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Plant Physiology

Activity of Peroxidase, Catalase and Content of Total Proteins in Leaves of some Herbaceous Plants of High Mountains of the Caucasus

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ABSTRACT. The influence of environmental conditions on the activity of antioxidative enzymes peroxidase and catalase and on the content of total protein in leaves of medicinal herbs: jointweed (*Polygonum alpinum* All.), hellebore (*Veratrum lobelianum* Bernh.), St. John's wort (*Hypericum perforatum* L.), valerian (*Valeriana tiliifolia* Troitzki), and sorrel (*Rumex crispis* L.), growing at different altitudes of the Minor Caucasus (1750 m a. s. l. – lower forest zone, and 2450 m a. s. l. – alpine zone), and in Kazbegi region (Central Caucasus, 1750 m a. s. l.), has been investigated. In extreme conditions of high mountains the change of enzymatic activity was revealed as a specific peculiarity of plants. In the alpine zone of the Minor Caucasus the activity of peroxidase was higher in comparison with the lower timber zone species, and the tendency of protein accumulation in leaves was evident. In all tested plants high activity of enzyme catalase was revealed. The comparatively dry climate and heightened UV radiation of Kazbegi region caused an increase of the content of total proteins in plants, in comparison with lower forest and alpine zone species of the minor Caucasus. The activation of protein synthesis in plants, in combination with the antioxidative system, plays a protective role under the stressful conditions of high mountains. *© 2011 Bull. Georg. Natl. Acad. Sci.*

***Key words**: altitudinal gradient, peroxidase, catalase, total proteins*

High mountain environmental conditions characterized by heightened intensity of solar ultraviolet (UV) radiation, sharp seasonal and daily differences of temperature, low atmospheric pressure, decreased concentration of CO_2, short period of vegetation, etc. [1] lead to the accumulation of chemically active molecules and free radicals in plant cells, capable of changing the direction of metabolic process [2,3]. However, plants possess sufficient stability, thanks to their highly active antioxidant system, which inhibits free radical processes [4, 5]. It is shown that under extreme conditions the protective mechanism of antioxidant system is activated. The higher is the antioxidant activity, the more resistant is the species toward the stressor [6].

The protective antioxidant system consists of high and low-molecular substances [5]. Enzymes peroxidase and catalase are high-molecular, which are capable of eliminating the hydrogen peroxide formed during nonenzymatic or enzymatic dismutation [3].

The solar UV radiation increases proportionally with the altitude. The greatest part of UV radiation is absorbed by cells and may cause heavy damage of biomembranes and different biomolecules [7]. It is accompanied by changes of the secondary metabolism of plant tissues

including accumulation of crioprotective proteins (polyamines), which protect cellular structures and DNA from damage [8]. It is established that increased doses of UV radiation inhibit the synthesis of the majority of ordinary proteins, whereas synthesis of «stress» ones is induced [9, 10].

The purpose of the present paper was to study the influence of environmental conditions of Caucasian high mountains on the activity of antioxidative enzymes peroxidase and catalase, as well as to determine the total proteins in leaves of medicinal herbs.

Investigations were performed at different altitudes of the Minor Caucasus – the environs of v. Bakuriani (1750 m a. s. l., lower forest zone) and Tskhra Tsqaro pass (2450 m a. s. l., alpine zone), and in the Central Caucasus, in Kazbegi region, environs of v. Sno (1750 m a. s. l. – subalpine zone).

The lava plateau of Bakuriani (southern Caucasus upland) is characterized by cold, temperate but damp enough climate, with warm, short summer and frosty winter. The mean annual temperature of air is 4°C, relative humidity – 75%, total amount of precipitation per year is 838 mm. In the alpine belt, on Tskhra Tsqaro Kodiani pass the climate is cold, typical of woodless high mountains; mid-annual temperature of air is 1°C, relative humidity – 84%, annual amount of precipitation is about 1200 mm. The whole region is characterized by high humidity and by fog in spring and summer [11].

The area of Kazbegi (northern macroslope of the central Caucasus) is characterized by cold winter and cool summer. Mid-annual temperature of air is 5°C, total amount of precipitation per year is 700-800mm. Very often in the second half of summer there occurs a drought: the temperature rises to 28-30 °C, relative humidity of air significantly falls (to 20-30%), the water potential of soil sharply decreases [12]. Thus, the climate of Kazbegi region is comparatively dry with relatively high level of UV radiation. The vegetation period of meadow plants of Kazbegi is quite long which makes it different from other high mountains [13].

