Activity of a catalase enzyme in plants from the burned areas of the Vidlič Mountain beech forest.

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ACTIVITY OF A CATALASE ENZYME IN PLANTS FROM THE BURNED AREAS OF THE VIDLIC MOUNTAIN BEECH FOREST

M. S. MARKOVIC[a]*, B. S. ILIC[b], D. L. MILADINOVIC[b], S. M. STAMENKOVIC[a], R. TRAJKOVIC[c], V. P. STANKOV-JOVANOVIC[d], G. T. DJELIC[e]

[a]*Department of Biology and Ecology, Faculty of Science and Mathematics, University of Nis, 18 000 Nis, Serbia*
E-mail: *marijam@pmf.ni.ac.rs*
[b]*Department of Pharmacy, Faculty of Medicine, University of Nis, 18 000 Nis, Serbia*
[c]*Department of Biology, Faculty of Science and Mathematics, University of Kosovska Mitrovica, 38 220 Kosovska Mitrovica, Serbia*
[d]*Department of Chemistry, Faculty of Science and Mathematics, University of Nis, 18 000 Nis, Serbia*
[e]*Faculty of Science and Mathematics, University of Kragujevac, Institute of Biology and Ecology, 34 000 Kragujevac, Serbia*

ABSTRACT

In 2007, a catastrophic fire on the Vidlic Mountain in south-east Serbia occurred. It burned down nearly 1000 ha of forest. Study of biochemical and physiological parameters in plants which inhabit post fire areas and their comparison with control is of essential importance in estimating the impact of fire on plant characteristics and potential applications. After the fire pioneer and indigenous plants from habitats affected by fire have a characteristic metabolism.

In this comparative study, the activity of enzyme catalase (EC 1.11.1.6, H_2O_2: H_2O_2 oxidoreductase) was determined in plant species from a habitat affected by fire and the same plant species from the forest which had not been affected by fire as a control. Assessment of enzyme activity was carried out on the root, leaves and flowers of plant species *Geranium macrorrhizum*, *Doronicum columnae*, *Aegopodium podagraria*, *Fagus moesiaca*, *Tussilago farfara*, *Glechoma hirsuta*, *Chelidonium majus* and *Primula veris.* The first group of plant samples used for determination was obtained from the habitat that was affected by fire two years ago. Catalase activity was

* For correspondence.

measured using the gasometric method and the values obtained for this activity were expressed as ml of O_2. The presented results show a significant increase in catalase activity in individuals from habitats affected by fire in relation to the control group. Increased catalase activity is a consequence of oxidative stress caused by chemical changes in soil that were generated by fire.

Keywords: enzyme catalase, forest affected by fire, Vidlic Mountain, oxidative stress.

AIMS AND BACKGROUND

From the year 2003 to 2007, there were 579 wild fires registered in Serbia. The largest number of these fires (370) was recorded in the summer of 2007 (Ref. 1), including the fire on the Vidlic Mountain. The vegetation of the forest, rocks, shrubs and grassland formations burned in the fire. After the fire was completely put out, it was estimated that more than 2500 ha of low vegetation, scrublands and forests burned down[2]. Approximately 1000 ha of beech forests were burned.

Numerous studies examined the effect of fire on the physiological functions of different plants. Plants have different defense response mechanism to stress that occurs as a result of fire. After a forest fire, the temperature and amount of light increase, which causes the change in the anatomical and physiological characteristics of plants[3]. Knapp et al.[4] discovered that plants on the burnt area are thicker and have wider leaves, higher specific leaf weight and a higher density of stoma. The increased thickness of leaves means an increase of the mesophyll relative to the entire surface of the leaf and leads to an increased CO_2 usage and degree of photosynthesis[5]. Fleck et al.[6,7] indicated a higher level of photosynthesis in the leaves of the *Quercus ilex* after the fire.

From the areas that were burned and also the areas that were not affected by the fire from the Vidlic Mountain was determined the content of: organic acids[8], heavy metals in soil and plant samples[9], chloroplast pigments[10]. In order to assess what kind of impact does fire have on the antioxidant properties of plants, plant species extracts from the burnt and the non-affected areas were tested[11,12]. The impact of fire on the antimicrobial and antioxidant activities of some plant species was examined[13]. The activity of the enzyme catalase was determined in root and shoot system plant parts of the species *Geranium macrorrhizum* of the burnt and non-affected areas[14]. Moreover, an examination and comparison of the chemical composition and antioxidant activity of the essential oil species *Ajuga chamaepytis* from the burnt and non-affected areas of Vidlic was made[15].

