

CYTOGENETIC AND BIOCHEMICAL EFFECTS INDUCED BY THE TREATMENT WITH ASCORBIC ACID AND CITRIC ACID ON *PICEA ABIES* (L.) KARST.

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Keywords: *Picea abies* (L.) Karst., ascorbic acid, citric acid, mitosis, aberrations, antioxidant enzymes

Abstract: This paper presents the influence of ascorbic acid and citric acid upon the mitotic division, and to the activity of some antioxidant enzymes to *Picea abies* (L.) Karst. The treatment was made through the germination of seeds in ascorbic acid and citric acid. We observed the stimulator or inhibitor effect of ascorbic acid and citric acid to the mitotic index and estimated the aberrations appearance. Comparative the control, the mitotic index increased at 0.1 % concentration ascorbic acid and decreased at 0.25 % and 0.5 % concentration of the same substance. The citric acid induced a decrease in the dynamics of mitotic index comparative the control. Also, we observed an increase of aberrations appearance to the treatment with citric acid. We established the activity of catalase, peroxidase and superoxide dismutase and the influence of ascorbic acid and citric acid to the activity of these antioxidant enzymes. After statistical interpretation emerged that these substances (except 0.25 % ascorbic acid) induced an inhibition of catalase activity and a stimulation of peroxidase and superoxide dismutase activity.

INTRODUCTION

The investigation of cell division is a permanently preoccupation, this process having a major importance in development of the individual biological systems. Choosing *Picea abies* L. (Karst.) as the investigation material is motivated by a small number of existed studies, and the dates that will be obtained can be used to improve plants. Also, the ascorbic acid and citric acid are alimentary additives and we considered interesting to test these substances.

We found in literature similar studies to *Secale cereale* which was treated with tartrazine (E102), carmoisine (E122), patent blue (E131) and acid green (E142). The effect of vitamin C on plants was studied with sterile plant cultures and observed an increase of growth [Hausen, Synnöve, 1935]. A few researchers made correlations between concentrations of SO₂ and O₃ like pollutants factors and the increase of antioxidant in conifers. They observed that the maximum effect is to the exposure of trees to the combination SO₂ and O₃ [Mehlhorn, Seufert, Schmidt, Kunert, 1986]. We found in a study that the superoxide dismutase and ascorbate peroxidase activities were sufficient to cope with a higher production of toxic oxygen species and that adaptation was necessary for the antioxidant substrates and their regeneration systems [Pole, Rennenberg, 1992].

In this approach we treated *Picea abies* L. seeds with solutions of ascorbic acid (E300) and citric acid (E330) in three different concentrations: 0.1 %, 0.25 % and 0.5 %. We followed the cytogenetic effects of ascorbic acid and citric acid to the cells division and to the activity of some antioxidant enzymes. Also we correlate the dates obtained with the control.

MATERIALS AND METHODS

Proceeding for the cytogenetic studies

Biological material used in this experiment is represented by seeds of *Picea abies* (L.) Karst. from the forest Department of Piatra Neamț.

The seeds were put to germination in ascorbic acid and citric acid solutions in laboratory conditions. When the roots reached up to 5 - 10 mm in length were sampled for the treatment.

Substances: ascorbic acid (vitamin C, E 300), citric acid (E 330): 0.1 %, 0.25 %, 0.5% (c %);

Treatment: germinated seeds in ascorbic acid and citric acid with three concentrations: 0.1 %, 0.25 % and 0.5 %. Except this variants, there also used a control and in this case no treatment were applied to the radicular meristems.

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

The radicular meristems were displayed using squash technique (Cîmpeanu and other, 2002).

The microscopically examination was carried out using the optic microscope Novex K-Range.

The microphotography's were made with digital camera Canon.