A and B sections of UV radiation were measured at all points of research by the UVX -Radiometer (UVP inc., USA) (Table 1).

Plant material was collected at the end of July and beginning of August in the flowering phase. Herbaceous plants: jointweed (*Polygonum alpinum* All.), hellebore (*Veratrum lobelianum* Bernh), St. John's wort (*Hypericum perforatum* L.), valerian (*Valeriana tiliifolia* Troitzki), sorrel (*Rumex crispis* L) served as test odjects.

Peroxidase activity was determined spectrophotometrically by measuring the optical density of guaiacol oxidation products during particular intervals of time [14]. Catalase activity was studied gasometrically, measuring volume of released oxygen after adding the water extract of experimental leaves to hydrogen peroxide [15]. The content of total proteins was determined after Lowry, using Folin reagent [16]. Fresh leaves were picked from 10 fully expanded plants. The results obtained represent mean values of three replicates with standard deviation.

According to the experimental results it is clear that stress conditions typical of high mountains caused changes in plant metabolism. Investigation of the activity of antioxidative enzyme peroxidase has revealed specific peculiarities among the plants studied. High activity of the enzyme was detected in leaves of jointweed (*Polygonum alpinum* All.), valerian (*Valeriana tiliifolia* Troitzki) and sorrel (*Rumex crispis* L). Comparatively low was the activity of enzyme in leaves of hellebore (*Veratrum lobelianum* Bernh) and St. John's wort (*Hypericum perforatum* L.). This pattern was characteristic of plants at all three points (Fig. 1).

Among the experimental plants of the Minor Caucasus the tendency of peroxidase activity to increase was revealed in plants of the alpine zone in comparison with timber zone species. It is known that peroxidase plays a special role in the total metabolism of plant due to its polyfunctional activity [17, 18, 19]. Enhancement of enzyme activity under stress conditions may be the result of shifts in metabolism for the realization of the protective and adaptive abilities of plants [20, 21].

High activity of antioxidative enzyme catalase was revealed in all experimental plants from both Minor and Central Caucasian regions (Fig. 2). In the alpine zone of the Minor Caucasus (2450m) activity of catalase increased by 20% in leaves of sorrel (*Rumex crispis* L) and jointweed (*Polygonum alpinum* all.), by 40% in leaves of hellebore (*Veratrum lobelianum* Bernh). The activity of the enzyme slightly diminished in leaves of St. John's wort (*Hypericum perforatum* L.), while in valerian (*Valeriana tiliifolia* Troitzki) – by 20%. Rise of the activity of antioxidative enzymes plays a protective role under high mountain con-

Table 1.

Intensity of A and B sections of the solar UV (2 o'clock pm)

Place	Intensity of UV radiation, $\mu W \cdot cm^{-2}$	
	A	B
Bakuriani	2.6	2.2
Tskhra Tsqaro	3.0	2.5
Kazbegi	3.3	2.7

