# Gamma Radiations Effects on Catalase and Assimilatory Pigments in False Acacia Seedlings Grown in Forestry Nursery

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## Abstract

*Low radiation doses effects on the species* ***Robinia pseudoacacia*** *(false acacia) have been studied in this paper. Biochemical analyses were focused on the catalase activity as well as the assimilatory pigment contents in the leaves of some common acacia seedlings from a forestry nursery situated in the river Prut valley. Radiation influence was tested performing exposures to a Co source with a very small activity equivalent to local temporal variations of atmospheric radioactivity, susceptible of influencing natural variability of vegetal species. Catalase activity was considerably diminished under the level of the control in all samples while chlorophyll* ***a*** *was significantly affected by short exposure times, suggesting that irradiation can be also a tool in tree biotechnology.*

**Keywords**: *Robinia pseudoacacia*, gamma radiation, catalase activity, assimilatory pigments

## Introduction

Chemical and physical factors were much utilized as efficient meanings in modern biotechnologies, radiations being admitted as incontestable tools for the generation of mutants with new biochemical features in plants, animals and microorganisms.

Among the successful investigations of Romanian researchers preoccupied by forestry trees amelioration, the yielding of a mutant with enzymatic activity modification in the species *Pseudotsuga menziesii* var. Douglas, after radiation treatment, should be mentioned [1]. The biochemical mutant was characterized by a small catalase content and a high peroxidase content during winter, conferring him a good adaptability to could winter climate. Other results related to the influence of ionizing radiations in enzymes were reported by Ferdeş and Ferdeş, 1992 [2] which found that enzymatic reaction rates modify as well as the values of Michaelis-Menten constant and the specificity of some amylases, proteases and isomerases in irradiated bacteria and yeasts. The study of radiation effects on young vegetal organisms in early ontogenetic stages may be considered further a direction of actuality in tree biotechnology related sciences since radioactivity variations may appear spontaneously in atmosphere and can influence natural variability of certain species. Vegetal species from the atmospheric superior level are directly exposed to the radiation flows delivered either from cosmic sources or from artificial sources, assuring a partial protection to animal and vegetal species from inferior levels of forestry biocenoses [3-4].

Though radioactive pollution as the chemical one may be caused by the water absorbed from the soil, however direct exposure of leaves to electromagnetic radiation flows from the atmosphere and to the air currents bearing radioactive dust particles represents the main modality of radioactive pollution of tree leaves. Biochemical tests accomplished during this study intended to evidence the changes in catalase activity and assimilatory pigment contents from the leaves green tissues of black locust seedlings. *Robinia pseudoacacia* represents o species of high importance to the ecological reconstruction in plain and hill regions (Boring, 1984, [5] Hanover, 1989, [6], Keresztesi, 1988 [7]) where dry summers and soil structure do not allow other species plantation. Forestry nurseries from Iasi area intent the seed multiplication of biological material selected from the natural local stands (**Figure 1**). Investigations presented below are focused on black locust seedlings where it is expected that the sensitivity to radiations is higher than in adult trees (**Figure 2**).

## Material and Methods

False acacia seedlings aged 6 months have been picked up from the forestry nursery of Sculeni-Iasi in veseels containing adequate quantity of surrounding soil being then kept for two days to accomodate to the laboratory microclimate (24 Celsius degree temperature, light/dark cycle 12hours/12 hours). For the exposure to radiations a Cobalt source with very low activity (100 microCurie) was utilized, exposure times being of 1; 2; 6 and 12 hours. The day after exposure leaves catalase activity and assimilatory pigment contents were measured. Catalase activity was determined iodometrically in enzyme extract obtained with 0.1 M $Na_2HPO_4$ solution [8]. Assimilatory pigment contents were measured by spectrophotometrical assay after extraction with acetone 90% [9]. For each seedling five green tissue samples were taken for analysis, average value and standard deviation being calculated.

