PEROXIDASE, SUPEROXIDE-DISMUTASE AND CATALASE ACTIVITY IN CORN PLANTS DEVELOPED UNDER THE INFLUENCE OF POLYPHENOLIC COMPOUNDS AND DEUTERIUM DEPLETED WATER

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Abstract: In this experiment we studied the role of deuterium depleted water and spruce bark (*Picea abies L.)* polyphenolic extracts in the activity of some enzymatic systems involved in the metabolism of plants of maize (*Zea mays* L.). Thus, we evaluated the activity of peroxidase, catalase and superoxide dismutase in the leaves and roots of maize, developed in the different experimental variants. It was found that the peroxidase activity decreases in the roots treated with deuterium depleted water and increases when the plants are treated with the polyphenolic extract derived from spruce bark. Also, it was observed an increase in catalase activity in maize plants treated with polyphenolic extract from spruce bark.

INTRODUCTION

Polyphenolic compounds are the must important classes of secondary metabolites that play an important role in the biosynthesis process. Natural bioactive compounds have a broad spectrum of both the plant as a whole and on tissues and organs, interfering in the metabolic processes.

Since plant activity is intimately correlated with the biochemical processes taking place in the all vegetative organs, a series of experiments in the literature aimed at studying the influence of polyphenolic products of enzymatic systems involved in carbohydrate metabolism and cellular respiration (Anghel, 2004; Tudose, 2002). Thus, for seedlings of *Phaseolus vulgaris* L, in the concentration range of 50-100 mg / L bioactive polyphenolic compounds, were found to increase germination capacity and yield of plant biomass biosynthesized correlated with increased activity of amylase, polyphenol oxidase and ascorbatoxidase on average 15-25% compared with control, while decreasing catalase activity (Anghel, 2004). From the results obtained in the study of the influence of global polyphenolic extract of vine wood, which contains biologically active principle, the activity of some enzymes of carbohydrate metabolism was found and polyphenolic extract positively affects the germination process in general. There is a major action on α-amylase activity in the first 24 hours of germination process. Also, there was an intense activity of enzymes (α-amylase, β-amylase, catalase, peroxidase and protease) involved in the germination process that is an argument of a normal functioning of the process. Moreover, it was found that the sequence of enzymes in the germination proces was kept (Tudose, 2002). Through, the characteristic biological activity, natural polyphenols are essential compounds in the stimulation of plants growth and development. The stimulation or inhibition capacities on the plant growth and development is closely correlated with concentrations of polyphenolic compounds applied and activities of enzymatic systems (Tanase et al. 2013).

The changes that occur in normal water characteristics lead to significant changes in the fundamental processes of the cells and therefore, DDW was used as tracer to characterize wholetree water transport and storage properties in individual trees belonging to the coniferous species. The use of DDW appears suitable for answering some questions regarding relative differences in water use among trees, water redistribution among neighbours and internal water transport and storage processes in plants. Recent research has shown that spruce bark extract and DDW have a great influence on plant growth and development. through studies carried out (Tanase et al. 2013).

By the studies carried out it has been found thart enzymatic activity of peroxidase was found to be higher in the maize callus developed in the presence of DDW and polyphenolic extract. Intensity changes of the enzyme activity in the presence of DDW and polyphenolic extract from spruce bark show their involvement in the regulation of metabolic processes in cells and the role of the tested solutions in maintaining the balance between the resulted and depleted free radicals (Tanase et al. 2013).

The aim of this study was to evaluate the effect of spruce bark aqueous extract and deuterium depleted water (DDW) as bioregulators on the activities of enzymatic systems such as peroxidase, superoxide dismutase and catalase.

MATERIALS AND METHODS

Deuterium depleted water or light water is a microbiological pure distilled water, with an isotopic concentration of 25 ppm, obtained by isotopic distillation in vacuum of natural water with an isotopic concentration of 145 ppm D / (D + H). Deuterium depleted water (DDW) was purchased from Romag Prod, Severin (Halanga), a manufacturer of heavy water used for the reactors of the Cernavoda nuclear power plant. Spruce bark (*Picea abies* L.) was purchased from the Alpine LTD Timber Company, Vatra Dornei. The bark was dried at room temperature under normal aeration conditions, grounded and subjected again to a drying process.

Maize seeds were purchased from Unisem Company, Romania. Germination tests were carried out going through a standard procedure, using increments of 5 petri dishes for each solution studied (Table 1). On a filter paper were placed every five soybean seeds, carefully selected to no present major damage. For starters, the vegetal material has undergone a process presterilizare, which consisted of submerged seed absolute ethanol for 10 seconds, following the sterilization in the presence of sodium hypochlorite 10% for 20-30 minutes (Cachita et al., 2004). The volume of solution added was 10 mL / dishes. Petri dishes thus prepared were incubated in the dark in a thermostat set at 27 º C. After a period of seven days Petri dishes were taken out and the roots, stems and leaves are separated for enzymatic analyzes.

The spruce bark aqueous extracts were characterized from the point of view of dry matter content, total polyphenolic content, total content of tannins, flavonoids, flavonols and antocyanins using selected samples with about the same content in total polyphenols. These results as well as the extraction method were published in our previous work (Tanase et al. 2013).

