ACTION OF CAFFEINE AND SODIUM AZIDE ON ACTIVITY OF SOME ANTIOXIDATIVE ENZYMES IN *Carum carvi* L. SEEDLINGS

ELENA MAXIM[1], LĂCRĂMIOARA OPRICĂ[2], GABRIELA CĂPRARU[3], ELENA TRUŢĂ, VLAD ARTENIE[2]

Keywords: catalase, peroxidase, superoxide dismutase, caffeine, sodium azide

Abstract: Caffeine and sodium azide treatments determine variations of oxidative stress implied enzymes, depending both on used concentration and treatment duration. The catalase activity intensifies in 6 hours treatment, in direct relation with caffeine concentration increase, fact proving the accumulation of a bigger amount of hydrogen peroxide that it must be neutralized by catalase. Peroxidase do not displays significant variations in the applied treatments, while superoxide dismutase has a more intense activity in 12 hours treatment.

INTRODUCTION

Oxygen radicals are among the most recognized and widely investigated mediators of cell damage (Kehrer, 1993). Chemical species such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-), and nitric oxide (NO) derived from incomplete reduction of molecular oxygen are highly reactive toward key constituents of living cells, including DNA, proteins, and lipids. Genotoxicity, protein denaturation, compromised enzymatic activities, and lipid peroxidation all represent consequences of cell exposure to excess amounts of reactive oxygen species (ROS), a condition usually indicated as oxidative stress. Both exogenous oxygen radicals and oxygen species endogenously derived as a byproduct of mitochondrial respiration, drug metabolism, or any other intracellular redox reaction can exert deleterious effects on cell function and viability, depending on cellular antioxidant defences and capability to repair oxidative damage (Galeotti et al., 1990).

The plants have specific enzymes, known as *antioxidative enzymes* because their ability to annihilate ROS negative effect. The plant antioxidative enzymes are the catalase (CAT), superoxide dismutase (SOD), peroxidase (POX), ascorbate peroxidase (APX), and glutathione S transferase (GST). Out of destructive effect generated by ROS in plants, these radicals seem also to have a critical role in transduction signals. These are the reasons for which it is very important to maintain the equilibrium between the formed free radicals and those removed/annihilated.

In this paper, our objective is to evidence the activity of most important enzymes of oxidative stress, namely catalase, peroxidase, and superoxide dismutase.

Catalase is universally found in nature, its activity being evidenced in all aerobic micro organisms, in plant and animal cells. Catalase diminishes the hydrogen peroxide level in peroxisomes, but it is missing in chloroplasts. Hydrogen peroxide is the most stable between reactive oxygen species, because it is a very strong nucleophyllic oxidant. It is responsible for enzyme inhibition in Calvin pathway.

Peroxidase (donor: hydrogen peroxide-oxidoreductase) is a bicomponent enzyme belonging to hemoproteins. It accomplishes a special role in oxidoreducing processes associated with plant and animal respiration and it catalyzes the oxidation of several substrata of determined chemical nature, in the presence of hydrogen peroxide.

Superoxide dismutase (superoxide oxidoreductase, SOD) catalyzes the dismutation of superoxide free radical, O_2^- , in oxygen and hydrogen peroxide.

$$2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$$

SOD is an antioxidant enzyme protecting living cells against noxious effects of superoxide free radicals which are one of the most noxious forms of reactive oxygen species. The superoxide radical is generated by the O_2 univalent reduction during enzymatic reactions or under the action of ionizing rays. Because of the fact that SOD is present in all aerobic organisms and in almost all subcellular compartments that generate activated oxygen, this oxidoreductase is considered as having a central role in the protection against oxidative stress associated with generation of reactive oxygen species.

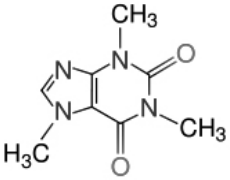
Three different types of SOD were identified and classified depending on metal present in their structure. Certain Cu (II) – Zn (II) – SOD isozymes were discovered in cytosol and in chloroplasts of evolved plants. Mn (II) – SOD is localized in mitochondria of eukaryotic cells, while the iron containing enzyme was found in bacteria and plants.

