*Journal of Plant Development* ISSN 2065-3158 print / e-ISSN 2066-9917
Vol. **31**, Dec **2024**: 37-52 Available online: www.plant-journal.uaic.ro
DOI: 10.47743/jpd.2024.31.1.948
**Research Article**

# EVALUATION OF ANTIOXIDANT ACTIVITY IN OAK LEAVES FROM THE REPUBLIC OF MOLDOVA EXPOSED TO HEAT STRESS

Petru CUZA

Department of Geosciences and Forestry, Moldova State University,
MD 2009, Chișinău – Republic of Moldova.
E-mail: petrucuza@mail.ru, ORCID: 0000-0003-0192-4427

**Abstract**: The total activity of antioxidant substances, oxidases, and catalases was evaluated in the leaves of three oak species – pedunculate oak (*Quercus robur* L.), sessile oak (*Q. petraea* Liebl.), and downy oak (*Q. pubescens* Willd.) from the Republic of Moldova, subjected to thermal shock at 50 °C for durations of 20, 40, and 60 minutes. The study aimed to identify variations as well as common and divergent responses of these species according to ecological zone, thermal shock duration, and recovery period, in order to assess their thermotolerance and specific adaptations to environmental conditions. The results showed that during the recovery period, leaves of pedunculate oak and downy oak from the center and southern regions, as well as sessile oak from the center region, exhibited high total antioxidant activity. Additionally, pedunculate oak and sessile oak from the center and southern regions displayed increased oxidase activity, while sessile oak from the center region demonstrated high catalase activity, indicating the activation of adaptive processes to thermal shock. Conversely, pedunculate oak and sessile oak from the northern region exhibited a decrease in total antioxidant and oxidase activity during the recovery period, suggesting an increase in leaf thermotolerance. Moreover, the downy oak from the northern region demonstrated an increase in thermotolerance, reflected in the activity of all types of antioxidant substances after extended recovery periods. The study demonstrated that the investigated oak species enhance their thermotolerance in the northern region by developing specific adaptive strategies to thermal shock. These oak species adjust their antioxidant and enzymatic activity in accordance with the specific environmental conditions of the ecological zone in which they grow.

**Keywords**: *Quercus robur* L., *Q. petraea* Lielb., *Q. pubescens* Willd., leaves, thermal shock, recovery, adaptation, thermotolerance.

## Introduction

Currently, extreme temperatures during the summer, along with prolonged heat waves, have expanded their impact to approximately 10% of the Earth's surface, compared to just 1% recorded in the 1960s [HANSEN & al. 2012]. Estimates suggest an increased probability of both the frequency and intensity of heat waves rising in the 21$^{st}$ century [YAO & al. 2013], which has already led to a 0.5 °C increase in the global average temperature [COUMOU & ROBINSON, 2013; COUMOU & al. 2013]. This trend aligns with forecasts indicating that global warming will be partially driven by increasingly frequent and intense high-temperature conditions [COUMOU & ROBINSON, 2013; DULIÈRE & al. 2013].

Moreover, climatic conditions, particularly temperature and photoperiod, significantly influence plant species distribution through direct physiological constraints related to growth and reproduction, as well as indirectly through ecological factors such as competition for resources. Climate change, including temperature variations over the past century, has resulted in significant adverse effects on the geographic distribution, abundance, phenology, and

***Received***: 7 September 2024 / ***Revised***: 6 December 2024 / ***Accepted***: 10 December 2024

physiological state of numerous species [CHEN & HILL, 2011]. Simulation models indicate that climate change can induce extensive modifications in terms of the geographic distribution and survival of species. This underscores the urgent need for comprehensive strategies to mitigate climate change effects on both plant species and broader ecosystems.

Species capable of rapid migration and adaptation to higher altitudes gain survival and competitive advantages, and under specific conditions, they may experience geographic expansion [THOMAS & al. 2004; MENENDEZ & al. 2006]. However, such shifts in species distribution can jeopardize their abundance by fragmenting migration corridors and reducing dispersal distances due to barriers arising from the multifunctional land use. These constraints ultimately contribute to a decline in population numbers [GASTON, 1994].

High temperatures associated with drought can induce metabolic dysfunctions in organisms distributed in a particular area, such as most plants, a phenomenon that can affect multiple physiological processes and trigger stress conditions. This combination of factors can trigger heat stress in plants, initiating the synthesis of antioxidant enzymes and related metabolic pathways to reduce the excessive accumulation of unwanted and harmful reactive oxygen species. Among the most common reactive oxygen species are singlet oxygen ($1O_2$), superoxide radical ($O_2^{*-}$), hydrogen peroxide ($H_2O_2$), and hydroxyl radical (OH*), which are responsible for oxidative stress [ASADA, 2006]. Uncontrolled increase in the concentration of reactive oxygen species can cause cellular damage to plants, including lipid peroxidation, protein damage, and disruption of cellular membranes. These processes can lead to physiological dysfunctions and weaken the viability of plants.