The high intensity light and UV-B radiation causes oxidative stress and damage in plants[16]. These conditions can be achieved in the areas where the vegetation was completely burned by the fire. The functioning of the antioxidant system and the degree of oxidative damage can be evaluated successfully on the basis of determining the biochemical parameters – indicators of the oxidative stress[17,18]. One of the indicators of oxidative stress in plants is the activity of the enzyme catalase. Changes in the biotic and abiotic factors at the burnt area cause morphological-physiological changes in plants. As a result of a group of changes in their environment, plants alter their metabolism. First of all, an acceleration of the plant metabolism occurs which hence leads to an acceleration of the enzymatic activity. Significant changes were recorded because concentrations of some enzymes in plants increased. These enzymes, whose concentrations significantly increase in stressful conditions, are catalase and peroxidase. These two enzymes remove free radicals whose concentration in a cell increases in stressful conditions[19]. Increased concentrations of the enzyme catalase indicate oxidative stress in plants that grow on burnt areas. Catalase (EC 1.11.1.6, H_2O_2: H_2O_2 oxidoreductase) is one of the most powerful enzymes known. The reactions which this enzyme catalyses are essential for plant life. Catalase breaks down the toxic hydrogen peroxide into water and molecular oxygen. By using hydrogen peroxide, which breaks down into water and molecular oxygen, the catalase further oxidises toxic molecules which include phenol, formic acid, formaldehyde and alcohol.

Numerous authors have found a point of comparison between the activity of the enzyme catalase and the content of chlorophyll[20–22]. They have noticed that the catalase activity is several times higher in etiolated seedlings and leaves with chlorosis than in green leaves. They interpret the similar behaviour of catalase and chlorophyll as having closely linked ways of biosynthesis, which was shown in results obtained by Mikhlin and Mutuskin[23].

EXPERIMENTAL

Determination of plant material for analysis was performed by using the key for the regional flora[24,25].

Plant material for determining the activity of the enzyme catalase was collected based on the conditions of the area in the spring of first and second year after the fire in the burnt area of the beech forest on the Vazganica location. Plants collected at the same time from a nearby area not affected by the fire were used as the control plant group. Herbarium samples of the analysed plants were placed in the herbarium of University of Belgrade, Faculty of Biology (BEOU) and their voucher numbers are given in Table 1. Immediately after being picked, plant samples were put into liquid nitrogen in which they were transported and then put into a freezer kept at –20ºC where they were stored until the analysis. Before the analysis, the root and shoot system plant parts were separated and cut into little pieces.

Beech samples (*Fagus moesiaca*), besides being collected and stored in a freezer until analysis like all of the other samples, were amassed the first year after the fire. Newly sprouted seeds, along with the soil from the burnt area, were taken to a laboratory where the seedlings were kept in a plastic container for several weeks until the analysis of the catalase enzyme activity.

Table 1. Inventory numbers of plants and the coordinates of the location where the plants were collected for determining the activity of catalase enzyme

Inventory number	Plant species	Location	Habitat	Date	Coordinates	Elevation (m)
16422	*Fagus moesiaca* (K. Maly) C z e c z.	Vazganica	burnt area of beech forest	29.04.2008	43°10′36.6″ N 22°43′35.4″ E	1120
16423	*Aegopodium podagraria* L.	Vazganica	burnt area of beech forest	22.06.2008	43°10′37.2″ N 22°43′29.5″ E	1140
16424	*Glechoma hirsuta* W a l d s t. & K i t.	Vazganica	burnt area of beech forest	11.05.2008	43°10′43.2″ N 22°43′30.4″ E	1110
16425	*Chelidonium majus* L.	Vazganica	burnt area of beech forest	21.06.2008	43°10′39.1″ N 22°42′34.1″ E	1115
16426	*Doronicum columnae* T e n.	Vazganica	burnt area of beech forest	29.04.2008	43°10′37.0″ N 22°42′28.8″ E	1080
16427	*Tussilago farfara* L.	Vazganica	burnt area of beech forest	30.03.2008	43°10′41.2″ N 22°42′37.1″ E	1070
16428	*Primula veris* L.	Crni vrh	rocky surface	28.04.2008	43°10′22.6″ N 22°39′19.6″ E	840
16431	*Geranium macrorrhizum* L.	Vazganica	burnt area of beech forest	31.05.2008	43°10′41.1″ N 22°42′49.1″ E	1190

The catalase enzyme activity was determined by a gasometric method[26]. This method was based on assessing the amount of oxygen being released after reacting with H_2O_2 which was added to the plant extract that contained catalase.