MATERIAL AND METHODS

The biological material was represented by 21 days old caraway seedlings resulted by germination of the seeds treated with caffeine (0.10%; 0.25%; 0.50%; 1.00%) and sodium azide (10^{-7}, 10^{-5}, 10^{-3} M/l), for 6 and 12 hours. The

determination of catalase activity was spectrophotometrically performed (Sinha method). Peroxidase was determined by method of L.V. Gudkova and R.G. Degtiari, and superoxide dismutase activity was estimated by the method of Winternbour, Hawkins, Brian and Carrell, modified by Artenie (Artenie et al., 2008).

Caffeine ($C_8H_{10}N_4O_2$) is a white powder with density=1.23, molecular weight=194.19, moderate solubility in organic solvents, and melting point 234-239^{0}C. It has the following chemical structure:



Sodium azide (NaN_3) is a chemical mutagen that directly acts on resting nucleic acids (Butnaru, 1985). It has the following chemical structure:

$$N\equiv N^{+}—N^{-2}Na^{+}$$

RESULTS AND DISCUSSIONS

Together with deoxyadenine, 5-iododeoxyuridine, cytosine arabinoside, 5-brom-uracil, 5-iodo-uracil, teophylline, teobromine, nebularine, the caffeine belongs to the analogues of nitrogenous base, these compounds having the characteristics to substitute adenine and guanine and to be incorporated in DNA macromolecule, so provoking errors. At the next replication, the initial nitrogenous base will be changed with an analogue base, as result of some incorporation errors. These compounds act during period of nucleic acid replicative biosynthesis. The mutagenic effects of caffeine have been studied in prokaryote and eukaryote organisms, but the results were very different, inciting to controversies and discussions. Concerning the mutagenicity, in literature, even the results for the same organism are occasionally antagonistic (Laranja et al., 2003). Caffeine induced mutations were evidenced in bacteria, fungi, insects, as well as chromosome aberrations in onion, *Vicia faba*, hemp, human tissue cultures.

The testing of caffeine effects in various biological systems not resulted in identical data. For example, this purine derivative (methyl xanthine) determined the increase of dividing cells frequency and of ana-telophase chromosome aberration frequency in some species of cereals and technical plants. In dill, the caffeine treatment induces a slight diminution of mitotic index and an increase of the incidence of aberration containing cells (Maxim et al., 2009a). The caffeine action mechanism is not entirely deciphered. It is possible that like other methylated purines caffeine act as a solubilizing agent, able to form molecular complexes , because of the fact that a close relationship exists between the solubilizing potential of a chemical compound and its ability to produce chromosome aberrations.

NaN_3 is known as a strong mutagen agent in cereals and vegetables, as a respiration inhibitor, a catalase and peroxidase inhibitor, but a stimulant factor of germination in cultivated barley (Lenoir et al., 1986). Sodium azide do not has mutagen effect and do not induce sterility in *Arabidopsis*, but in combination with N-nitroso-N-methyl-N'-nitrosoguanidine, N-nitroso-N-methyluretane, tri-2-chlorethylamine, X rays, this compound causes numerous mutations and determines *Arabidopsis* sterility. Sodium azide is considered an effective and efficient mutagen in barley, it inducing preponderantly point mutations (Kleinhofs et al., 1978). In caraway, sodium azide determines a decrease of frequency of mitotic cells and an increase of the number of cells with chromosome aberrations (Maxim et al., 2009b), while in *Secale cereale* this substance induce micronuclei formation (Kaymak, 1994).

1. Catalase activity

Caffeine treatment applied for 6 h exerted an intensification of catalase activity proportionally with alkaloid concentration increase, the maximum value being registered in 1 % caffeine variant that is almost two times higher than the control (32.9881 comparatively to 16.8331). The extension of the treatment to 12 h determines an unsignificant increase of catalase activity in 0.25 % caffeine treated variant (with approximately 3 % comparatively to control), the other variants showing slightly lower values or close o those registered for control. In the variant treated for 12 h with the highest caffeine concentration (1%), the seed germination was inhibited in a very high degree and the biological material was not in a sufficient quantity to realize these biochemical determinations (Fig. 1).