The reaction centers of photosystem I and II in chloroplasts represent primary sources of reactive oxygen species generation, although these can also be generated in other organelles such as peroxisomes and mitochondria [SOLIMAN & al. 2011]. Scientific data indicate that thermal damage to the photosystems under the influence of high temperatures results in reduced photon absorption [HALLIWELL, 2006]. Under stress conditions, when the photon intensity absorbed by photosystem I and II is excessive, this surplus energy is considered as an excess of electrons, thus serving as a source for reactive oxygen species [HALLIWELL, 2006].

Under stress conditions, characterized by high temperatures and drought, plants activate antioxidant mechanisms to mitigate the harmful effects of thermal stress and maintain metabolic homeostasis. These defense mechanisms against oxidative stress are achieved through molecules, including both enzymatic and non-enzymatic components. Key components of this system include enzymes such as superoxide dismutase, peroxidase, catalase, polyphenol oxidase, and alternative oxidase essential in metabolic pathways for the elimination of reactive oxygen species and the control of lipid peroxidation [BOECKX & al. 2015; SAHA & al. 2016]. Increasing the concentration of these enzymes represents an efficient strategy for cell protection against the harmful effects of oxidative stress, thereby ensuring the prevention of excessive accumulation of reactive oxygen species.

By predominantly localizing in mitochondria, the function of alternative oxidase is closely linked to the mitochondrial respiratory chain, playing an essential role in energy generation within cells and providing an alternative pathway for electron transfer in the respiratory chain. Thus, alternative oxidase limits the generation of reactive oxygen species, such as superoxide ($O_2^{*-}$) and hydrogen peroxide ($H_2O_2$). This mechanism contributes to maintaining a redox balance within the cell and protecting it against potential damage caused by reactive oxygen species, thereby regulating oxidative stress in cells [SAHA & al. 2016].

In the forests of the Republic of Moldova, indigenous oak species such as *Quercus robur*, *Q. petraea*, and *Q. pubescens* exhibit sensitivity to stressors generated by high temperatures and heat waves. Their vulnerability is largely due to the fact that, over an extended period, the regeneration of forest stands has been predominantly through shoots, a process that has led to pronounced negative selection. This selection has involved the elimination of valuable trees and stands from populations, both in terms of dendrometric and qualitative aspects. Additionally, these species face excessive habitat fragmentation [CUZA, 2001; DASCALIUC & al. 2005]. Although oak species fulfill multiple essential ecological functions, significantly impacting environmental protection, they also hold considerable economic importance. They provide timber used in furniture production, flooring, wine barrels, and more. However, threats such as prolonged droughts and increasingly frequent heat waves can lead to succession phenomena or alterations in the distribution of indigenous oak species.

Given the current context, studies on the thermotolerance of oak species become highly relevant to anticipate and sustainably manage forests in the future. In this regard, conducting in-depth research to assess the adaptive capacity of oak species to climate change and to develop effective strategies for conservation and sustainable forest management is essential. Additionally, it is important to mention that in the Republic of Moldova, there is an ongoing campaign to expand the forest area through afforestation. In this context, the proper use of species, considering the habitat's specificity, is a particular concern within the efforts for the conservation and sustainable development of forest resources.

## Material and methods

Leaf samples for laboratory experiments were collected in July, with fifteen specimens taken from the lower and southern sections of the tree crowns for each species and provenance. The selected species included pedunculate oak (*Quercus robur*), sessile oak (*Q. petraea*), and downy oak (*Q. pubescens*). These trees are located within the Filimon Carcea Forestry District (northern region), Cociulia Forestry District (southern region), and the “Plaiul Fagului” Scientific Reserve (central region).

In the laboratory, leaves from each species and location were subjected to heat shock at 50 °C for intervals of 20, 40, and 60 minutes. Following the heat shock treatment, the leaves were transferred to desiccators, where they were maintained under controlled conditions: a temperature of 25 °C, relative humidity of 85%, illumination of 20 lux, and a photoperiod of 16 hours of light and 8 hours of darkness.

The antioxidant activity in the leaves was assessed using the method described by DASCALIUC & al. (2018), as detailed below. On days 1, 3, and 5 after the heat shock treatment, leaf samples were taken from the desiccators for biochemical analysis to determine total oxidase activity. Leaf samples, weighing 0.1 g each, were cold-macerated, and the resulting material was extracted for 30 minutes at 25 °C in a 0.2 M Tris buffer solution (pH 7). The extract was then centrifuged for 15 minutes at 4000 g.

To determine the total capacity for reducing free oxygen, reflecting both the direct reduction potential of antioxidant substances and enzymatic activity, 40 µl of the supernatant obtained after centrifugation was added to 1.6 ml of buffer solution to ensure a stable testing environment. The mixture was then incubated at 25 °C.

In all experiments, the dynamics of oxygen content reduction in the experimental solutions were measured using a YSI oximeter (USA), with a control solution (containing only 1.6 ml of buffer solution) used for comparison. After 15 minutes of incubation at 25 °C, the oxygen content in the experimental solution reached a stationary phase, at which point the rate of oxygen consumption equaled the rate of its diffusion into the solution.

