# THE ACTIVITY OF SOME OXIDOREDUCTASES IN HORDEUM *VULGARE* L. PLANTS TREATED WITH ETHYL–METHANE-SULFONATE AND *ROSMARINUS OFFICINALIS* L. HYDRO-ALCOHOLIC EXTRACTS

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**Abstract**: This paper focuses on the activity of some oxidoreductases (catalase, peroxidase, superoxide-dismutase) in barley seedlings (*Hordeum vulgare* L.) after 6 hours of seeds treatment with different concentrations (0,01 – 0,50%) of ethyl-methane-sulfonate and 12 hours with hydro-alcoholic 0,5% rosemary (*Rosmarinus officinalis* L.) extract (EHR). The EMS treatments led to an obvious increase of the superoxide dismutase, catalase and peroxidase activity in plants, while the application of the hydro-alcoholic rosemary extract, after the EMS treatment, led to a significant decrease of the activities of these enzymes, since the rosemary extract has an obvious antioxidant effect.

## INTRODUCTION

In order to counteract the oxidative stress, the plants have developed intracellular defense strategies. These strategies are represented by an enzymatic and a non enzymatic antioxidant system. The non enzymatic system includes ascorbic acid, ά-tocopherol, carotenes, polyphenols, flavones and the enzymatic system includes superoxide dismutase, catalase, peroxidase, ascorbate oxidase, glutathione reductase and polyphenol oxidase. The function of these antioxidant systems relies in the prevention of formation or in the destruction of toxic radicals formed during the oxidative stress, thus ensuring the survival of plants in improper conditions.

In the last decades there has been a great interest in emphasizing the antioxidant properties of some medicinal and aromatic plants. The antioxidant effect of some aromatic plants would be the result of the presence of the hydroxyl groups from the phenol compounds, (Shahididi and Wanasundara, quoted by Faixova and Faix, 2008). Among the antioxidant compounds, the polyphenols and the flavones represent the object of various plant studies (Blaschek et al., 2007; Wichtl, 2009; Hasani-Ranjbar et al., 2009).

Some Lamiaceae species, such as rosemary, oregano, sage and others, have a strong antioxidant effect, (Dragland et al., 2003; Wang S. Y., 2003). The antioxidant effect of some rosemary active principles has been proven in various studies concerning the volatile oils, phenol compounds, flavonoides and diterpenes (Armatu et al., 2010; Papageorgiou et al., 2008; Stefanovits-Banyai et al., 2003; Schwarz and Ternes, 1992). Also, the use of the rosemary extracts in food industry is based exactly on their antioxidant effect that prevents the degradation of the food products containing fats (Cuvelier et al., 1996; Fadel and El-Massry, 2000; Schwarz and Ternes, 1992).

On the other hand, the ionizing radiations and some chemical mutagens (such as alkylating agents) lead to a strong oxidative stress in living organisms (Ghiorghiţă and Corneanu, 2002) by over producing of reactive oxygen species (ROS).

Considering these facts, our paper focuses on the capacity of hydro-alcoholic rosemary extracts (EHR) to decrease the oxidative stress induced to the barley plants by the treatment with ethyl-methane-sulfonate (EMS). In this respect, we investigated the activity of some oxidoreductases in barley seedlings after the treatment with EMS and EHR.

## MATERIAL AND METHODS

To complete the experimental part, the barley seeds (*Hordeum vulgare* L.), *Mădălin* cultivar, have been treated for 6 hours with different concentrations (0,01%; 0,025%; 0,05%; 0,10%; 0,50%) of ethyl-methane-sulfonate solutions (EMS). After these treatments, the seeds were well washed in water, in order to remove the mutagen agent, and then treated for 12 hours with hydro alcoholic rosemary (*Rosmarinus officinalis* L.) extracts (EHR).

The rosemary alcoholic extract was obtained at cold, using the plant/solvent report of 1:7 and a concentration of ethanol of 70%. The barley seeds were then treated with a 0,5% diluted solution of the initial extract and, after the germination and the hydroponics cultivation, the 14 days old seedlings were submitted to enzymatic analyses. The activity of some oxidoreductases (superoxide-dismutase, peroxidase and catalase) was evaluated.

The determination of the superoxide-dismutase was done by the method of Winterbourn, Hawkins, Brian and Carrell, adapted by Vlad Artenie, the catalase activity by iodometric titration and the peroxidase by the ortho-dianisidine method (Artenie et al., 2008; Cojocaru et al., 2009). The results were statistically analyzed, a series of statistic indicators being calculated, such as average, standard error and deviation, variation coefficient, average safety coefficient, superior and inferior limits of the confidence intervals. The results of our investigations are presented in Table 1 – 6.