Thus, by using standard methods we evaluated the activity of peroxidase, catalase and superoxide dismutase in the leaves and roots of maize developed in the 8 experimental versions, shown in Table 1.

Table 1 - Experimental variants

Experimental variant	Abbreviation	Total polyphenolic content (mg GAE/L)
Control	Control	-
Deuterium depleted water	DDW	-
Deuterium depleted water and spruce bark polyphenolic extract (1:1)	DDW+M1	96
Spruce bark polyphenolic extract	M1	191
Spruce bark polyphenolic extract	M2	130

Enzyme activity assays

The radicle and leaves resulted after 8 weeks of germination were suspended in 5 mL of cold 50 mM phosphate buffer (pH 7.8) and sonicated three times for 30 sec (Ultrasonic Processor CPX130, Cole-Palmer, Instruments, Illinois, USA). The slurry was then centrifuged for 10 min at 5000 rpm. The supernatant was used further for enzyme activity assays.

Peroxidase activity assay (EC 1.11.1.7)

The activity of peroxidase was monitored using hydrogen peroxide as substrate acceptor and *o*-dianisidine as donor. The absorption was recorded at 436 nm (Tanase et al. 2013).

SOD activity assay (EC 1.15.1.1)

Activity of superoxide dismutase (SOD) was determined by measuring its ability to inhibit photochemical reduction of nitro blue tetrazolium (NBT) (Artenie et al. 2008; Dhindsa et al. 1981).

Catalase activity assay (CAT, EC 1.11.1.6)

The activity of catalase was assayed by the method of Sinha (1972). Catalase was allowed to split H_2O_2 for different periods of time. The reaction was stopped at different time intervals by the addition of dichromate and acetic acid mixture and the remaining peroxide was determined by measuring chromic acetate amount, at 570 nm, after heating

the reaction mixture. The activity of catalase was expressed as μM H_2O_2/g protein/min. One unit of catalase activity **(U)** was defined as the amount of enzyme that converts one micromole of substrate to product in one minute (Tanase et al. 2013).

Quantitative determination of proteins using Bradford assay

In order to calculate the enzymatic specific activities of extracts, the total protein concentration was estimated (Artenie et al. 2008). All UV-Vis absorption spectra were recorded using a Libra single beam Spectrophotometer (Biochrom, UK) and quartz cuvettes (Hellma/Müllheim).

RESULTS AND DISSCUSSIONS

Determination of total protein. It was found that the total protein concentration is the same for all experimental variants. Given these observations it was concluded that the enzyme activity will have the same trend as that of the specific activity (ratio of enzyme activity calculated the total amount of protein). Thus, the total enzyme activity was calculated only for the three enzymes analyzed.

Peroxidase activity. By determination of the enzymatic activity of peroxidase, the roots of maize plants we found that it is reduced in the presence of deuterium depleted water up to 21% compared to the control (Figure 1). A reduction in the activity of peroxidase was recorded in variants when in growth medium was added DDW in combination with the spruce bark polyphenolic extract (DDW+M1) or spruce bark polyphenolic extract with concentration of 191 mg GAE / L (M1). A small increase in enzymatic activity of peroxidase in roots of maize was recorded for M2 variant.

As concerning the enzymatic activity of peroxidase in maize leaves, there is a reduction as compared to the roots of all the variants analyzed. Compared to the control there is an increase in peroxidase activity (over 10%) in the leaves of maize plants that have been developed in the presence of deuterium depleted water in mixture with spruce bark polyphenolic extract (DDW+M1). For the other alternatives do not have significant differences compared with the control.

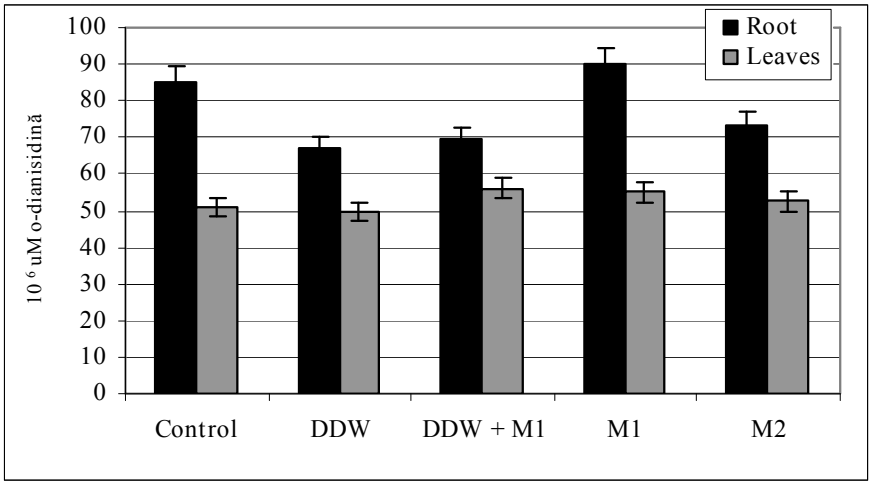

Figure 1 - Variation of peroxidase activity in the roots and leaves of maize depending on growth conditions

Superoxide dismutase activity. By analyzing the figure 2 we can see that in the roots of maize takes place an inhibition of the activity of superoxide dismutase, as compared with the activity in leaves. Regarding the enzymatic activity of SOD in plants developed in the different experimental variants we didn't find significant differences compared with the control samples (Fig. 2). In the case of variant M1 there is an increase in enzymatic activity compared to the control (15% to 12% for roots and leaves). For other variants is a slight reduction in superoxide dismutase activity in both roots and the leaves.

