THE PEROXIDASIC ACTIVITY IN THE EMBRIONIC SMALL ROOTS OF *TRITICUM*, *LOLIUM* AND *BROMUS*, AFTER THE GERMINATION OF CARIOPSES ON SUBSTRATUM MOISTURED WITH WATERY EXTRACT PREPARED FROM THE METAMORPHOSED ROOTS OF HORSE RADISH (*ARMORACIA RUSTICANA* LAM.), AT THE TIME OF BLOSSOMING OF THE PLANT

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ABSTRACT. In our experiment, we studied the peroxidasic activity in *Triticum aestivum* L., *Lolium perene* L., and *Bromus inermis* L. roots of plantlets, in the 7th day of cariopse germination on filter paper substratum moistured with aquaeous extract, which was prepared in different concentrations (5, 15, 25, 50, 75 and 100%), from the metamorphosed roots of horse radish (*Armoracia rusticana* Lam.) picked up at the time of blossoming of the plant. The peroxidasic activity was intensified at the smaller concentrations of extract (5-15%), but at high concentrations (25-100%) the enzyme activity was diminished.

Keywords: germination, stimulation, peroxidases activity

INTRODUCTION

The peroxidases, very widely spread enzymes in the vegetal realm, are involved in a multitude of physiological processes, such as the germination and growth of plantlets, in the formation of the buds and flowers, in the ripening of fruits, in tuberogenesis, in the defense mechanisms, are used as tests in the analyses of cell viability, etc.

The peroxidases play major roles in growth, particularly by the control or by their participation to the catabolism of auxine, an important growth phyto-hormone (Cachiţă et. al., 1976; Negruţiu et. al., 1979; Gaspar et. al., 1982, 1991), and in the development process, along the formation of roots, buds and flowers, the activity of the anodic peroxidases is increased, growth associated with a continuous process of lignification in the cells of the xylem (Thorpe et. al, 1978; Gaspar et. al, 1979).

Bohm et. al. (2006) stated that the peroxidases respond to the stress factors, such as the *alelopatic substances*, compounds entering the category of secondary metabolism products. These chemical substances are present in different organs of many vegetal species, such as the leaves, flowers, fruits and seeds, but also in stems and roots, especially of the perrennial ones (Miró et. al., 1998; Delachiave, 1999).

The horse radish (*Armoracia rusticana* Lam., family *Brassicaceae*), is a herbaceous, perrennial plant, originary from the neighbouring regions to the Black Sea and in Europe it is grown both as a vegetable and as a medicinal plant. In our country it is grown in all regions, especially around the big cities, but it also grows spontaneously, as a common plant, besides the fences in humid places (**Ciofu** et. al., 2003).

The horse radish has in the soil a thick root, vertical, strong and branched out, which penetrates the soil deeper than 70 cm, coloured grey-yellowish on the outside and sheer white on the inside (*Flora Republicii Populare Române*, 1955). On its length it presents numerous dormant buds, which makes – particularly when the plants are young – that each portion from the root placed in proper conditions to produce a new plant (Ciofu et. al., 2003) ensuring the vegetative multiplication of this species exclusively on a vegetative way (Pârvu, 1991).

Chemically, the root contains proteic substances (2%), glucides (6%), sodium mineral salts (5mg%), potassium (295mg%), calcium (55mg%), phosphorus (35mg%) and iron (0,7mg%), vitamins, sulphured glicosids, mirozine, small quantities of volatile oil, hydrochloric acid, sulphuric acid, carbonic acid, silicic acidand antibiotic substances (fitoncide) (**Pârvu,** 1991). The content in peroxidases, sulphured glicozids, fitoncide and volatile oils determine the piquant taste and the characteristic flavour. (**Ciofu** et. al., 2003).

The alelopatic potential of the species of Brasicacee is attributed to the releasing in the surrounding environment of the *alil izotiocianate,* volatile compound (Choesin; Boerner, 1991), a derivative of *sinigrine*.