## RESULTS AND DISCUSSIONS

It is well known that, in living organisms, the oxidative stress leads to the production of the reactive oxygen species with severe disruptive effect upon the cellular metabolism and the development of some processes that are often assigned to the alteration of the pattern of gene expression.

In plants, antioxidant enzymes such as superoxide-dismutase, peroxidase and catalase are seen as the „defensive team", playing the role of protecting the cells from the injuries caused by oxidative stress (Mittler, 2002). The increase of the activity of these enzymes represents the most common pathway that leads to the elimination of the reactive oxygen species.

The action of superoxide-dismutase (SOD) consists in converting the superoxide radical in hydrogen peroxide. In our investigations, we observed that the SOD activity obviously increased in barley plants after the single treatments with EMS, regardless the concentration, (Table1), reaching maximum values in case of the samples treated with 0,05 and 0,10% EMS (24,86 - 25,46 USOD/ml/min), as compared to 8,45 USOD/ml/min at control. At higher doses of the mutagen, (0,50%), the SOD activity slightly decreased (21,34 USOD/ml/min).

The intensification of the SOD activity suggests that the EMS has determined the increase of superoxide radicals (O2●-) concentration in the tissues of the barley plants. These radicals are strongly reactive and toxic and must be inactivated Since they cause severe oxidative degradations in the cells, the evolution of all aerobe organisms became dependant to the development of some effective defense mechanisms, meant to remove them.

Table 1. The activity of superoxide-dismutase in the barley seedlings from the EMS treated seeds

| Variant | Medium activity (USOD/ml/min) | S $\bar{x}$ | S (σ) | CV% | m% | LS | LI |
|---|---|---|---|---|---|---|---|
| Control plant | 8,45 | 0,30 | 0,52 | 5,96 | 3,34 | 8,64 | 8,26 |
| 0,01% EMS | 22,45 | 0,29 | 0,51 | 0,45 | 0,26 | 22,64 | 22,27 |
| 0,025% EMS | 22,68 | 0,30 | 0,52 | 0,34 | 0,19 | 22,87 | 22,49 |
| 0,05% EMS | 24,86 | 0,15 | 0,27 | 0,11 | 0,06 | 25,05 | 24,67 |
| 0,10% EMS | 25,46 | 0,91 | 1,58 | 0,42 | 0,24 | 25,65 | 25,27 |
| 0,50% EMS | 21,34 | 0,21 | 0,37 | 3,11 | 1,79 | 21,53 | 21,15 |

S $\bar{x}$ = average standard error, S (σ) = standard deviation, CV% = average variation coefficient, m% = average precision coefficient, LS = superior limit of the coefficient interval, LI = inferior limit of the confidence interval

Table 2. The activity of superoxide-dismutase in the barley seedlings from the EMS and EHR treated seeds

| Variant | Medium activity (USOD/ml/min) | S $\bar{x}$ | S (σ) | CV% | m% | LS | LI |
|---|---|---|---|---|---|---|---|
| Control plant | 8,45 | 0,30 | 0,52 | 5,96 | 3,34 | 8,64 | 8,26 |
| 0,01% EMS +EHR | 9,52 | 0,26 | 0,46 | 4,46 | 2,57 | 11,53 | 7,50 |
| 0,025% EMS +EHR | 9,45 | 0,12 | 0,21 | 3,14 | 1,81 | 11,47 | 7,44 |
| 0,05% EMS +EHR | 10,34 | 0,34 | 0,59 | 0,53 | 0,31 | 12,36 | 8,33 |
| 0,10% EMS + EHR | 10,25 | 0,15 | 0,27 | 0,15 | 0,09 | 12,27 | 8,24 |
| 0,50% EMS +EHR | 9,23 | 0,55 | 0,95 | 0,22 | 0,13 | 11,25 | 7,22 |

S $\overline{x}$ = average standard error, S (σ) = standard deviation, CV% = average variation coefficient, m% = average precision coefficient, LS = superior limit of the coefficient interval, LI = inferior limit of the confidence interval

The hydro-alcoholic rosemary extract (EHR) added after the mutagen treatments determined a normalization of the SOD activity in case of all the treatment variants, which makes us appreciate that the EHR had a clear antioxidant effect. The average intensity of the SOD activity varied in case of the combined treatments (EMS + EHR) between 9,23 and 10,34 USOD/ml/min, compared to 8,45 USOD/ml/min in control plants, (Table 2). The role of SOD in the cells was probably taken in this case by other defense mechanisms against superoxide radicals.

For each treatment variant, three parallel determinations were made and the results were statistically analyzed. Thus, the average, the standard error and deviation, the average and safety coefficient there were calculated, as well as the limits of the confidence intervals that, as the values show, are pretty close, which makes us conclude we have kept constant the extraction and determination conditions.

Another objective of this study was the determination of the catalase activity in plants, considering the fact that the value of this biochemical parameter also changes in the oxidative stress.