## Results and Discussions

The values obtained for catalase activity are given in **Figure 3**. A drastic reducing of enzymatic activity in all samples in comparison to the control can be seen. It may be observed that in samples corresponding to exposure times of 1 and 2 hours, catalase activity enzymatic activity is of about 25% from the control value while in samples corresponding to exposure times of 6 and 12 hours it was further decreased two times more. In **Figure 4** a negative correlation between catalase activity and irradiation time may be seen, experimental data fitting on a logarithmic function (correlation coefficient R=0.98), meaning that changes recorded by us are not random fluctuations. In **Figure 5 a,**

**b, c,** it is given the situation of assimilatory pigment contents in the investigated samples. Chlorophyll ***a*** content is progressively reduced (Figure 5a) for irradiation times of 1 and 2 hours, remains stationary for the time of 6 hours and is restored closed to control for the time of 12 hours irradiation.

In Figure 3b one can see that for chlorophyll ***b***, a diminishing in the samples corresponding to irradiation times of 2 and 6 hours occurred, followed by a restoration until a value somewhat higher than in control for the exposure time of 12 hours. It is notable that these variations are smaller than in the case of chlorophyll ***a*** (6-14% in comparison to 13-27%). The same situation was characterizing the variations recorded for carotene content. In the samples corresponding to 2, 6 and 12 hours negative variations of 5-15% were obtained in comparison to the control while in the sample corresponding to 1 hour exposure time an increase of 5% was recorded (Fig. 3c). The average value of the standard deviation was equal to 4.5% for both chlorophylls but it was doubled for carotene, so one may conclude that only the variations recorded in chlorophyll ***a*** are actually significant. So, all three types of pigment from the seedling leaves are affected by low radiation doses which induced inhibitory effects with a tendency to restoration for high exposure times. No mathematical functions were found to approach with a good correlation coefficient the variations of pigment contents to the increasing of the radiation time.

Catalase activity remarkable decreasing for the exposure times tested within this experiment suggested that radiations can work in the direction of enzyme molecule damage (by disorganization of secondary and tertiary structures), though protection mechanisms of the cell against the ionizing radiation effects may have, in the same time, an influence (stimulation or inhibition), on the biosynthesis in the molecule [10-11]. Indeed it is known that water radiolysis in the cell may determine an increase of hydrogen peroxide, against the toxic effect of this one cell defending by means of catalase. It seems that in the case studied inhere the two concomitant phenomena have as result the decreasing of catalase activity (analogous to the oak seedlings grown in forestry nursery, studied in similar conditions [12]).

As for the assimilatory pigment contents it is notable that the tendency of decreasing corresponding to relatively short exposure times is followed by a contrary tendency, of restoration control values for longer exposure times (especially 12 hours). This reveals that, in the above hypothesis, the balance of the two radiation action directions, which is first unfavorable, changed later becoming favorable to photosynthesis.

## Conclusions

False acacia seedlings grown in nursery are sensitive to the action of ionizing radiations such as those delivered by a Cobalt source with a very low activity. Enzymatic activity of catalase seems to be diminished because of the radiation effects on the secondary and tertiary structures, which predominate on the possible phenomenon of stimulation of this enzyme biosynthesis for the cell protection against the action of hydrogen peroxide generated, indirectly by radiations. A negative logarithmic correlation can be revealed to approximate experimental data with a very good correlation coefficient (R=0.989). Assimilatory pigment contents present a reduction both at the level of chlorophylls and of the carotene pigments. The principal assimilatory pigment, chlorophyll a is most influenced by radiations while secondary pigments suffer significantly smaller influences.