The difference in oxygen percentage between the control solution (without extract) and the experimental solution (with extract) was determined based on the capacity of antioxidants and oxidases to bind oxygen. Consequently, the activity of antioxidants and oxidases led to a reduction in oxygen content in the solution during the stationary phase, compared to the control variant.

To determine catalase activity, 40 µl of the solution was added to 1.46 ml of buffer solution, along with 60 µl of 0.05% $H_2O_2$, followed by incubation at 25 °C. The dynamics of oxygen content change were influenced by the activity of oxygen removal through the degradation of hydrogen peroxide by catalases, as well as the activity of oxygen binding by oxidases. The combined outcome of these processes was evaluated based on the oxygen percentage in the solution at the stationary phase. Thus, using the oximetry method, the activities of oxidases and catalases were determined separately, along with the combined effect of these enzymatic activities.

To illustrate the specificity of processes within certain antioxidant components, the graphs in Figures 1-3 depict the oxygen utilization activity by antioxidants and oxidases as negative values, while the oxygen removal activity by catalases is represented by positive values. The studies were conducted in three replicates, with the mean value and standard deviation of the mean determined [GIURGIU, 1972].

## Results

### Variability in the activity of antioxidant substances, oxidases, and catalases in the leaves of oak species

### 1. Activity of antioxidant substances, oxidases, and catalases in pedunculate oak (*Quercus robur*) leaves

The data presented in Figures 1a, 1b, and 1c indicate that, over a 5-day recovery period following heat shock, the oxygen-binding processes in the extracts from pedunculate oak leaves exhibited distinct processes. Specifically, the activity of antioxidant substances and oxidases differed from that of catalases in terms of oxygen removal. These variations highlight the differential responses of these biochemical components to heat stress.

In the northern region, the overall activity of antioxidant substances and oxidases was comparable and elevated on the first day following thermal shock, with an increase observed as the treatment period extended. This activity was significantly higher than that in the control sample, indicating a strong response of the oak trees to thermal stress. Additionally, catalase activity in the leaf extracts was high, surpassing that of antioxidant substances and oxidases. However, despite the duration of thermal shock exposure, catalase activity remained lower than in the control sample. This suggests that recovery processes are more advanced for catalases, while antioxidant substances and oxidases continue to exhibit increased activity compared to the control.

After three and five days of thermal shock, a trend towards reduced activity of antioxidant substances and oxidases was observed, with levels reaching those comparable to the control sample and lower than on the first day post-shock. In contrast, catalase activity increased somewhat after 20 and 40 minutes of shock, and after five days, it was similar to that of the control sample.