Sinigrine belongs to the class of glucosinolates, which are sulphure compounds produced by the

secondary metabolism of plants. It has a molecular weight of 397,45 and in the body of the plants composing it, it is found under the form of crystals, having the melting point of 127^0C. It is easily soluble in water, in warm alcohol, insoluble in benzene and chloroform. In the horse raddish root one can find *mirozinaza*, enzyme which decomposes the *sinigrine* in the *alil izotiocianate*, glucose and acid potassium sulphate, according to the following reaction: (Neamţu, 1983).

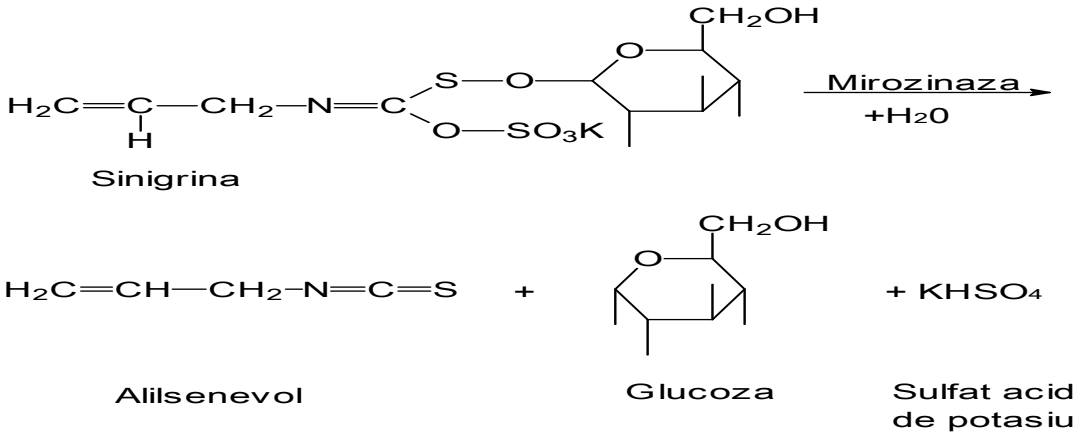

Fig. 36. Sinigrine hydrolysis (according to **Neamţu**, 1983)

In order to study the would-be effects produced by the compounds with alelopatic action present in the watery extract obtained from the metamorphosed root of horse radish, picked up at the time of blossoming of the plant, we have set as an aim to test its action (in various dilutions) upon the *peroxidasic* activity in the embrionic small roots of the **wheat** (*Triticum aestivum* L.), **ryegrass** (*Lolium perene* L.) and **brome grass inaristata** (*Bromus inermis* L.) plantlets.

MATERIALS AND METHODS

The vegetal material used as an object of study in the present experiment was represented by the embrionic small roots of the plantlets of **wheat**, **ryegrass** and **brome grass inaristata**, after seven days of germination of the cariopses on a substratum moistured with watery extract prepared from fresh root of horse radish picked up at the time of blossoming of the plant, respectively summer (in the month of June).

The basic extract from metamorphosed root of horse radish was prepared as follows: 250g of fresh roots were grated by a plastic material shredder, and the vegetal material obtained was covered with 500 ml distilled water; the mixture was left to macerate for 12 hours, after which the supernatant was decanted, and the extract was filtered through filter paper and preserved until usage in the dark at the temperature of 4^o C. from the watery extract obtained, considered 100%, there were prepared five dilutions, namely 5%, 15%, 25%, 50%, and 75%. Thus, outside the proof lot (V_0) represented by cariopses placed to germinate on substratum humidified water there were made 6 experimental variants: V_1= extract 5%; V_2= extract 15%; V_3= extract 25%; V_4= extract 50%; V_5= extract 75%; V_6= extract 100%.

The germination of cariopses was performed in colourless plastic casseroles, maintained at the laboratory temperature (22^o ±2^oC), in indirect light, with specific day variations.

Determining the peroxidasic activity was analysed by the *colorimetric method*, which is based on the oxidation reaction of the reactive p-phenilendiamine; the intensity of violet coloration which appears further to oxidation, was appreciated *spectophotometrically*, with filter of 483nm (Lück, 1974).