First, 0.5–1 g of plant material were measured. Afterwards, the material was extracted in a crucible with 0.5 g of $CaCO_3$ and 20 ml of distilled water which was added gradually. After that, the extract was transferred into an Erlenmeyer flask with a port on the side and using the apparatus the activity of the enzyme catalase was measured. The Erlenmeyer flask was sealed with a stopper which was pierced with a needle with an attached syringe. The syringe contained 5 ml of 3% H_2O_2. The H_2O_2 was injected in the Erlenmeyer flask at the beginning of the experiment.

The content was stirred for a minute and after waiting 1 min stirred again for one minute. After these 3 min, the amount of freed oxygen could be read on the burette scale. Afterwards, the recalculation for 1 g of material was made.

Each analysis was repeated three times and the average value was calculated. Catalase activity was expressed indirectly through the freed oxygen volume.

RESULTS AND DUSCUSSION

The stress in the living environment can extremely inhibit the enzymes, directly or indirectly through physiological and biochemical processes, as well as in terms of enzyme activation which will catalyse their decomposition. A group of enzymes, which includes catalase and peroxidase, is important for the physiology of resistance and endotoxicology[27]. Hydrogen peroxide is the substrate on which these enzymes act, which is indicated by the fact that their presence in the medium encourages increased activities of these enzymes – substrate induction[28,29]. The hydrogen peroxide forms during the course of various metabolic processes as a reduced form of oxygen and can cause a number of metabolic changes in the tissues of the plant. Because of the high toxicity to living cells, it is necessary to remove or degrade it. The activity of the enzyme catalase breaks it down into products that are not harmful to plants[27].

Catalase activity was measured in root and shoot system parts of plants from the burnt areas in the beech forests of the Vidlic Mountain and from the closest beech forest area not affected by the fire which represent a control group. The given results show that the catalase activity is different in certain plant species and shows that the root and shoot system plant parts of the tested plants are uneven (Tables 2 and 3).

It was noticed that, in the first year after the fire, the activity of the enzyme catalase in the species *Geranium macrorrhizum*, *Aegopodium podagraria* and *Fagus moesiaca* increased both in the root and shoot system plant parts of the burnt area (experimental group) compared to the non-affected surface (control group), while only the catalase activity in the root system of the species *Doronicum columnae* and *Tussilago farfara* increased in relation to the control group (Table 2). Therefore, the activity of catalase in all underground parts of the plants increased the first year after the fire.

Table 2. Activity of enzyme catalase in the root and shoot system plant parts on the not burned area and the burnt area of Vidlic Mountain, the first year after the fire, converted to 1 g of fresh matter

Plant species	Control group (not burned area) (ml O_2)	Experimental group (burnt area) (ml O_2)
Geranium macrorrhizum, shoot system plant part	6.19	6.68
Geranium macrorrhizum, rhyzome with roots	6.35	7.75
Doronicum columnae, flower heads	33.17	26.5
Doronicum columnae, stems	–	13.77
Doronicum columnae, leaves	–	19.69
Doronicum columnae, rhyzome with roots	12.35	21.13
Aegopodium podagraria, shoot system plant part	8.18	8.61
Aegopodium podagraria, rhyzome with roots	6.69	8.97
Fagus moesiaca, shoot system plant part under laboratory conditions	13.76	19.16
Fagus moesiaca, root under laboratory conditions	6.80	9.09
Fagus moesiaca, shoot system plant parts	9.66	9.84
Fagus moesiaca, roots	11.37	11.70
Tussilago farfara, shoot system plant parts	20.10	17.78
Tussilago farfara, rhyzome with roots	15.95	17.44