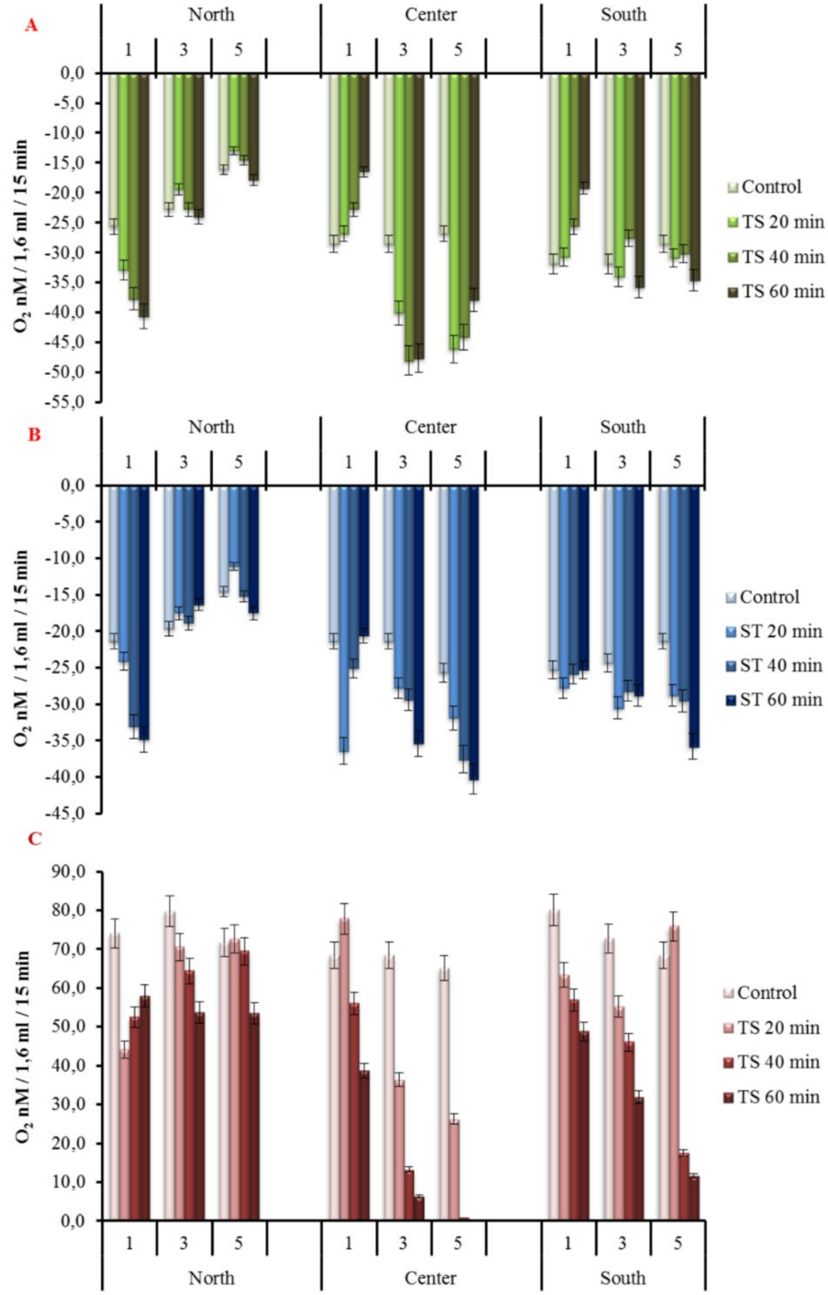


**Figure 1.** Dynamics of changes in the activity of antioxidant substances (A), oxidases (B), and catalases (C) in extracts from the leaves of pedunculate oak, exposed to thermal shock at 50 °C for 20, 40, and 60 minutes. 1, 3, 5 – recovery of antioxidant components' activity on the first, third, and fifth day after the application of thermal shock

In the center region, the analysis of the overall activity of antioxidant substances, oxidases, and catalases in leaf extracts of pedunculate oak trees reveals a clear trend of reduction following the first day of thermal shock. This trend is observed in conjunction with an increase in the duration of thermal shock exposure, with activity levels becoming lower than those in the control sample as the thermal shock duration extends. These results indicate that exposure to thermal shock induces recovery processes among antioxidant substances. On the third and fifth days post-exposure, there was an increase in the overall activity of antioxidant substances and oxidases, while the control sample activity was significantly lower. This suggests adaptive processes in the trees, activating antioxidant defense mechanisms to mitigate the effects of thermal shock. In contrast, catalase activity showed a different trend, significantly decreasing throughout the post-shock period with prolonged exposure to thermal shock. These changes suggest that the enzymatic system of pedunculate oak trees undergoes complex alterations in response to thermal stress, with catalase activity potentially being affected differently compared to other antioxidant enzymes.

In the southern region, a high and relatively constant activity of antioxidant substances and oxidases was observed, indicating that the antioxidant mechanisms of oak trees in this area are active and effective in managing thermal stress. Additionally, the analysis of overall antioxidant activity and oxidase levels during the recovery period, specifically after three and five days, revealed an increase compared to the control leaf extracts. This suggests that exposure to thermal shock stimulates the activity of these antioxidant substances in response to the stress, demonstrating the trees' ability to mobilize their defense mechanisms to mitigate the negative effects of thermal stress. In contrast, catalase activity decreased with the extension of the recovery period following exposure to thermal shock, indicating a specific regulation of these enzymes in the trees' response to thermal stress in the southern region. Pedunculate oak trees in this area appear to be well-adapted to thermal stress, possessing efficient antioxidant mechanisms to cope with it, along with a specific regulation of catalase activity during the recovery period.

### 2. The activity of antioxidant substances, oxidases and catalases in the leaves of the sessile oak (*Quercus petraea*)

Figures 2a, 2b, and 2c provide a detailed illustration of changes in the activity of antioxidant substances, oxidases, and catalases in the leaves of sessile oak trees under thermal shock in three distinct regions: north, center, and south.

In the northern region, the overall activity of antioxidant substances and oxidases extracted from leaves one day after thermal shock is moderate. However, a significant increase is observed with extended thermal exposure. This increase clearly surpasses the corresponding activity levels in the control leaf extracts (Figures 2a and 2b). These processes suggest that the response of sessile oak leaves to thermal shock initiates the trees' protective mechanisms to alleviate the generated oxidative stress. After three and five days of recovery post-thermal shock, the overall activity of antioxidant substances and oxidases decreased significantly compared to the first day post-shock. Simultaneously, their activity remained at levels comparable to those of the control samples. These observations indicate that the antioxidant system of the trees may experience a significant reduction in efficiency as the recovery time progresses. Nevertheless, the maintenance of activity at the level of the control samples could also suggest the plants' capacity to activate compensatory mechanisms to mitigate the negative effects of thermal stress and maintain internal homeostasis.