For preparing the enzimatic extract of **wheat,** in the seventh day of germination, there were grounded 10 small roots; at **ryegrass** and at **brome grass inaristata** their number has been increased to 20, the small roots of these plantlets being smaller than those at wheat. Grinding the small roots was made with well washed sand, sterilized in advance by heat in drying closet at 120^oC. Over each homogenous product obtained by grinding the small roots there were added 4ml dilluted buffer prosphate. The samples were centrifuged at 6000 rotations/minute, for 20 minutes and the supernatant obtained collected in test tubes and preserved among ice cubes, in the fridge until the photocolorimetric determination of the peroxidasic activity. Reading the samples was made at a spectophotometer type Spekol 11, with a filter adjusted at a wavelength of 483nm.

For determining the peroxidasic activity, in the vat of the spectophotometer there was introduced 0,5 ml enzyme extract, for each sample, to which there was added 1ml ultra-dilluted oxigenated water 0,05 ml buffer concentrated phosphate and 0,05ml solution p-phenilendiamine 1% (freshly prepared solution).

The intensity of the violet colouring of the mixture resulted further to the contact of peroxidases from the extract with the p-fenilendiamină solution from the reaction vat, was determined at *30 seconds* from the homogenization of the mixture, since its colour rapidly intensifies, which constitutes a proof of the fact that the peroxidasic activity in the small roots is very intense.

The data regarding the peroxidasic activity determined in the embrionic roots of **wheat**, **ryegrass** and **brome grass inaristata,** after the germination of the cariopses on substratum humidified with watery extract obtained from metamorphosed root of horse radish, were related to the values of the respective parameter identified in the small roots of the proof lot,

cariopses germinated on filter paper humidified with distilled water. The average values of the extinctions were measured at every third sample, for each experimental variant taken by us into study; the statistic significance of the results was established with the help of test „t" (Steinback, 1961).

RESULTS AND DISCUSSION

The results of the research have traced the effect of the phyto-toxic substances contained in the watery extract of metamorphosed root of horse radish, picked up at the time of blossoming of the plant, upon the *peroxidasic activity* in the embrionic small roots of the three species of *monocotyledonous* plants taken into study, are presented in table 1 and in the histograms from figure 1. The pluses or minuses registered are marked in figure 1 with regard to the values of the respective parameter measured at the proof, graphically marked with 0.

The spectophotometric determinations performed at *seven days* from placing the cariopses to germinate on substratum humidified with watery extract prepared from metamorphosed root of horse radish, have outlined the fact that the activity of peroxidases is in close connection with the *concentration* of the extract used in experiments, as well as with th *species* of plant analysed.

Examining the experimental data synthetized in Table 1 and in figure 1, (V_1) one can notice the *activating* of peroxidases in the small roots of the *monocotyledonus* plantlets analyzed at the action of the horse radish in small concentrations, of *5%*. Thus, in the small roots of the plantlets of **ryegrass** there were emphasized non-significant stimulations of the peroxidasic activity ($p>0,05$)**,** the increase as to the proof being of 10,58%, while at **wheat** and at **brome grass inaristata** these stimulations, of 17,78% and of 22,78% (fig. 1, V_1) were significant from a statistical point of view ($p<0,05$).

The data obtained by biochemical determinations regarding the activity of peroxidases in the embrionic roots of *monocotyledonus* plantlets taken into study, present a maximum intensification of the activity of these enzymes on the variant with watery extract prepared from the metamorphosed root of horse radish, picked up at the time of blossoming of the plant, in concentration of *15%* (V_2). We exemplify the things stated by the average values of the extinctions registered in the extracts prepared from the embrionic roots of the plantlets of **wheat**, **ryegrass** and of **brome grass inaristata,** germinated and grown on substratum humidified with watery extract of metamorphosed root of horse radish, in concentration of *15%* (V_2), which were of 1,503, of 0,397 and of 0,501, which related in percentage to the corresponding biochemical parameter, of 1,187, 0,312 and of 0,360 (table 1) determined in the extracts prepared from the proof small roots, represents a more increased enzimatic activity with 26,62% ($p<0,05$) at **wheat**, with 21,47% ($p<0,05$) at **ryegrass** and with 39,17% ($p<0,05$) at **brome grass inaristata** (fig. 1,V_2), significant stimulations from a statistical point of view.