The results show an increase of the catalase activity in the barley plants after single EMS treatments, which suggests the presence of important amounts of hydrogen peroxide in the plants.

The activity of catalase is 2-3 times bigger than in the untreated plants (8,54 mg $H_2O_2$/g/min). Although different EMS concentrations (0,01-0,5% EMS) have been used, the differences in the catalase activity are not significant, varying between 20,37 mg $H_2O_2$/g/min (the sample treated with 0,05% EMS) and 26,69 mg $H_2O_2$/g/min (the sample treated with 0,50% EMS), (Table 3).

Generally, at high concentrations of mutagen solutions (0,05 - 0,50%), we identified a high catalase activity (24,65 – 26,69 mg H2O2/g/min).

Table 3. The activity of catalase in the barley seedlings from the EMS treated seeds

| Variant | Medium activity (mg $H_2O_2$/ml/30min) | S $\overline{x}$ | S (σ) | CV% | m% | LS | LI |
|---|---|---|---|---|---|---|---|
| Control plant | 8,54 | 0,08 | 0,14 | 4,93 | 2,84 | 8,66 | 8,41 |
| 0,01% EMS | 23,29 | 0,05 | 0,08 | 0,92 | 0,53 | 23,33 | 23,24 |
| 0,025% EMS | 20,37 | 0,29 | 0,51 | 4,16 | 2,40 | 20,52 | 20,21 |
| 0,05% EMS | 26,35 | 0,37 | 0,65 | 2,26 | 1,30 | 26,77 | 25,92 |
| 0,10% EMS | 24,65 | 0,03 | 0,09 | 1,83 | 1,06 | 24,69 | 24,60 |
| 0,50% EMS | 26,69 | 0,32 | 0,56 | 11,67 | 6,74 | 26,84 | 26,53 |

S $\overline{x}$ = average standard error, S (σ) = standard deviation, CV% = average variation coefficient, m% = average precision coefficient, LS = superior limit of the coefficient interval, LI = inferior limit of the confidence interval

Table 4. The activity of catalase in the barley seedlings from the EMS and EHR treated seeds

| Variant | Medium activity (mg $H_2O_2$/ml/30min) | S $\overline{x}$ | S (σ) | CV% | m% | LS | LI |
|---|---|---|---|---|---|---|---|
| Control plant | 8,54 | 0,08 | 0,14 | 4,93 | 2,84 | 8,66 | 8,41 |
| 0,01% EMS +EHR | 6,29 | 0,23 | 0,41 | 1,41 | 0,81 | 6,54 | 6,03 |
| 0,025% EMS +EHR | 6,63 | 0,44 | 0,76 | 1,11 | 0,64 | 7,65 | 5,60 |
| 0,05% EMS +EHR | 7,31 | 0,34 | 0,59 | 1,43 | 0,82 | 8,39 | 6,22 |
| 0,10%+ EMS EHR | 7,31 | 0,19 | 0,33 | 0,77 | 0,44 | 7,56 | 7,05 |
| 0,50% EMS +EHR | 7,31 | 0,11 | 0,19 | 1,23 | 0,71 | 8,39 | 6,28 |

S $\overline{x}$ = average standard error, S (σ) = standard deviation, CV% = average variation coefficient, m% = average precision coefficient, LS = superior limit of the coefficient interval, LI = inferior limit of the confidence interval

The administration of the hydro-alcoholic rosemary extract (EHR) after the EMS treatment induced an important decrease in the activity of catalase, the values being very close to those registered in the case of the untreated plants. So, considering a catalase activity of 8,54 mg $H_2O_2$/g/min (in the control plants), in case of combined treatments (EMS and EHR), the catalase activity oscillated only between 6,29 and 7,31 mg $H_2O_2$/g/min, (Table 4). This behavior can only be explained by the intervention of the antioxidant compounds from the hydro-alcoholic rosemary extract and their scavenger quality.

Another enzyme whose activity has been analyzed in our investigations was peroxidase, enzyme that plays an important role in the detoxification processes, being a regulator of the electronic flow in the cell respiration, but also a „trap" of the free radicals. As we know, peroxidase appears in case of lower quantities of $H_2O_2$, while catalase is stimulated by higher quantities of this oxidizing agent.

After the EMS treatments, the peroxidase activity increased in the barley seedlings, being about 3 times higher than the untreated plants. Thus, compared to 0,84 UP/g/min in the case of the control, the EMS treated samples had an intensity of the peroxidase activity between 2,54 and 2,71 UP/g/min, (Table 5).

As the analysis of the experimental results shows, the concentration of the mutagen agent did not sensible affect the activity of peroxidase, the differences between the variants being non significant, which makes us suppose that the stress caused by the application of the mutagen agent was extremely strong in all the experimental concentrations.

