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THE STUDY OF *ASTRAGALUS ONOBRYCHIS* VAR. *CHLOROCARPUS* (GRIS.) STOJ. ET STEF. (LEGUMINOSAE) ANTIOXIDANT SYSTEM

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Abstract: Live organisms during evolution have developed the defence system against toxic oxygen forms. Plant antioxidant system constitutes antioxidant enzymes [Superoxide Dismutase (SOD), Peroxidase (P), Catalase (CAT), etc.] and antioxidant compounds [Glutathione (GSH), flavonoids, tocopherols, ascorbic acid, pigments, etc.]. The aim of the research was to investigate the content of lipid peroxidation (LP), GSH and total flavonoids, as well as the selenium accumulation and distribution in overground part of *Astragalus Onobrychis* Var. *Chlorocarpus* (Gris.) Stoj. et Stef. (Leguminosae). Finally, a survey of some enzymes [SOD, P and Glutathione Peroxidase (GSH-Px)] has been given.

Introduction

The occurrence of oxygen in atmosphere caused the enveloping of live organism protection mechanism, which make impossible oxygen penetration from outside into a cell. It is well known that oxygen can be toxical in the following forms: reduced, activated, sometimes even molecular form [1]. These oxygen forms can cause metabolic disorders in each live cell and their death, in some cases. Oxygen toxic forms have bad influence on different plant tissue, specially on chloroplaste [2]. Polyunsaturated fatty acids, present in chloroplaste are easily oxidised by reduced and activated oxygen forms [3]. In the course of lipid peroxidation many gas products can occur (pentane, ethane, ethene), as well as the products with aldehyde group (malonaldehyde, hydroxytransnoenale) [4]. These products can be connected with appropriate protein groups and perform protein inhibition [5].

Antioxidant enzymes have a primary role in regulation of reduced form oxygen amount. In this work three enzymes will be considered: SOD, P and GSH-Px. They catalase following reactions [6]:

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$$O_2^{\cdot} + O_2^{\cdot} + 2H^+ \rightarrow H_2O_2 + O_2 \quad (1)$$

$$\text{Donor} + H_2O_2 \rightarrow \text{Oxidised donor} + H_2O \quad (2)$$

$$ROOH + 2GSH \rightarrow ROH + H_2O + GSSG \quad (3)$$

The most important compounds that are in the second plant defence line are: GSH, flavonoids, tocopherols, ascorbic acid, carotenoids, etc. GSH prevents enzymes thiol group's oxidation [7]:

$$2GSH + 1/2O_2 \rightarrow GSSG + H_2O \quad (4)$$

Glutathione can react with H_2O_2 [8]:

$$H_2O_2 + 2GSH \rightarrow GSSG + 2H_2O \quad (5)$$

It has been shown that LP can be inhibited by flavonoids acting as strong $O_2^{\cdot}$ scavengers [9]:

$$ROO^{\cdot} + AOH \rightarrow ROOH + AO^{\cdot} \quad (6)$$

This research has two aims. One is an introduction of the plant antioxidant system. The other is considering of possibility for obtaining a natural plant antioxidants and their utilisation in medicine [10].

Experimental

General. All spectrophotometric determinations were taken on Perkin Elmer lambda 15 spectrometer. Selenium level was determined by elecrothermal atomic absorption spectrometry (AAS-ETA), using a Perkin Elmer M-1100 atomic absorption spectrophotometer.

Plant material. *Astragalus Onobrychis* Var. *Chlorocarpus* (Gris.) Stoj. et Stef. (Leguminosae) fresh and air-dried leafs were collected in the May 1993., October 1994., and Jun 1995., on Seličevica mountain (near Doljevac).

Measurement of total flavonoids. Air-dried plant material (from Jun 1995.) was extracted (1 hour) with mixture (wather-methanol-atcetic acid). After filtration, extract was diluted. Solution of $AlCl_3$ was prepared by dissolving 0,001 mole in 100 ml of deionization water. The yellow complex was monitoring at the wavelength of 430 nm, using quercetin dehydrate as the standard.

Measurement of selenium content. Plant material from May 1993. was use for selenium content determination. Samples were prepared as previously described [11].

Enzymes assay. For estimate of plant antioxidant availability leafs from October 1994. were used. The activities of antioxidant enzymes as well as the level of LP and GSH have been determined by spectrophotometry, using methods given in literature [12-16].

Results and discussion

The activity of SOD, P and GSH-Px in leaf of *A. onobrychis*. Var. *Chlorocarpus* L. (the October 1994.) is listed in Table 1.

Table 1. Activities of SOD, P and GSH-Px (U/ml)

SOD	P	GSH-Px
48,8-53,2	10,9-12,8	2,0-2,4

On the base of the results (Table 1), one can say that leaf of the researched plant is not in danger by O_2^- radical. The similar results were obtained by Gašić and co-workers [17]. This radical is normal metabolite of plant cells and is kept at low steady-state levels by the action of Superoxide Dismutase. Low values of Peroxidase activity (10,9-12,8 U/ml) point that there is no substrate for peroxidation. Gašić and co-workers, on the base of their research concluded: Enzyme Peroxidase is very useful for demonstrate expansion or diminish tendency of any plant genus. Namely, low values of Peroxidase activity according to expansion tendency and contrary. Investigated species shows expansion tendency [18] what correspond to low Peroxidase activity.

Table 2. Selenium accumulation and distribution of *A. onobrychis*. Var. *Chlorocarpus* L. (μg/kg)

Flower	Leaf	Stalk
178,0	152,8	115,4

The activity of the third antioxidant enzyme, GSH-Px has been higher in comparison to the literature [17]. It can be explained in this way: in our plant the content of selenium (cofactor of GSH-Px) is higher (Table 2.).

Results of GSH, LP and total flavonoids content determination are given in Table 3.

Table 3. The content of GSH (μmole/ml), LP (μmole/g) and total flavonoids (mg/100g, dry wt) in leaf of *A. onobrychis*. Var. *Chlorocarpus* L.

GSH	LP	Total flavonoids
2,1-2,4	48,8-56,4	70

The content of GSH, another scavenger of superoxide radical, is not high [19], what can be proof that examined plant is adapted to ecology of natural habitats. This fact is in connection with level of LP. Our results are similar to those in literature [19]. The content of total flavonoids points that *A. onobrychis* Var. *Chlorocarpus* L. is a typical flavonoid plant and can be used for obtaining of phenol compounds.

Conclusion

Plant antioxidant defence system constitutes two subsystems: antioxidant enzymes and nonencymatic compounds. On the base of the our results it can be concluded that the defence system of *Astragalus Onobrychis* Var. *Chlorocarpus* (Gris.) Stoj. et Stef. (Leguminosae) against toxic oxygen forms successfully functions. Also, on the base of the amount of total flavonoids, it can be said that the researched plant is a potential source for obtaining natural antioxidants (in form of phenol compounds)

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PROUČAVANJE ANTIOKSIDANTNOG SISTEMA BILJNE VRSTE ASTRAGALUS ONOBRYCHIS VAR. CHLOROCARPUS (GRIS.) STOJ. ET STEF. (LEGUMINOSAE)

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Sadržaj: U radu je proučavan antioksidantni sistem biljne vrste Astragalus Onobrychis Var. Chlorocarpus (Gris.) Stoj. et Stef. (Leguminosae). Određena je specifična aktivnost antioksidantnih jedinjenja (SOD, P I GSH-Px), zatim sadržaj GSH i ukupnih flavonoida, kao i nivo lipidne peroksidacije. Konačno, ispitivana je akumulacija i distribucija selena u biljci.