In the case of the experimental variant with watery extract prepared out of metamorphosed root of horse radish, picked up at the time of blossoming of the plant, in concentration of *25%* (V_3), the peroxidasic activity in the embrionic small roots of the plantlets of **brome grass inaristata** was slightly *stimulated*, with 10,28% ($p>0,05$) (fig. 1, V_3), statistically non-significant increases with relation to the proof regarded as 100%, however, comparative to the enzymatic activity from the small roots of these plantlets grown on substratum humidified with watery extract in concentration of 15%, the activity of these enzymes was *diminished*.

In the embrionic small roots of **wheat** and **ryegrass** resulted from the cariopses germinated on substratum humidified with an extract prepared out of metamorphosed root of horse radish, in concentration of *25%* (V_3), the peroxidasic activity was *inhibited,* the extinction being of 1,185 and of 0,300 comparatively with the value of 1,187 and of 0,312 (table 1) which reflects the peroxidasic activity from the proof small roots (V_0), there being registered a minus of 0,17%, respectively of 3,85% (fig. 1, V_3) of the activity of this enzyme, however, insignificant values from a statistical point of view ($p >0,05$). The decrease of the peroxidasic activity in the small roots of the **wheat**, **ryegrass** and **brome grass inaristata** plantlets is due to the phyto-toxic effect of the watery extract prepared out of horse radish root, which is made concrete by brown small roots, which we regard as a beginning of necrosis affecing the other physiological processes.

The superior concentrations, of *50%*, *75%* and of *100%,* of the watery extract obtained out of metamorphosed root of horse radish were entirely inhibiting for all the three vegetative species taken into study, blocking the germination and plantlets growth, reason for which the enzymatic activity could not be determined. This diminishing of growth could be explained by the fact that the small roots, under the effect of phyto-toxic substances contained in the watery extract of metamorphosed root of horse radish have entered a process of senescence, of general stopping of their vital activity.

Conclusively, after *seven days* from placing the seed material to germinate, on substratum moistured with watery extract prepared out of metamorphised root of horse radish picked up at the time of blossoming of the plant, in concentration of *5%* (V_1) and of *15%* (V_2) the peroxidasic activity was *stimulated* in the embrionic small roots of the plantlets of **wheat**, **ryegrass** and **brome grass inaristata,** while the growth in length of the plantlets was strongly inhibited. At a higher concentration of the extract, of *25%* (V_3), the enzymatic activity in the small roots of the plantlets of **wheat** and **ryegrass,** as to the similar parameter determined at the samples prepared from the small roots of the proof plantlets, was inhibited, whereas at **brome grass inaristata** was slightly stimulated.

Stimulating the capacity of peroxidasis indicates high levels of H_2O_2 generated, probably, by the toxicity of the alelo-chemical substances contained in the watery extract obtained from the root of horse radish

picked up at the time of blossoming of the plant and an attempt of the plants of protecting themselves against the oxidative stress.

In the reactions of aerobic oxido-reduction, among other products, oxigenated water is formed as well. For the living cells, the accumulation of oxigenated water is harmful, since the oxignated water is a strong oxidant. The accumulation in the body of H_2O_2 leads to the disturbing of certain metabolic processes, because of the fact that it produces a non-enzymatic oxidation of certain substances with vital functions for the cell. This accumulation is prevented by specific enzymes, such as the peroxidases and catalases. The peroxidases decompose the oxigenated water in products useful to the metabolic processes. The decomposing of the oxigenated water is done in the presence of a hydrogen donor, according to the following scheme:

$$AH_2 + H_2O_2 \rightarrow A + 2H_2O$$

Table 1

The average values of the extinction, revealing the intensity of the *peroxidasic activity* at the proof small roots of **wheat** (*Triticum aestivum* L.), ryegrass (*Lolium perenne* L.) and **brome grass inaristata** (*Bromus inermis* L.), and in those generated from embryos germinated on dampened substratum with watery extract of horse radish root, picked up at the time of blossoming of the plant (*Armoracia rusticana* Lam.), at *seven days* from placing the seed material to germinate (wherein: V_0.-proof lot germinated on filter paper moistured with distilled water; V_1= extract 5%; V_2= extract 15%; V_3= extract 25%; V_4= extract 50%; V_5= extract 75%; V_6= extract 100%)