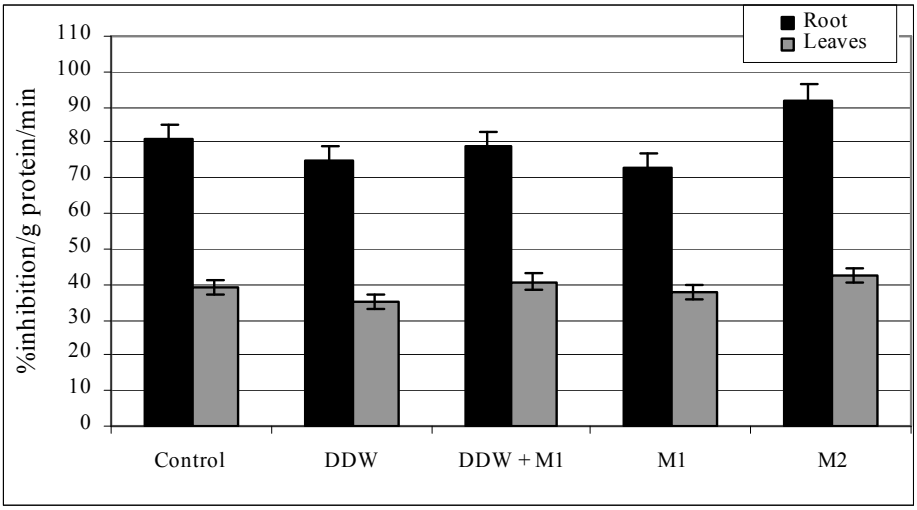

Figure 2 - Variation of superoxide dismutase activity in the roots and leaves of maize depending on growth conditions

Catalase activity. The observations made in the maize plants show the action of the enzymatic activity of catalase added to the solution to increase (Fig. 3). Thus, there is an increase in catalase activity in leaves compared with the control. Significant differences were recorded in DDW variants (103%) and M1 (122%). In case of the root it was observed a decrease in the enzymatic activity for the plants developed in the variants DDW and DDW + M1, and a slight increase in M2 and M1 variants plants.

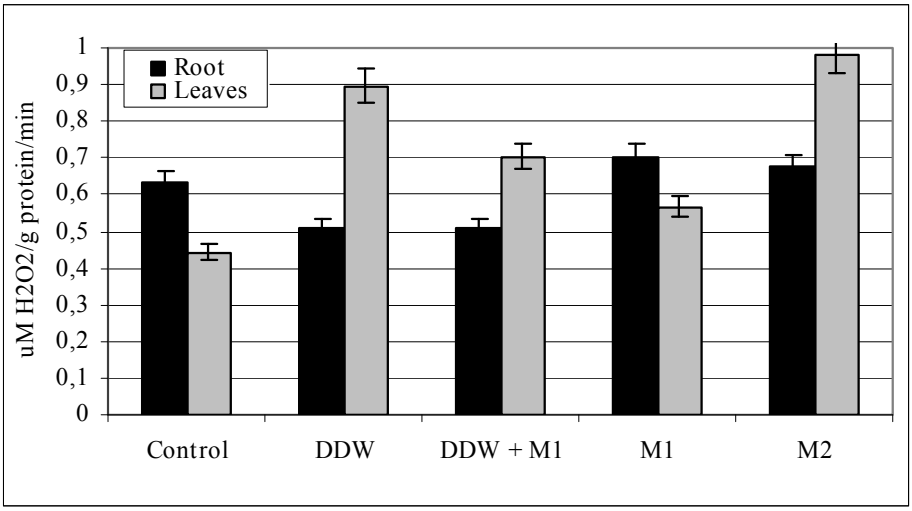

Figure 3 - Variation of catalase activity in the roots and leaves of maize depending on growth conditions

CONCLUSIONS

It was found that the total protein concentration is the same for all experimental variants. By analyzing the enzymatic activity of peroxidase in the roots of maize plants, we found that it is reduced in the presence of deuterium depleted water and extract obtained from the spruce bark. Compared to the control an increase in peroxidase activity in the leaves of maize plants were developed in the presence of deuterium depleted water mixed with polyphenolic extract from spruce bark. The observations made in the maize plant for the enzymatic activity of catalase, showing the action of the solutions added to the growth medium. Thus, there is an increased activity of catalase in leaves compared with control in the presence of deuterium depleted water and spruce bark polyphenolic extract. At the root is a decrease in the enzymatic activity of catalase variants DDW and DDW + M1 and its slight increase in M2 and M1 variants plants.

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