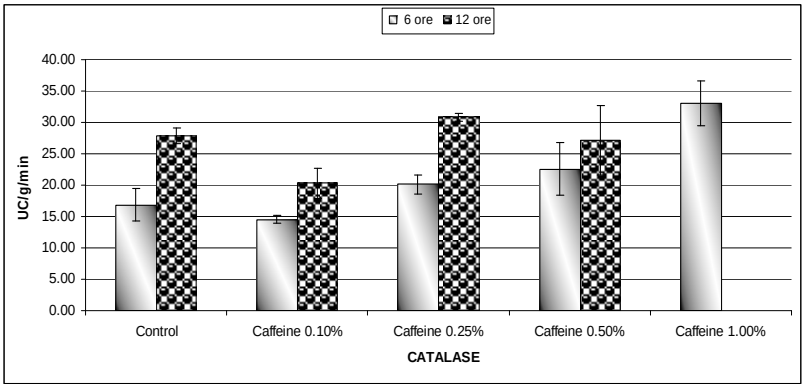


Fig. 1. Variation of catalase activity in caraway seedlings after caffeine treatment

For sodium azide, the catalase activity presented variations depending especially on treatment duration. Thus, after 6 h treatment, the enzyme intensity is superior to control (10^{-7}M/l, 10^{-5} M/l) or close to its value (10^{-3}M/l). On the contrary, in 12 h treatment, the catalase presented an activity inferior to that of control in all treatment variants (Fig. 2).

The increase of catalase activity is a consequence of a more intense H_2O_2 generation, reason for which it is necessary to assure a continuous monitoring of the concentration of this metabolite by a higher catalase activity.

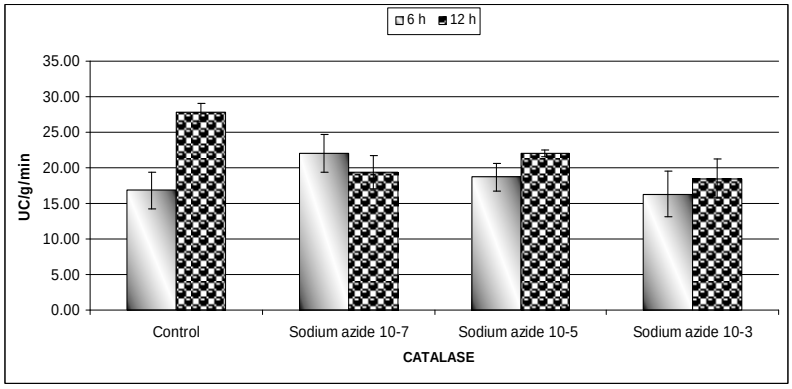


Fig. 2. Variation of catalase activity in caraway seedlings after sodium azide treatment

2. Peroxidase

As shown in Fig. 3, in 6 h control and in 12 h control, the peroxidase activity registers very close values, namely 9.9499 UP/g/min, respectively 10.0421 UP/g/min. In caffeine treatment, the highest increase of peroxidase activity is noted in 0.25 % caffeine treatment, both in 6 h and 12 h treatment. Also, in sodium azide treatment, the peroxidase activity not varies in a marked manner comparatively to control (Fig. 3 and 4). This behaviour can be a result of the high lability of plant peroxidases in conditions of heating, lighting or even centrifugation.

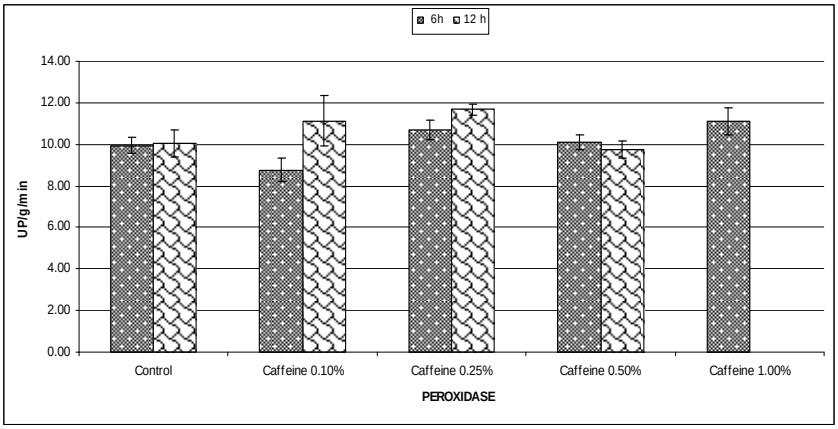


Fig. 3 Variation of peroxidase activity in caraway seedlings after caffeine treatment