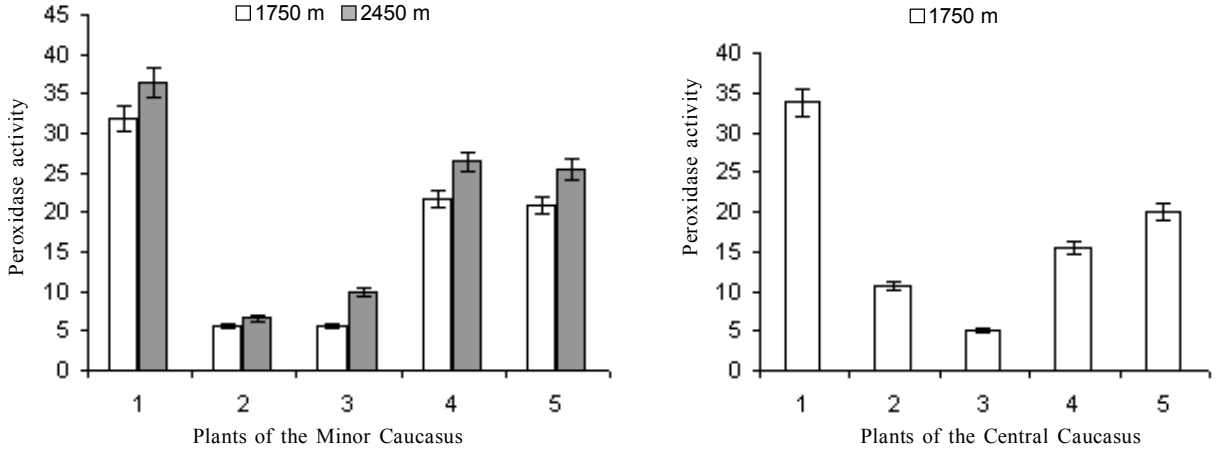

Fig. 1. Influence of altitude on the activity of peroxidase (conditional units per g of fresh weight) in leaves of medicinal plants. 1. *Polygonum alpinum* All. 2. *Veratrum lobelianum* Bernh. 3. *Hypericum perforatum* L. 4. *Valeriana tiliifolia* Troitzki. 5. *Rumex crispis* L.

ditions. According to some authors, the rise of the activity of antioxidants with altitude is due to the combined effect of low temperature and intensive radiation [22].

Comparison of the enzymatic activity of experimental plants has revealed the following regularity: decrease of the activity of one enzyme was accompanied by activation of other one. Abatement of catalase activity in leaves of St. John's wort (*Hypericum perforatum* L.) and valerian (*Valeriana tiliifolia* Troitzki) from the alpine zone of the Minor Caucasus correlated with the rise of peroxidase activity. The same regularity was revealed in the enzymatic activity of plants from Kazbegi region. This points to a rearrangement in the activity of antioxidative enzymes. According to some authors, one and the same enzyme may have different level of resistance to stressors in different species leading to decrease or increase of enzymatic activity [23].

Işil Öncel et al. [24] have studied the role of antioxidative protection and biochemical adaptation among high mountain plants. Significant differences in antioxidative activity were established on family level. The authors concluded that, though the antioxidative system plays an important role in alpine plants, additional accumulation of soluble proteins and proline is significant in the resistance of plants to stress.

Determination of the content of total proteins in experimental plants of the Minor Caucasus has revealed the tendency to their accumulation in alpine zone plants, compared with the timber zone species. Specific peculiarities were revealed both in protein content and ability of their accumulation. This index in leaves of Kazbegi region plants was higher compared with the same species of Bakuriani and Tskhra Tsqaro (Table 2).The highest content of proteins was detected in leaves of jointweed (*Polygonum alpinum* all.) and St. John's wort (*Hypericum perforatum* L.). The index was 4.5% higher in leaves of jointweed (*Polygonum alpinum* All.) compared with alpine zone

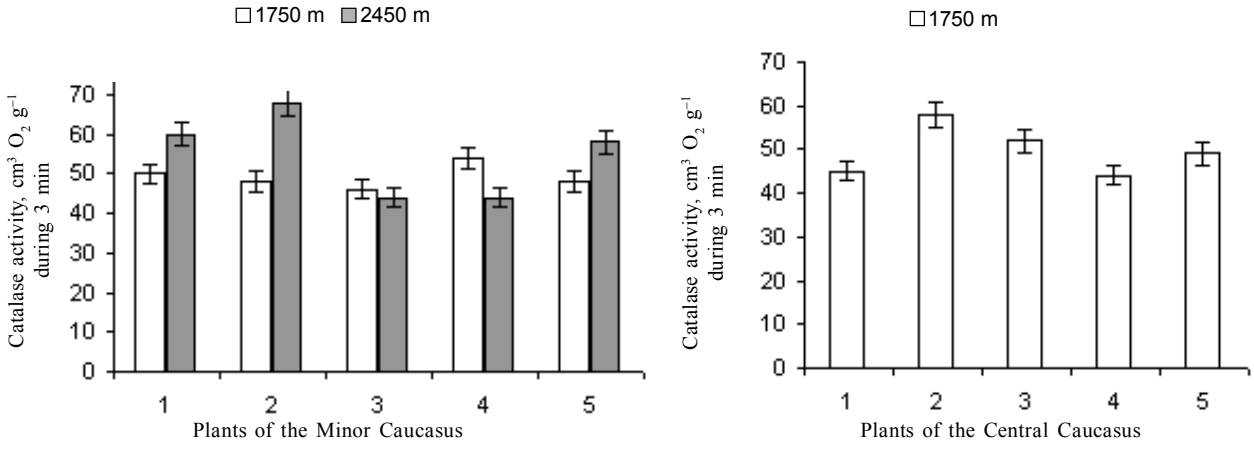

Fig. 2. Influence of altitude on the activity of catalase (cm^3 $O_2 \cdot g^{-1}$ during 3min) in leaves of medicinal plants. 1. *Polygonum alpinum* All. 2. *Veratrum lobelianum* Bernh. 3. *Hypericum perforatum* L. 4. *Valeriana tiliifolia* Troitzki 5. *Rumex crispis* L.