Proceeding for de biochemical studies

For estimated the antioxidant enzyme activity (superoxide dismutase, peroxidase and catalase), we made a few biochemical analyses. We followed the energizing or inhibitor effect of ascorbic acid and citric acid on these enzymes and the action to the cellular level. For accuracy, to each of used concentration we realize three repetitions. We obtained an enzymatic extract by immix the vegetable material and extract him with 0.1 M disodic phosphate solution. After this, the material was centrifuged at 3000 rot / min for 15 minutes. In the supernatant obtained we measured those three enzyms conform the protocol.

The catalase activity was established by titrimetric method with sodium thiosulfate [Artenie, Tănase, 1981]. Like catalasic unity was considered that quantity of enzyme which decompose a micromole of oxygenated water on minute.

The superoxide dismutase activity was determinated using the Winterbourn, Hawkins, Brian and Carrell method [Artenie et all, 2008]. This method is based on inhibited of NBT reduction from the superoxid anion made by riboflavin photoreduction. One unity of SOD is that enzyme quantity which produced 50 % inhibition.

The peroxidase activity was estimated by spectrophotometric method with o-dianisidine [Gudkova, Degteari, 1968]. The activity of this enzyme is expressed in enzymatic unities (micromoles of oxygenated water decomposed per minute). The experimental studies were statistical processed after Student test [Văleanu, Hâncu, 1990].

RESULTS AND DISSCUSIONS

After microscopically investigations and biochemical analyzes we obtained a several dates which compared the control. We'll try to found correlations between the action of ascorbic acid and citric acid to the mitotic index, frequency of aberrations appearance and to the action of antioxidant enzymes: catalase, peroxidase and superoxide dismutase. Statistical interpretations of biochemical and cytogenetic analyzes allow us a large precision of results correlation.

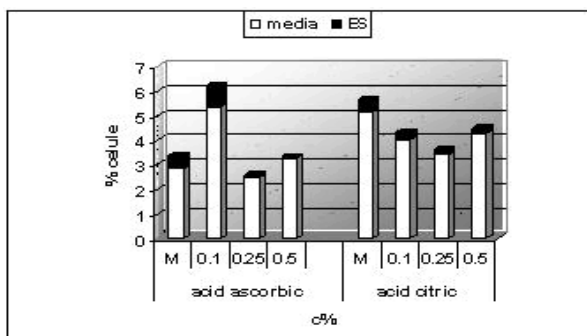


Fig.1. Dynamics of mitotic index after the treatment with ascorbic acid and citric acid at *Picea abies* L.

Mitotic index (IM %) represent the number of cells in division related to the number of all cells multiplication with 100. We observed that the mitotic index increase almost double at 0.1 % concentration of ascorbic acid and also to 0.5 % for the same substances. After the treatment with citric acid the mitotic index decrease at all concentrations, much more at 0.25 % concentration (fig.1).

After the treatment, we observed through aberrations, the presence of ana-telophases with simple and multiple bridges (fig. 3, 5), ragged bridges (fig. 4), ana-telophases with retardatory and expelled chromosomes (fig. 6). The frequency of aberration appearance has a

maximum at 0.5 % concentration to both treatments (more to citric acid). For the treatment with citric acid 0.1 % and 0.25 % induced mutations much more comparative ascorbic acid (fig.2).

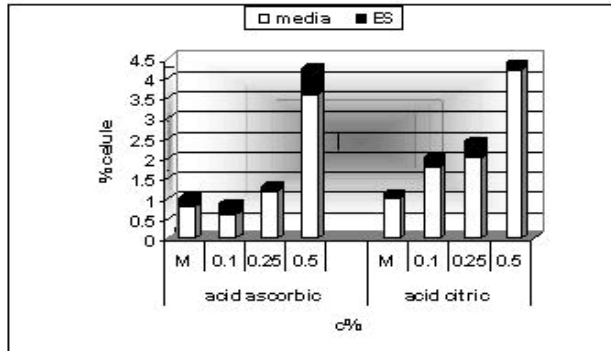


Fig.2. Frequency of aberrations appearance induced by the treatment with ascorbic acid and citric acid at *Picea abies* L.