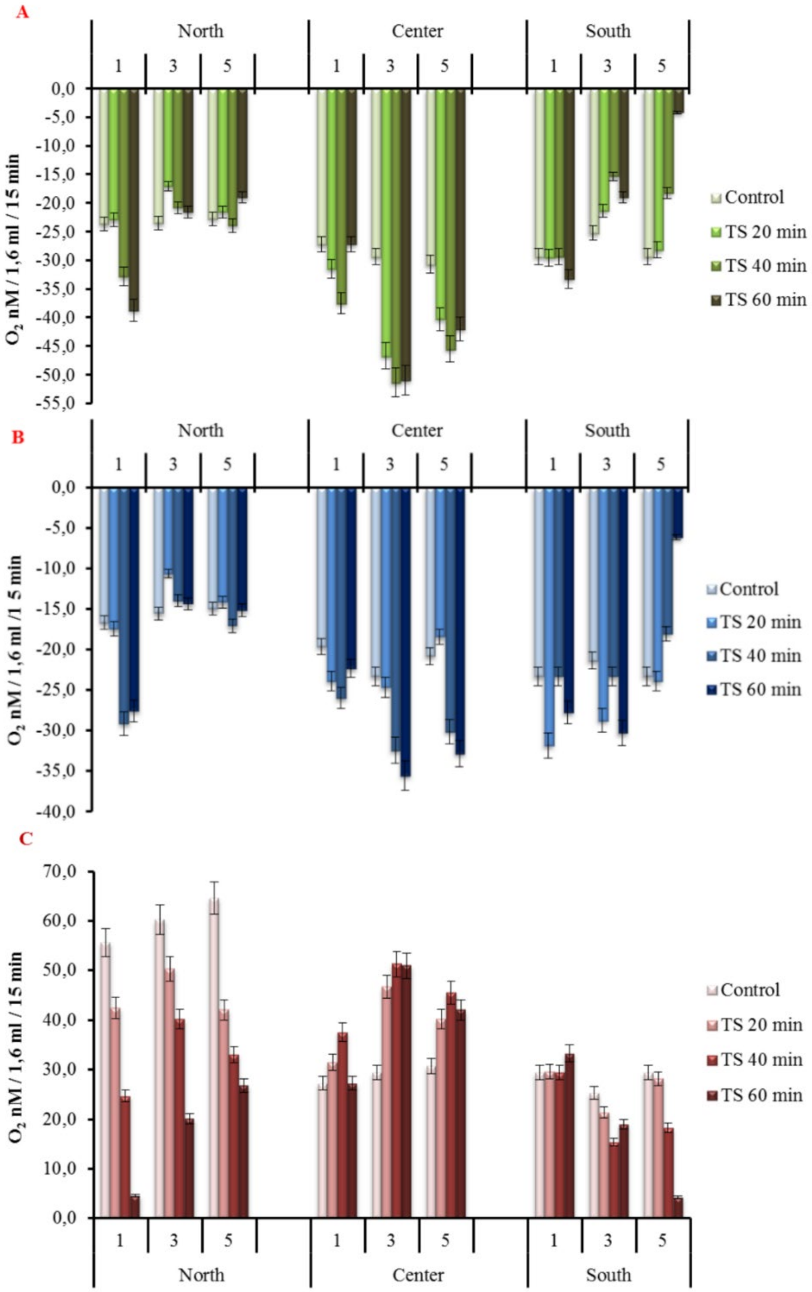


**Figure 2.** Dynamics of changes in the activity of antioxidant substances (A), oxidases (B), and catalases (C) in extracts from the leaves of sessile oak, exposed to thermal shock at 50 °C for 20, 40, and 60 minutes. 1, 3, 5 – recovery of antioxidant components' activity on the first, third, and fifth day following thermal shock application

In contrast, catalase activity exhibits an opposing trend over the five-day recovery period, showing a progressive decrease as the duration of thermal shock exposure increases, remaining significantly lower than in the control sample. This suggests that catalase activity may have adverse consequences as the post-shock recovery time advances.

In the center region, antioxidant substances and oxidases show moderate activity on the first day after thermal shock exposure, with antioxidant substances exhibiting a slightly higher level. Figures 2a and 2b clearly illustrate that, in both cases, the activity of these antioxidant components exceeds the levels observed in the control leaf extracts, indicating that their activity is directed towards strengthening protective mechanisms and controlling reactive oxygen species.

However, the overall activity of antioxidant substances and oxidases notably increases after three and five days of thermal shock exposure, significantly surpassing the control sample. The same trend is evident for catalase activity. The observed phenomenon demonstrates that, during an extended recovery period, sessile oak trees exhibit an adaptive response, highlighting protective mechanisms that include metabolic and enzymatic processes of antioxidation and detoxification, which intervene to mitigate the negative effects of thermal stress.

In the southern region, on the first day post-shock, the activity of antioxidant substances, oxidases, and catalases remains at a moderate level. However, the overall activity of antioxidant substances and catalases generally shows levels similar to those in the control sample, while the activity of oxidases in the thermal shock-exposed leaf extracts is noticeably higher compared to the control. As the recovery period and thermal dose increase, all antioxidant compounds show a trend towards decreased activity, with levels being lower five days after the shock compared to the control sample. This suggests either a “fatigue” of the antioxidant system or a reduction in the processes generating reactive oxygen species, indicating an improvement in the physiological state of the sessile oak trees.

The results indicate significant variations in the enzymatic and non-enzymatic responses of sessile oak leaves to thermal shock, suggesting that the impact of this thermal perturbation depends on geographic location.

### 3. The activity of antioxidant substances, oxidases and catalases in the leaves of downy oak (*Quercus pubescens*)

The data presented in Figures 3a, 3b, and 3c reflect the dynamics of changes in the overall activity of antioxidant substances, oxidases, and catalases in the leaves of downy oak across the three ecological regions, five days after exposure to thermal shock.