Table 2.

Influence of altitude on the content of total proteins in leaves of herbaceous plants (in%)

Region, altitude above sea level, m	Plants				
	Polygonum alpinum All.	*Veratrum lobelianum* Bernh.	*Hypericum perforatum* L.	*Valeriana tiliifolia* Troitzki	*Rumex crispis* L.
Bakuriani 1750	7.50	5.44	13	7.0	7.0
Tskra Tsqaro 2450	9.9	6.80	15	7.6	8.3
Kazbegi region 1750	12.0	7.0	16.0	7.8	10

plants of Bakuriani and 2.1% higher – compared with Tskhra Tsqaro species. Comparatively high temperature and increased background of UV radiation in Kazbegi region are responsible for protein accumulation, which is presumably an adaptive reaction of plants to extreme conditions of the given region.

Prakash et al. [25] noted the role of high level of soluble proteins at high altitude. It was established that accumulation of protein, sugars and proline plays a significant role in the survival of plants under high mountain conditions [26]. In our early experiments the structural and functional peculiarities of leaves of dandelion, plantain and clover, growing under high mountain conditions of the Minor Caucasus revealed the tendency of protein accumulation in alpine zone plants [27]. The supposition was offered that the rise of protein amount may be linked to the accumulation of shock proteins as a result of increased UV radiation.

According to the obtained data on the activity of antioxidative enzymes catalase and peroxidase, and content of total proteins in leaves of medicinal plants from the Minor and Central Caucasus, it may be concluded that adaptation of plants to special climatic conditions of the given regions is expressed through the specific peculiarities of metabolic changes leading to regulation of antioxidative enzymes along with accumulation of protective proteins.

მცენარეთა ფიზიოლოგია

პეროქსიდაზას, კატალაზას აქტივობა და ჯამური ცილების შემცველობა კავკასიონის მაღალმთიანეთის ზოგიერთი სახეობის ბალახოვან მცენარეში

ე. ჩხუბიანიშვილი*, ნ. კაჭარავა*, გ. ბადრიძე*, შ. ჭანიშვილი*, თ. ქურდაძე*

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(წარმოდგენილია აკადემიკოს ნ. ნუცუბიძის მიერ)

**შესწავლილია გარემო პირობების გავლენა ანტიოქსიდანტური ფერმენტების – პეროქსიდაზას და კატალაზას აქტივობაზე და ჯამური ცილების შემცველობაზე მცირე კავკასიონის სხვადასხვა სიმაღლეზე (1750 მ ზ.დ. – ტყის ქვედა სარტყელი და 2450 მ ზ.დ.– ალპური სარტყელი) და ცენტრალურ კავკასიონზე (ყაზბეგის რაიონი – 1750 მ ზ.დ.) მოზარდი სამკურნალო ბალახოვანი მცენარეების (*Polygonum alpinum* All., *Veratrum*

***lobelianum* Bernh., *Hypericum perforatum* L., *Valeriana tiliifolia* Troitzki, *Rumex crispis* L.) ფოთლებში. მაღალმთიანეთის ექსტრემალურ პირობებში ფერმენტული აქტივობა ცვალებადობს მცენარეთა სახეობრივი სპეციფიკურობის მიხედვით. მცირე კავკასიონის ალპური ზონის მცენარეთა ფოთლებში ტყის ქვედა სარტყლის სახეობებთან შედარებით გამოვლენილია ცილების დაგროვების ტენდენცია პეროქსიდაზას გააქტიურებასთან ერთად. ყველა შესწავლილი რეგიონის მცენარეებისთვის დამახასიათებელი აღმოჩნდა ფერმენტ კატალაზას მაღალი აქტივობა. ცენტრალური კავკასიონის შედარებით მშრალმა კლიმატმა და მომატებულმა ულტრაიისფერმა რადიაციამ გამოიწვია ჯამური ცილების რაოდენობის მატება მცირე კავკასიონის შესწავლილი რეგიონების მცენარეებთან შედარებით. ცილის სინთეზის გააქტიურება, ანტიოქსიდანტურ სისტემასთან ერთად, დამცველობით ფუნქციას ასრულებს მაღალმთიანეთის სტრესულ პირობებში.**

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