Variants / Species	V_0	V_1	V_0	V_2	V_0	V_3	V_0	V_4	V_0	V_5	V_0	V_6
Triticum aestivum	1,187	1,398	1,187	1,503	1,187	1,185	-	-	-	-	-	-
	p<0,05		p<0,05		p>0,05		-		-		-	
Lolium perenne	0,312	0,345	0,312	0,379	0,312	0,300	-	-	-	-	-	-
	p>0,05		p<0,05		p>0,05		-		-		-	
Bromus inermis	0,360	0,442	0,360	0,501	0,360	0,397	-	-	-	-	-	-
	p<0,05		p<0,05		p>0,05		-		-		-	

Note: p = significance threshold

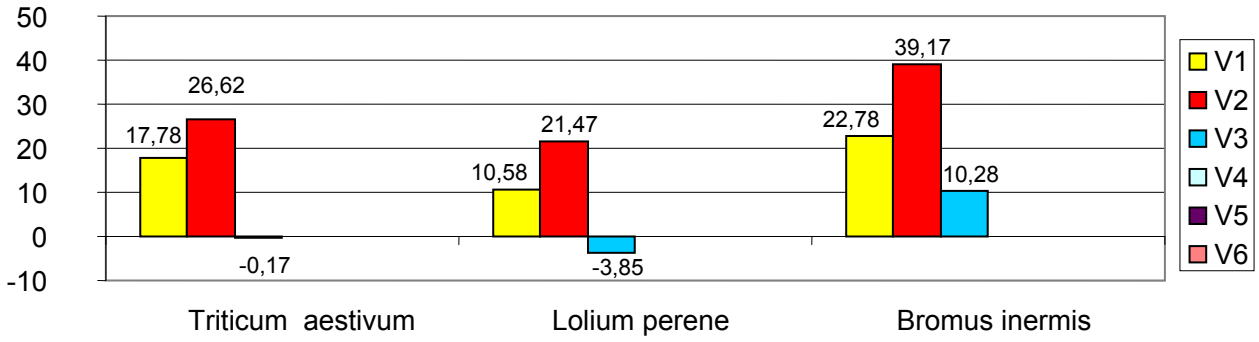

Fig. 1 Expressing in percentage values of positive or negative differences with relation to the proof (in the graph it was marked with 0), of the *peroxidasic* activity in the small roots of **wheat** (*Triticum aestivum* L.), ryegrass (*Lolium perenne* L.) and **brome grass inaristata** (*Bromus inermis* L.), generated from the embryos germinated on substratum moistured with watery extract prepared from metmorphosed root of horse radish picked up at the time of blossoming of the plant (*Armoracia rusticana* Lam.), at *seven days* from placing the seed material to germinate, with relation to the same parameter determined in the proof small roots, lot germinated on distilled water, values considered as reference values, respectively of 100% (wherein; V_1= extract 5%; V_2= extract 15%; V_3= extract 25%; V_4= extract 50%; V_5= extract 75%; V_6= extract 100%)

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The peroxidases present a relatively high specificity towards the acceptors; as a rule the acceptor is H_2O_2, seldom other hydro-peroxides as well, but they are less specific as to the donors. As donors there can function a series of phenols, amino-phenols, diamines, aminoacids etc. (Neamţu et. al., 1983).

CONCLUSIONS

The physiologically active substances contained within the watery extracts obtained out of the metamorphosed root of horse radish – depending of their concentration – causes changes of the peroxidasic activity.

The *peroxidasic* activity in the embrionic small roots of **wheat**, **ryegrass** and **brome grass inaristata** was *stimulated* at concentrations of *5%* and *15%* of the extract, followed by an abrupt decrease of the activity of this enzyme. Intensification of the peroxidasic activity is an attempt of plants to protect themselves against the oxidative stress.

At the concentration of *25%* of the watery extract of horse radish root, the enzimatic activity at **brome grass inaristata** has remained highly increased with relation to the similar parameter determined at the samples prepared out of the small roots of the proof plantlets, whereas at **wheat**, and **ryegrass** it was *inhibited*.