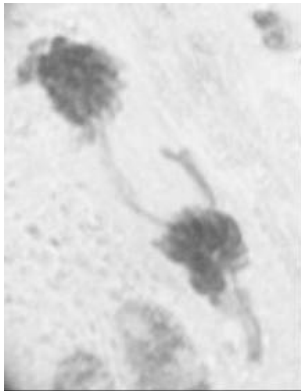


Fig.3. Ana-telophases with bridge, retardatory and expelled chromosomes (*Picea abies* L., ascorbic acid 0.25 %)

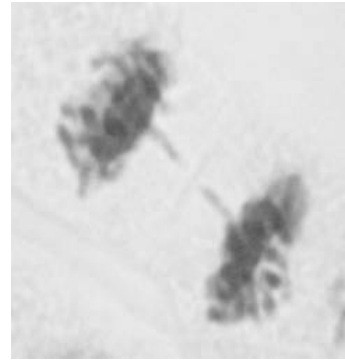


Fig.4. Ana-telophases with broken bridge (*Picea abies* L., ascorbic acid 0.25%)

The presence of ana-telophases with multiple bridges explained that the substances used blocked chromosomes migration.

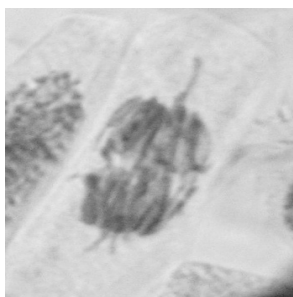


Fig. 5. Ana-telophases with bridges and expelled chromosomes (*Picea abies* L., citric acid, 0.5 %)



Fig. 6. Metaphases with expelled chromosomes (*Picea abies* L., citric acid, 0.5 %)

Thus, we can make a correlation between the increase of mitotic index and the antioxidant effect of ascorbic acid comparative citric acid.

Between those over two thousand enzymes known until now, about 25 % are enzymes that catalyzes varied redox reactions and belong to the oxidoreductases class. In these investigations we follow the activity of some antioxidant enzymes, catalase, peroxidase and superoxide dismutase after the treatment with ascorbic acid and citric acid (substances with reduction potential). We correlate the results obtained with the control.

Regarding catalase, the enzyme activity is inhibited at the treatment with ascorbic acid at 0.1 % and 0.5 % of substance concentration. The same enzyme is stimulated to 0.25% concentration of ascorbic acid. To the treatment with citric acid the activity of catalase decrease much more of all concentrations (fig.7).

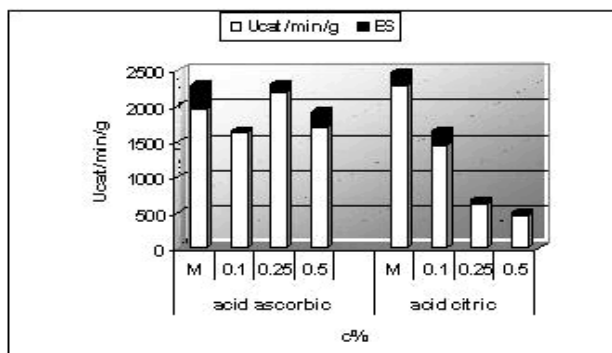


Fig.7. Catalase activity (Ucat/min/g) in *Picea abies* L. seeds germinated in ascorbic acid and citric acid

The calculations of relative activity demonstrate the same thing. We observed an inhibition of 17,218 % at 0.1 % ascorbic acid and 12,831 at 0.5 % ascorbic acid. Also the stimulation of 0.25 % concentration is 10,816%. From ascorbic acid, 0.1 % is extremely powerful statistical (0.25 % and 0.5 % are insignificant). To the citric acid the maximum

inhibition is 79,991 % to 0.5 % concentration (the inhibition of relative activity decrease proportional the concentration of citric acid with 43.424 % comparative 0.1 % concentration). The treatment with citric acid has an important statistical significance (fig.8).