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Figure 1. False acacia forestry nursery

13. Figure 2. False acacia seedlings transferred in laboratory

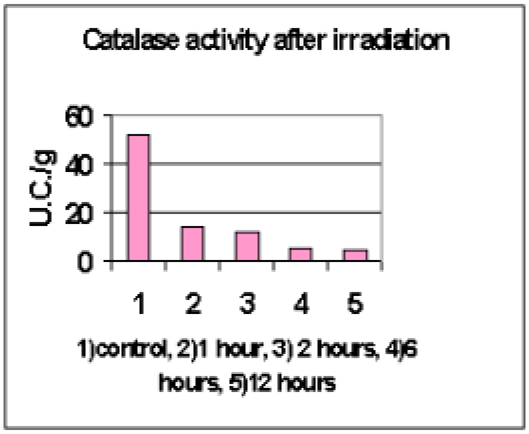


**Figure 3.** Catalase activity after exposure to radiations

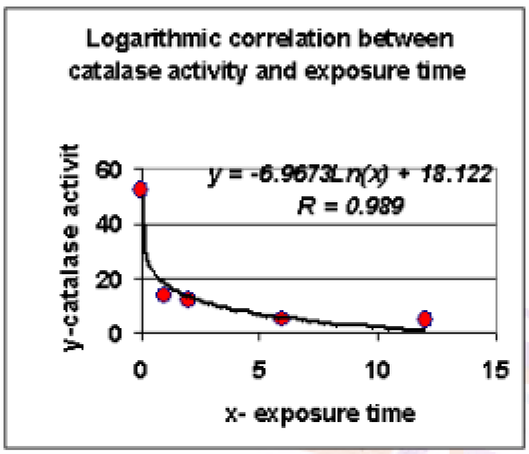


**Figure 4.** Logarithmic correlation between catalase activity and exposure time

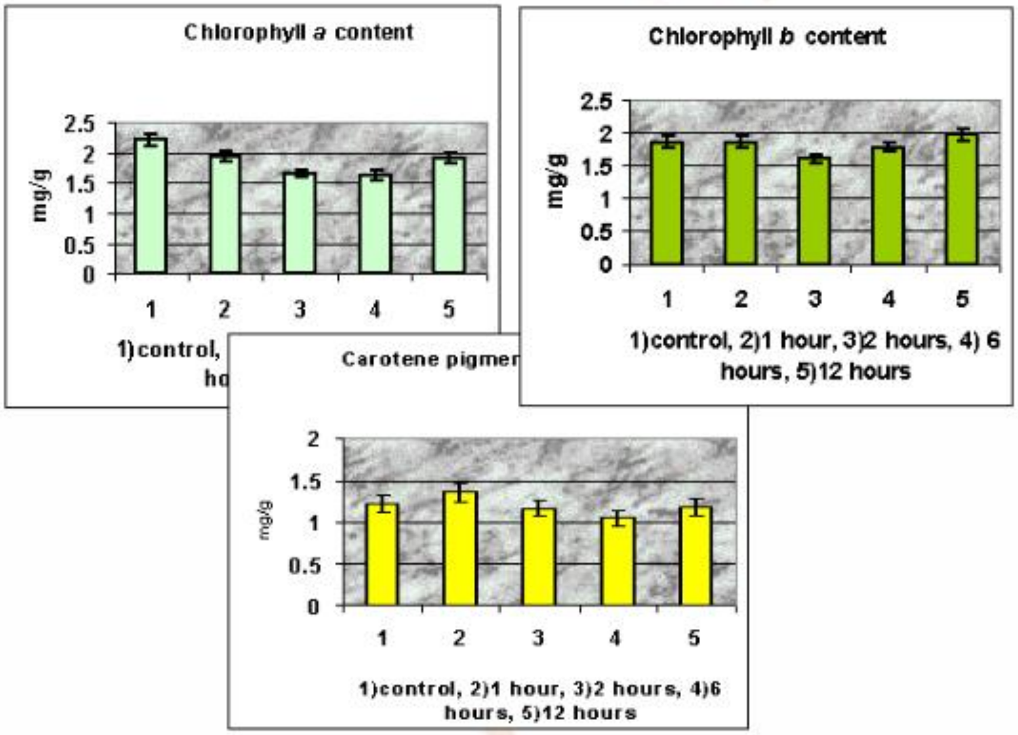


Figure 5. a. Chlorophyll ***a*** content ; b. Chlorophyll ***b*** content; c. Carotene pigment content