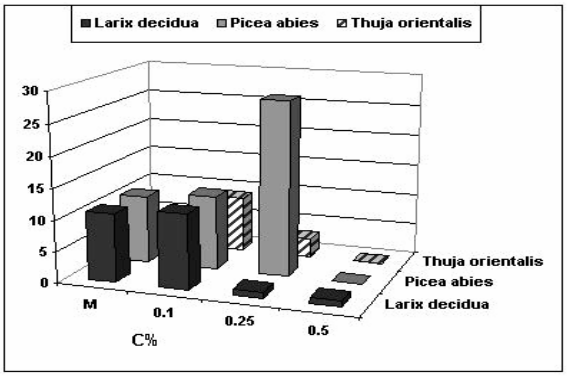

Fig.10.Aberrations frequency induced by the treatment with sodium bisulphit

Sodium bisulphit is a food additive known as E 222. He induces an increase of frequency aberrations at 0.1 % concentration at all species, than only to *Picea abies* at 0.25 % concentration of sodium bisulphit. Concentration of 0.5 %, generally, destroy the cells (Fig.10).

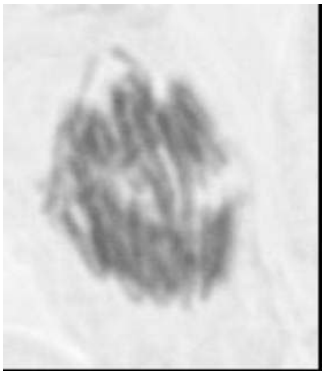

Fig.11. *Picea abies*, 0.1 %
A-T with multiple bridges

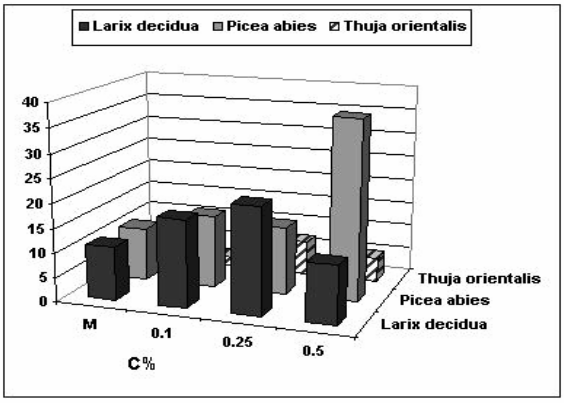

Fig.12. Aberrations frequency induced by the treatment with riboflavin

The treatment with riboflavin (and traces of citric acid) induces an increase of frequency of aberrations to all three species. *Picea abies* is more sensitive to the treatment than the other species studied (Fig.12).

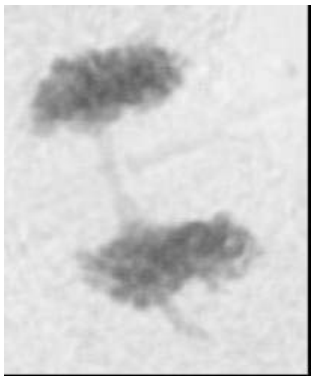

Fig.13. *Picea abies,* 0.5 %
A-T with bridge and
expelled chromatic material

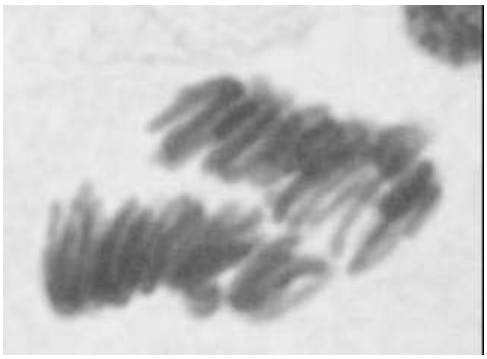

Fig.14. *Larix decidua,* 0.25 %
A-T with three polles

Fig.15. *Larix decidua,* 0.25 %
A-T with ragged bridge

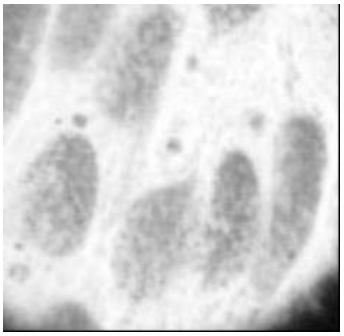

Fig.16. *Picea abies,* 0.25 %
Interphases with micronucleus

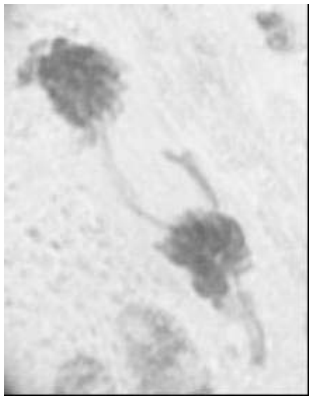

Fig.17. *Picea abies,* 0.25 %
A-T with bridge, retardatary chromosome
and expulsed chromatic material

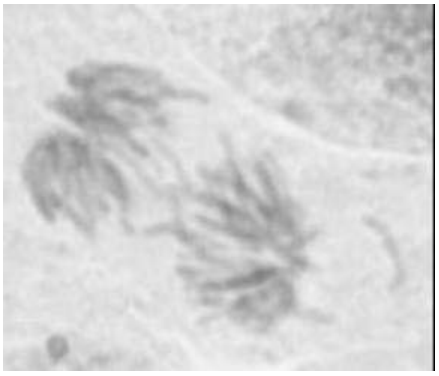

Fig.18. *Picea abies* 0.25 %
A-T with double bridges and retardatary
chromosome

CONCLUSIONS

The treatment was effectuated with substances from food additives category (E 300, E 330, E 101 and E 222).

The biggest frequency of cells in different division phases was registered after the treatment with ascorbic acid and riboflavin, which demonstrated that this substances stimulate the mitotic division.

Comparative with the frequency of aberrantes ana-telophases, the frequency of normal ana –telophases is decreased at seeds treated with ascorbic acid and citric acid, this indicated their inhibitor effect on mutations.

After the treatment with this substances with reduction potential, we observed through aberrations, the presence of A-T with simple, double, triple and multiple bridges, ragged bridges, A-T with retardatary chromosomes, with expelled chromosomes and interphase with micronucleus (especially at *Picea abies*, 0.25 %, riboflavin treatment).

At all the four treatments, the ana-telophases with multiple bridges present the highest percentage, which explain that the substances blocked chromosomes migration.

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