In the northern region, there is a significant increase in the overall activity of antioxidant substances and oxidases on the first day following thermal shock exposure compared to the control sample. As the duration of thermal shock exposure increases, the activity of these antioxidant substances appears to be positively influenced, continuing to rise with extended exposure of the leaves. This reaction indicates a strong response of downy oak trees to thermal shock.

In contrast, catalases activity decreases as the duration of thermal shock extends, reaching minimal levels after five days of recovery, particularly following exposure periods of 40 and 60 minutes. This phenomenon suggests a specific adaptation of downy oak trees to thermal stress, where the sensitivity of catalases plays a significant role.

The analysis of histograms presented in Figures 3a and 3b demonstrates that the overall activity of antioxidant substances and oxidases during a recovery period of three and five days shows a significant decrease compared to the first day post-thermal shock. These changes are influenced by both the duration of thermal shock and the recovery period of the plants, reflecting their regulatory capacity in response to thermal stress. This phenomenon highlights the ability of downy oak, classified as a heliophyte and thermophile species, to adjust the activity levels of antioxidant substances and oxidases during the post-stress recovery process in accordance with the ecological requirements of the trees.

In the center region, the overall activity of antioxidant substances and oxidases remained consistently high on the first and third days post-thermal shock, including in the control sample. A noticeable decrease began after five days of exposure to extended doses, particularly between 40 and 60 minutes. The specific activity of these antioxidant substances, maintained at high and relatively stable levels in the initial days post-stress, indicates their adjustment through protective mechanisms to control reactive oxygen species. Furthermore, the reduction in antioxidant activity after five days of recovery from prolonged thermal doses suggests a metabolic and biochemical stabilization of downy oak trees, indicating their adaptation to thermal shock conditions.

In contrast, catalase activity exhibited a significant reduction on the first, third, and fifth days of recovery, showing a decreasing trend from short thermal doses (20 minutes) to extended thermal doses (60 minutes). Throughout the recovery period, catalase activity remained at lower levels compared to the control sample (Figure 3b).

The significant decrease in catalase activity during the recovery period, especially after exposure to prolonged thermal doses, suggests a specific adaptation of the plants to thermal stress. This adaptation may involve the regulation of gene expression and metabolism to mitigate the negative effects of thermal shock on cellular function and to maintain homeostasis under post-shock conditions.

In the southern region, the overall activity of antioxidant substances remained high during the first three days of recovery post-shock, with values exceeding those of the control sample. This indicates a strong response of these components in the leaf extracts to thermal shock. On the fifth day, a decline in the overall activity of antioxidant substances was observed, with this decrease becoming more pronounced with the extension of the shock duration. Regarding oxidase activity, it was elevated on the first day but somewhat diminished by the third day of recovery. However, on the fifth day post-shock, a marked decrease in activity was noted, comparable to the control sample (Figure 3c).

Regarding catalases, activity demonstrated significant increases after the first day of shock with extended exposure durations, reaching high levels. However, by the third and fifth days of recovery, there was a notable decrease in catalase activity, particularly after prolonged thermal doses.