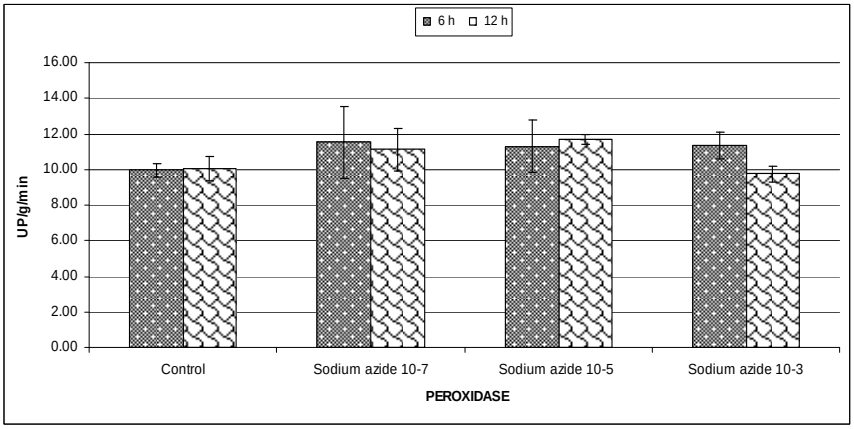


Fig. 4. Variation of peroxidase activity in caraway seedlings after sodium azide treatment

3. Superoxide dismutase

Superoxide dismutase realizes a control of superoxide anions level by preventing the starting of reactions generating noxious hydroxyl radicals and peroxynitrites (formed in reaction between superoxide anions and nitric oxide). The utilization of caffeine for 6 h determined, comparatively to control, the diminution of superoxide dismutase the exception being registered at minimum tested concentration of the alkaloid (0.1%). In the 12 h treated variant an increase of SOD

activity was induced, fact showing the generation of a more increased amount of noxious hydroxyl radicals which must be inactivated (Fig. 5).

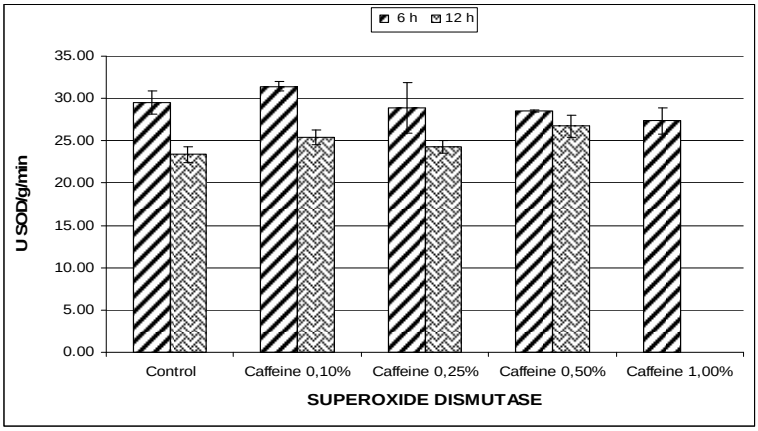


Fig. 5. Variation of superoxide dismutase activity in caraway seedlings after caffeine treatment

Similar behaviour was evidenced in the case of sodium azide. In 6 h treatment, the SOD activity is smaller that than of control, excepting 10^{-3} M/l variant, while after the 12 h treatment, in all used concentrations, SOD registers values superior to control (Fig.6).