Table 5. The activity of peroxidase in the barley seedlings from the EMS treated seeds

| Variant | Medium activity (UP/g/min) | S $\overline{x}$ | S (σ) | CV% | m% | LS | LI |
|---|---|---|---|---|---|---|---|
| Control plant | 0,84 | 0,13 | 0,22 | 1,21 | 0,70 | 0,98 | 0,69 |
| 0,01% EMS | 2,54 | 1,72 | 2,99 | 10,60 | 6,12 | 3,50 | 1,59 |
| 0,025% EMS | 2,67 | 0,11 | 0,19 | 3,22 | 1,86 | 3,69 | 1,64 |
| 0,05% EMS | 2,71 | 1,66 | 2,89 | 2,25 | 1,30 | 3,28 | 2,14 |
| 0,10% EMS | 2,65 | 0,87 | 1,51 | 1,23 | 0,71 | 3,67 | 1,62 |
| 0,50% EMS | 2,55 | 0,38 | 0,67 | 1,73 | 1,00 | 3,58 | 1,51 |

S $\overline{x}$ = average standard error, S (σ) = standard deviation, CV% = average variation coefficient, m% = average precision coefficient, LS = superior limit of the coefficient interval, LI = inferior limit of the confidence interval

For all the analyzed samples we calculated, according to the average values and the standard deviation, the superior and inferior limits of the confidence intervals, based on the critical value t (α, n-1), given by α = 0,05 and n-1 freedom degrees.

From the analysis of the values obtained for the variability intervals of the peroxidase, we can conclude that they generally have pretty small limits, the highest confidence intervals being noticed at the concentrations of the alkylating agent of 0,025%, and 0,1% (1,64 - 3,69 UP/g/min., respectively 1,62 - 3,67 UP/g/min.), while the tightest interval was at the untreated variant (0,69 – 0,98 UP/g/min).

Table 6. The activity of peroxidase in the barley seedlings from the EMS and EHR treated seeds

| Variant | Medium activity (UP/g/min) | S $\overline{x}$ | S (σ) | CV% | m% | LS | LI |
|---|---|---|---|---|---|---|---|
| Control plant | 0,84 | 0,13 | 0,22 | 1,21 | 0,70 | 0,98 | 0,69 |
| 0,01% EMS +EHR | 0,64 | 1,23 | 2,13 | 3,13 | 1,81 | 0,69 | 0,58 |
| 0,025% EMS +EHR | 0,62 | 0,47 | 0,82 | 1,11 | 0,46 | 0,85 | 0,38 |
| 0,05% EMS +EHR | 0,37 | 3,97 | 6,88 | 1,42 | 0,82 | 0,49 | 0,24 |

| Variant | Medium activity (UP/g/min) | S $\overline{x}$ | S (σ) | CV% | m% | LS | LI |
|---|---|---|---|---|---|---|---|
| 0,10%+ EMS EHR | 0,54 | 0,26 | 0,45 | 0,53 | 0,30 | 0,54 | 0,54 |
| 0,50% EMS +EHR | 0,69 | 1,70 | 2,95 | 3,57 | 2,06 | 0,72 | 0,66 |

S $\overline{x}$ = average standard error, S (σ) = standard deviation, CV% = average variation coefficient, m% = average precision coefficient, LS = superior limit of the coefficient interval, LI = inferior limit of the confidence interval

As well as the other two investigated enzymes, after the combined treatment (with EMS and EHR), the peroxidase activity registered much reduced values. While the peroxidase activity in the control plants was 0,84 UP/g/min, the enzyme activity in the rosemary extract treated samples after the EMS treatment had values between 0,37 (0,05% EMS + EHR) and 0,69 UP/g/min (0,50% EMS + EHR), (Table 6). The effect of the EHR upon the peroxidase activity did not depend on the EMS concentration associated with.

## CONCLUSIONS

The investigations related to the effects of the treatments with ethyl-methane-sulfonate (EMS) and the hydro-alcoholic rosemary extracts (EHR) on the activity of some oxidoreductases in barley plants (*Mădălin* cultivar) have led to the following conclusions:

EMS treatments produced an obvious increase in the superoxide-dismutase, catalase and peroxidase activities in plants, as a consequence of the oxidative stress caused by the mutagen agent.

EHR administration after the EMS treatments led to an obvious reduction of the activities of the analyzed enzymes in the barley plants. The similarity with enzyme activities in the control plants, in this case, shows a clear antioxidant effect of the compounds present in the hydro-alcoholic rosemary extract.

The EHR effect on the activity of the investigated enzymes did not evidently vary according to the concentration of the ethyl-methane-sulfonate solutions, probably because the oxidative stress was extremely aggressive even after the administration of small doses of the alkylating agent.

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