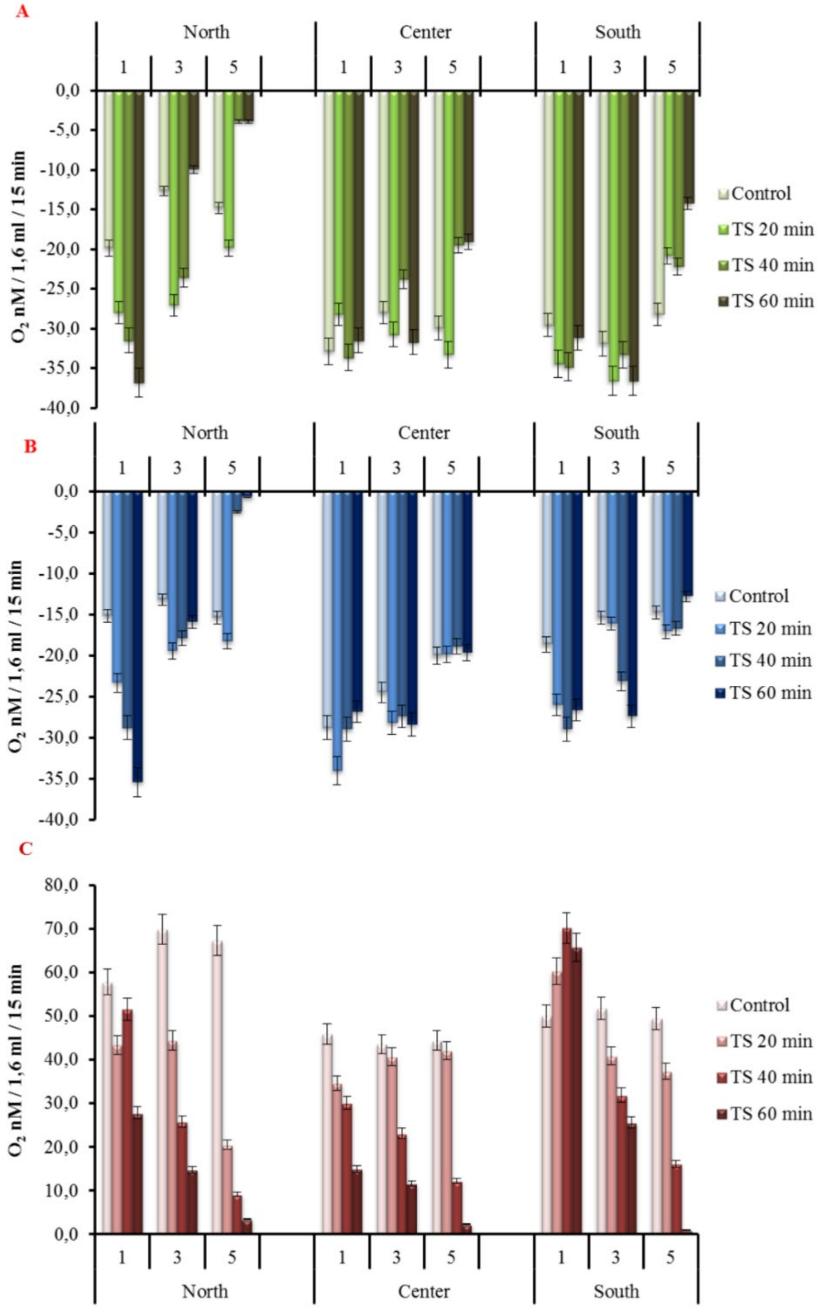


**Figure 3.** Dynamics of changes in the activity of antioxidant substances (A), oxidases (B), and catalases (C) in extracts from the leaves of downy oak, exposed to thermal shock at 50 °C for durations of 20, 40, and 60 minutes. Days 1, 3, and 5 – recovery of antioxidant components' activity on the first, third, and fifth day after thermal shock application

## Discussions

In recent decades, the health and survival of oak species have been alarmingly threatened, with oak dieback observed in forests worldwide [ALLEN & al. 2010; GENTILESCA & al. 2017]. Researchers believe that the decline and dieback of oak forests are caused by a range of factors, with climate change, prolonged droughts, and defoliating insects significantly contributing to this phenomenon [ROMAGNOLI & al. 2018]. Some studies suggest that tree species, including oaks, growing in regions with high precipitation are less resilient to high temperatures compared to those in arid regions characterized by prolonged droughts [HODGSON & al. 2015].

In our study, we compared three indigenous oak species growing in different ecological zones of Moldova, each characterized by distinct temperature and precipitation regimes. Oak leaves were subjected to thermal shock for varying durations at a constant temperature to elucidate their physiological responses, based on the overall activity of antioxidant substances, oxidases, and catalases. Measurements were conducted on leaf extracts at different recovery time points. We hypothesized that populations of these species develop optimal adaptations in specific habitats, where changing environmental factors positively influence their genotypes.

Our results highlight a robust response of pedunculate oak trees from the northern region to thermal shock, as evidenced by a significant increase in the overall activity of antioxidants and oxidases in leaf extracts on the first day post-shock. Interestingly, elevated levels of these substances are also observed in the leaves of oak trees from the center and southern regions, indicating a similar adaptation to thermal stress. These findings align with previous research demonstrating that plants can respond to prolonged thermal stress by increasing antioxidant activity [PINTÓ-MARIJUAN & al. 2013; ONO & al. 2021; NETSHIMBUPFE & al. 2023].

Additionally, we observed enhanced catalase activity on the first day after thermal shock in the leaf extracts of pedunculate oaks. However, following prolonged exposure to thermal shock, there is a significant decrease in catalase activity in oaks from the center and southern ecological zones of the republic. This observation suggests either a direct inhibition of catalase activity by high temperatures over an extended period or a reallocation of cellular resources toward alternative hydrogen peroxide detoxification pathways that may be more effective under prolonged thermal stress.

In a broader context, research on the plant *Arabidopsis thaliana* has revealed that prolonged thermal stress can lead to a significant accumulation of hydrogen peroxide in the plant leaves, with catalase activity being induced as a response to this stress. However, this adaptation does not appear to be sufficient to significantly enhance plant heat tolerance under the specific conditions of the study [ONO & al. 2021].

It is evident that the overall activity of oxidases in pedunculate oak leaf extracts, following thermal shock, is higher under conditions of effective recovery. This observation is supported by previous studies indicating that oxidase activity provides essential energy sources for cellular functions and helps to regulate reactive oxygen species within the mitochondrial electron transport chain during stress [FINNEGAN & al. 2004]. Thus, our study's results suggest a relationship between enzymatic activity and the efficiency of cellular recovery processes, providing a coherent explanation for the observed phenomena.

Analysis of the response of sessile oak to thermal stress reveals significant variations in the overall activity of antioxidant substances and oxidases in leaf extracts, influenced by specific ecological zones. In the center and southern regions, the activity of oxidases in sessile oak leaf extracts was elevated, surpassing the levels observed in control samples, and showed similar trends to those observed in pedunculate oak. This may reflect common adaptive responses of these oak species to increased oxidative stress levels.