Diminishing the activity of peroxidases is produced, probably, because of the fact that in the anti-oxidative cell system of the acceptor plants studied, the noxious effect of the species reactive to oxygen was not possible to be diminished, having in mind their highly reduced growth.

REFERENCES

Bohm PAF, Zanardo FML, Ferrarese MLL, Ferrarese-Filho O, Peroxidase activity and lignification in soybean root growth-inhibition by juglone. Biologia Plantarum, 2, pp. 315-317, 2006.

Cachiţă CD, Jacobs M, Negruţiu I, Gaspar Th, Comparative effects of procaine and 2,4-D on growth and peroxidases of Arabidopsis callus. Med. Fac. Landbouw. Rijksuniv. Gent., 41, pp. 1521-1526, 1976.

Choesin DN, Boerner REJ, Allyl isothiocyanate release and the allelopathic potenţial of Brassica napus (Brassicaceae). American journal of botany v., 78 (8), pp. 1083-1090, 1991.

Ciofu R, Stan N, Popescu V, Chilom P, Apahidean S, Horgoş A, Berar V, Lauer F K, Atanasiu N, 2003, Plante legumicole perene. În: Tratat de legumicultură, Edit. Ceres, Bucureşti, pp. 984-989.

Delachiave MEA, Rodrigues JD, Ono EO, Efeitos alelopáticoa de Losna (Artemisia absinthium L.) na germinação de sementes de pepino, milho, feijão e tomate. Revista Brasileira de Sementes, 21 (2), pp. 265-269, 1999.

Gaspar Th, Penel Cl, Hagége D, Greppin H, Peroxidases in plant growth, differentiation, and development processes. In: Biochemical, Molecular, and Physiological Aspects of Plant peroxidases. Ed. Lobarzewski, J., Greppin, H., Penel, Cl., Gaspar, Th., Edit. Univ. M. Curie-Sklodowska, Lublin, Poland and Univ. Geneva, Switzerland, pp. 249-280, 1991.

Gaspar Th, Penel Cl, Thorpe T, Greppin H, Peroxidases 1970-1980, A survey of their biochemical and physiological roles in higher Plants. Edit. University of Geneva, 1982.

Gaspar Th, Penel Cl, Tran Thanh Van M, Greppin H, Des isoperoxydases comme marqueurs de la différenciation cellulaire chez les végétaux. Edit. Xéme Rencontre De Meribel, pp. 175-196, 1979.

Lück H, Peroxidaza. In: Methods of Enzymatic Analysis. Ed. Bergmeyer, H.U., Edit. Academic Press New York, pp. 895-897, 1974.

Miró CP, Ferreira AG, Aquila MEA, Alelopatia de frutos de erva-mate (Ilex paraquariensis) no desenvolvimento do milho. Pesquisa Agropecuária Brasileira 33 (8), pp. 1261-1270, 1998.

Neamţu G, Interacţiuni biochimice între plantele superioare. În: Biochimie ecologică. Edit. Dacia, Cluj-Napoca, pp. 146-160, 1983.

Neamţu G, Popescu I, Lazăr ŞT, Burnea I, Brad I, Cîmpeanu GH, Galben T, Enzime. În: Chimie şi biochimie vegetală, Edit. Didactică şi Pedagogică Bucureşti, pp. 245- 246, 1983.

Negruţiu I, Jacobs M, Gaspar Th, Leaf formation and peroxidases from Arabidopsis callus, Z. Pflanzenphysiol., 91, pp. 119-126, 1979.

Pârvu C, Universul plantelor. Edit. Enciclopedică, pp. 209-210, 321, 356-357, 1991.

Steinbach M, Principii de interpretare statistică. În: Prelucrarea statistică în medicină şi biologie. Edit. Academiei Republicii Populare Române, pp. 216-253, 1961.

Thorpe TA, Tran Thanh Van M, Gaspar T, Isoperoxidases in epidermal layers of tobacco and changes during organ formation in vitro. Plant Physiol., 44, pp. 388-394, 1978.

*** Flora Republicii Populare Române, vol. III, Edit. Academiei Republicii Populare Române, pp. 253,1955.

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