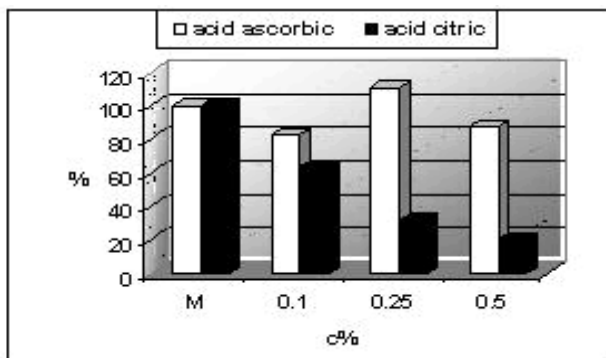


Fig.8. Relative activity (%) of catalase in *Picea abies* L. germinated in ascorbic acid and citric acid

The peroxidase activity increases at both treatments comparative the control. For the ascorbic acid treatment, maximum activity is at 0.25% concentration, and to citric acid increase proportional with concentration (fig. 9).

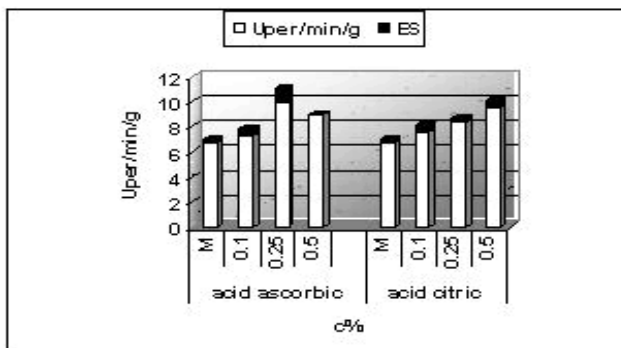


Fig.9. Peroxidase activity (Uper/min/g) in *Picea abies* L. seeds germinated in ascorbic acid and citric acid

For the peroxidase, ascorbic acid produced an absolute stimulation from 48.201 % at 0.25 % concentration. The treatment with ascorbic acid is powerful statistical significant at 0.25 % and 0.5 % concentration and insignificant statistical at 0.1 % concentration of acid. To the treatment with citric acid the stimulation of enzyme activity increase proportional with the concentration; maximum of 42.236 % at 0.5 % citric acid concentration (stimulation with 29.278 % comparative 0.1 % concentration). The treatment with citric acid has approximately the same statistical significance like ascorbic acid (fig.10).

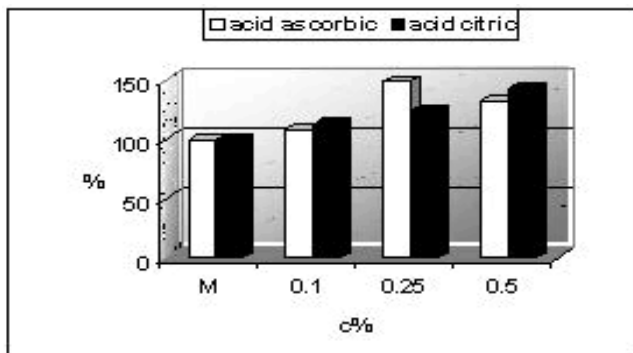


Fig.10. Relative activity (%) of peroxidase in *Picea abies* L. seeds germinated in ascorbic acid and citric acid

We observed that the activity of superoxide dismutase (SOD) increase visible at both treatment (like the peroxidase), proportional with the increase of concentration and comparative the control. These facts had the same signification, the cells reacting to the stress determinate by ascorbic acid and citric acid and protect cells (fig. 11).