In the second year after the fire, the activity of the enzyme catalase in the species *Geranium macrorrhizum* and *Primula veris* increased in both the shoot and root system parts of the experimental group, compared to the control group; in the *Doronicum columnae*, the catalase activity increased in the underground parts of the plant, stems and leaves of the experimental group in relation to the control group, and it decreased in the flower heads; in *Chelidonium majus*, the catalase activity was higher in the underground parts, leaves and stems in the experimental group than in the control plants, but it only decreased in relation to the control plants; in the *Glechoma hirsuta* species, the catalase activity was higher in the control than in the experimental plants (Table 3). The results show that the activity of the enzyme catalase has generally increased in plants from the burnt area of the beech forest compared to the plants from the non-affected area and it was almost always higher in the underground parts of the plants in relation to above ground parts. This can be explained by the fact that the root system plant parts are in direct contact with the chemical substances of the soil, which contains plenty of ash and has a different, qualitatively and quantitatively new chemical composition in relation to the land unaffected by the fire. Concentrations of compounds that are harmful to the plant increase in the soil and, consequently, in the underground parts of the plants in the burnt area of the beech forest, because it occurs during the metabolism of the plants under stressful conditions which occur after the fire. The catalase breaks down harmful compounds into harmless products, and they are found in a greater amount in the root system than in the shoot system of the experimental plants from the area affected by the fire, with the exception of the *Glechoma hirsuta*. This herbaceous plant has creeping sprouts that have thin wiry roots, which do not penetrate deep into the soil and has a small absorption surface. Therefore, it can be assumed that, in the burnt area, it accumulates less material harmful to the plant, meaning that the catalase activity does not increase in relation to the same plant from the non-affected area.

Table 3. Activity of enzyme catalase in the root and shoot system plant parts on the not burned area and the burnt area of the Vidlic Mountain, the second year after the fire, converted to 1 g of fresh matter

Plant species	Control group (not burned area) (ml O_2)	Experimental group (burnt area) (ml O_2)
Geranium macrorrhizum, flowers	12.12	17.17
Geranium macrorrhizum, leaves	7.90	10.89
Geranium macrorrhizum, rhyzome with roots	8.56	11.36
Doronicum columnae, flower heads	54.62	49.21
Doronicum columnae, stems	29.72	31.95
Doronicum columnae, leaves	37.99	48.92
Doronicum columnae, rhyzome with roots	19.95	26.61
Glechoma hirsuta, shoot system plant parts in bloom	30.44	23.54
Glechoma hirsuta, creeping sprouts with roots	24.56	15.96
Chelidonium majus, flowers	33.15	36.60
Chelidonium majus, leaves	11.22	12.19
Chelidonium majus, stems	41.02	40.01
Chelidonium majus, roots	10.36	11.33
Primula veris, leaves	27.84	38.59
Primula veris, flowers with flower stems	11.86	21.59
Primula veris, rhyzome with roots	10.06	10.68

Activity of the enzyme catalase in root and shoot system plant parts varies is uneven and varies among species. Increased or decreased activity of catalase depends on the morphological-anatomical structure of the plant leaves and roots, as well as a rich and diverse chemical composition, which is genetically determined and specie specific[27]. An increased catalase activity is a result of oxidative stress, which is caused by chemical changes in the soil influenced by fire[14]. Increasing the activity of the enzyme catalase in plants at the fire site is a metabolic form of breaking down harmful compounds, i.e. detoxification, which is a high-quality mechanism of resistance that responds to the changed conditions in the environment after the fire.

CONCLUSIONS

Plants that grow on the burnt areas of the Vidlic Mountain are highly adaptive plants, which have a characteristic metabolism and survival mechanisms. The changed conditions after the fire require an anatomical, physiological and biochemical adaptations from plants. The ability of plants to adapt to stressful conditions is crucial for their survival. Different plants respond differently to stress depending on different genetic backgrounds, different phenophases and different morph-anatomic characteristics of plants. An increased concentration of the enzyme catalase indicates oxidative stress in plants which grow in the burnt areas. Catalase breaks down harmful products of the plant metabolism at the burnt area into harmless compounds. On the basis of these results, we can conclude that the catalase is involved in the plant protective defense mechanisms against the toxic effects of the active forms of oxygen, which are formed during a reciprocal action of the harmful products of metabolism after the fire, with cell membranes and membranes of cell organelles.

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