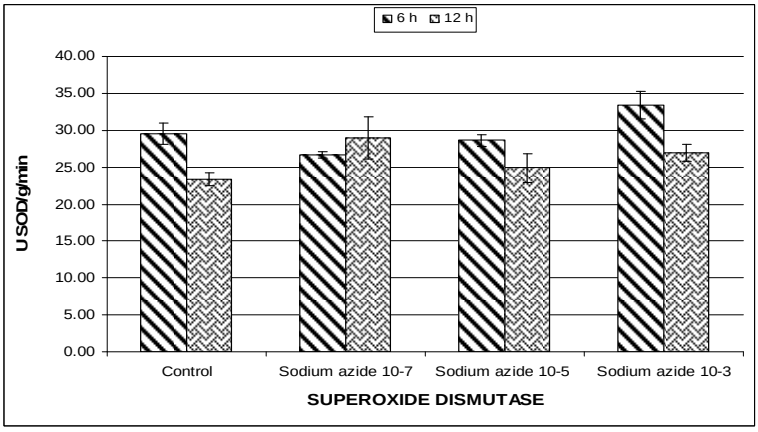


Fig. 6. Variation of superoxide dismutase activity in caraway seedlings after sodium azide treatment

Several studies (Olinescu, 1982; Simon and Dean, 1990) evidenced the competition existing between superoxide dismutase, catalase and glutathione peroxidase, in neutralization of H_2O_2 excess and of other peroxides, these enzymes intervening in regulatory systems of feedback type. The H_2O_2 generation is rigorously controlled in plant organism by the intervention of three

enzymes – catalase, glutathione peroxidase and other peroxidases, all having a very high reaction speed (Laszlo and Adam-Vizi, 2000).

CONCLUSIONS

The activity of the three antioxidative enzymes is variable depending both of utilized substance and tested concentrations.
Significant increases of catalase activity are registered in caffeine treatments, generally proportionally with the concentration increase.
Peroxidase do not significantly modifies probably because of the high lability of plant peroxidases in conditions of heating, lighting or even centrifugation.
Sodium azide caused a non significant increase of the three analyzed enzymes, this substance exerting a smaller oxidative stress for caraway plants. This behaviour can be correlated with the utilization of high dilutions of sodium azide or with the presence of some neutralization systems more efficient than those existing for caffeine.

REFERENCES

Artenie, V., Ungureanu, E., Negură, A. M., 2008. *Metode de investigare a metabolismului glucidic şi lipidic - manual de lucrări practice*, Ed. Pim, Iaşi.
Butnaru, G., 1985. *Genetica*, Editura Institutului Agronomic, Timişoara.
Galeotti, T., Borrello, S., Masotti, L., 1990. In: D. K Dass and W. B. Essman (eds.), *Oxygen Radicals: Systemic Events and Disease Processes*: 129 – 148.
Kehrer, J., 1993. *Crit. Rev. Toxicol*., 23, 21 – 48.
Kleinhofs, A., Owais W. M., Nilan R. A., 1978. *Mutat. Res*., 55, 165 - 195.
Laranja, A. T., Manzatto, A. J., Bicudo H. E. M. C., 2003. *Genet. Mol. Biol*., 26(4), 419 – 429.
Laszlo, T., Adam-Vizi, V., 2000. *Journal of Neuroscience*, 29, 8972-8979.
Lenoir, C., Corbineau, F., Côme, D., 1986. *Physiologia Plantarum*, 68(2), 301 - 307.
Maxim, E., Căpraru, G., Cîmpeanu, M. M., 2009a. *An. Şt. Univ. "Al. I. Cuza"*, Iaşi (serie nouă), Gen. Biol. Molec., tom X, fsc.1: 9 - 12.
Maxim, E., Căpraru, G., Axente F. M., 2009b. *An. Şt. Univ. "Al. I. Cuza"*, Iaşi (serie nouă), Gen. şi Biol. Molec., tom X, fsc.1: 13 - 16.
Olinescu, R., 1982. *Peroxidarea în chimie, biologie şi medicină,* Ed. Ştiinţifică, Bucureşti.
Simon, J. A., Dean, R. T., 1990. Free Radic. Res. Commun., 10, 303.

1 - "Mihai Eminescu" School, Vaslui
2 - Biological Research Institute, Iaşi
3 - "Alexandru Ioan Cuza" University, Iaşi
* - gabrielacapraru@yahoo.com