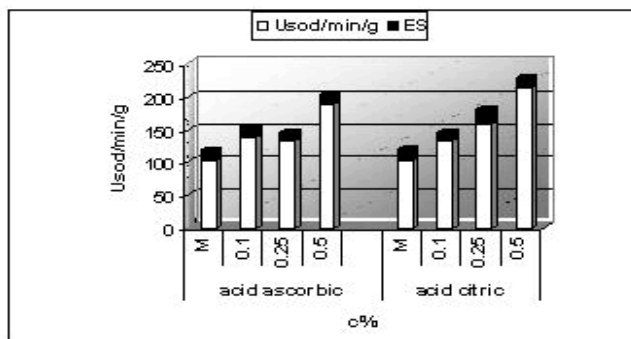


Fig.11. Superoxide dismutase activity (Usod/min/g) in *Picea abies* L. seeds germinated in ascorbic acid and citric acid

To the superoxide dismutase, the relative activity increase proportional with the increase of substances concentration, for the ascorbic acid is an activity stimulation of 79.748 % (an increase with 46.905 % comparative 0.1 % ascorbic acid concentration) and to citric acid the absolute value is 105.104 % (an stimulation with 79.048 % comparative 0.1 % citric acid concentration), both to 0.5 % concentration. Both 0.5 % concentration are powerful and extremely powerful statistical significant. The 0.1 % and 0.25 % concentration of both treatments are statistical insignificant (fig.12).

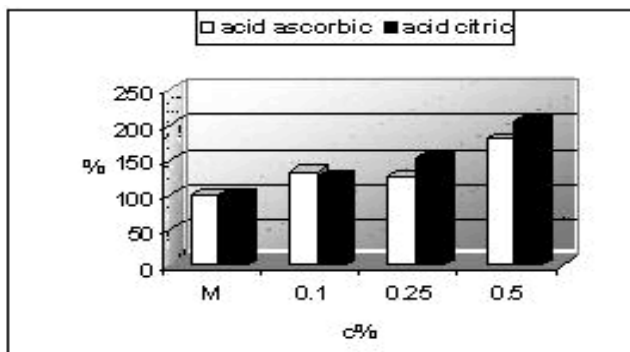


Fig.12. Relative activity (%) of superoxide-dismutase in *Picea abies* L. seeds germinated in ascorbic acid and citric acid

The peroxidase and superoxide dismutase activity in little plants of *Picea abies* L. treated with ascorbic acid and citric acid demonstrate an intensification of formation of the reactive forms of O₂ species, like peroxide and superoxide. The peroxides and activated forms of O₂ can produced damaged in plants or animals organism through inactivation of some enzymes and affection of some substances which are components of cellular organites [Olinescu, 1982].

CONCLUSIONS

The treatment with citric acid is more destructive for the cells comparative the treatment with ascorbic acid, which is demonstrate by the decrease of mitotic index comparative the control.

The frequency of aberrante ana-telophases is increase to citric acid treatment, which proved that citric acid is a stronger mutagen agent than ascorbic acid. Between aberration, the simple and multiple bridges present a highest percentage, which explain that the substances blocked chromosomes migration.

The catalase activity decrease comparativ the control at both treatment (except 0.25 % concentration of ascorbic acid) which determined a little stimulation of enzyme activity.

The peroxidase activity increase of both treatment,determined a high stimulation at 0.25 % ascorbic acid concentration.

The superoxide dismutase activity increase comparative the control at both treatments for all three concentrations, which explained that the cells are in oxidative stress and reacting through increasing of enzyme concentration.

To the ascorbic acid, the concentration which has an important statistical significance are 0.1 % for the catalase, 0.25 % and 0.5 % for peroxidase and 0.5 % for superoxide dismutase (SOD). At citric acid, statistical powerful are 0.1 %, 0.25 % and 0.5 % for catalase, 0.25 % and 0.5 % for peroxidase and 0.5 % for superoxide-dismutase (SOD). Generally, we observed that the high concentration induced a powerful inhibition or stimulation comparative the control.

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