In the southern region, a trend towards decreased overall activity of antioxidant substances and oxidases was observed in sessile oak leaf extracts, particularly after five days of recovery following extended thermal shock exposure. Catalase activity demonstrated a clear decline in the northern and southern regions following prolonged thermal exposure, whereas in the control samples, activity remained high, highlighting the recovery processes. The enhancement of these recovery processes in catalase activity appears to contribute to increased thermotolerance of leaves over time in these regions.

It is well-established that a plant's resistance to a stress factor depends on its ability to activate tolerance mechanisms and the presence of adaptive traits for avoidance, which are directly related to its habitat [LARCHER, 2004]. Specialized studies indicate that high temperatures can induce harmful effects on plants, including cellular membrane damage, protein inactivation, excessive production of reactive oxygen species, and disruption of key metabolic functions [RUELLAND & ZACHOWSKI, 2010]. Despite these investigations, recent research, such as that by ONO & al. (2021), has emphasized the importance of studying the specific plant responses to various thermal stress conditions, including the impact of thermal shock duration, whether short or prolonged.

In our studies on sessile oak, a species of both ecological and economic interest, we observed that prolonged exposure to thermal shock leads to a decrease in the activity of antioxidant substances and catalases. This variation in the metabolic and enzymatic response of sessile oak under different environmental conditions suggests the presence of complex and adaptive stress protection systems that are differentially activated depending on the duration of thermal shock and the recovery period.

In this context, the research conducted by WANG & al. (2023) on *Brassica campestris* is particularly relevant. Their study demonstrated that after exposure to thermal stress, there is a transiently induced expression of the BcWRKY22 gene, leading to the activation and significant increase in catalase activity. Concurrently, under thermal stress conditions, the expression of this gene showed the capability to reduce hydrogen peroxide accumulation. These findings highlight the crucial role of BcWRKY22 in modulating the response to thermal stress, positively impacting catalase activity and mitigating $H_2O_2$ accumulation through gene regulation. These results support our observations and suggest that the antioxidant protection mechanisms in sessile oak may be similar to those in other species, such as *Brassica campestris*, indicating a common adaptive strategy to thermal stress.

Another study conducted by SAHA & al. (2016) demonstrated that stress tolerance, through the attenuation and neutralization of reactive oxygen species, is mediated by the activity of the antioxidant enzyme alternative oxidase. This enzyme plays a crucial role in protecting leaves from oxidative damage through specific metabolic pathways, thereby contributing to the adaptation and survival of plants under abiotic stress conditions.

Our results highlight significant variability in the total activity of antioxidant substances, oxidases, and catalases, influenced by the trees' location, the duration of leaf exposure to thermal shock, and the recovery period. These findings underscore the complexity of protective mechanisms against thermal stress in sessile oak. Our observations are supported by similar studies conducted on other species [ONO & al. 2021; WANG & al. 2023], suggesting the existence of common adaptive processes in plant responses to thermal stress.

The dynamic analysis of the total activity of antioxidant substances and oxidases in the leaves of downy oak reveals significant aspects related to thermal stress adaptation across different ecological zones. In the northern zone, there is a notable increase in enzymatic activity on the first day of recovery, correlated with the intensity of the thermal shock. However, after three and five days of recovery, the activity of antioxidant substances progressively decreased. Conversely, in the center zone, there was an initially high activity of antioxidant substances and oxidases, but catalase activity diminished with prolonged exposure to thermal shock. In the southern zone, the activity of antioxidant substances, oxidases, and catalases declined after five days of recovery. These variations in enzymatic activity suggest that adaptive processes to thermal stress differ depending on the ecological zone and the duration of the recovery period.

The study conducted by DINAKAR & al. (2016) on *Pisum sativum* highlighted the importance of the alternative oxidase pathway in optimizing the photosynthetic process under thermal stress conditions in the mesophyll protoplasts of leaves. The capacity of the alternative oxidase pathway significantly increased with elevated intracellular levels of reactive oxygen species under suboptimal temperature and saturating light conditions. The authors demonstrated the crucial role of the alternative oxidase pathway in regulating reactive oxygen species and the antioxidant system's response to thermal stress.

When comparing our results with those from the *Pisum sativum* study, we observe that both emphasize the significance of oxidase activity, including the alternative oxidase pathway, in responding to thermal stress. While our study focused on how thermotolerance in downy oak leaves varies depending on the recovery period following exposure to thermal stress, the research on pea plants evaluated the role of the alternative oxidase pathway in optimizing photosynthetic activity. This difference in approach underscores the distinct responses of the two species to thermal stress, highlighting the enhancement of thermotolerance in downy oak and the optimization of photosynthetic activity in pea plants.

The findings presented here illustrate that the overall activity of antioxidant substances, oxidases, and catalases varies specifically depending on the oak species studied, taking into account the origin of the trees, the intensity of thermal shock, and the recovery period. The specific variation in the activity of oxidases and catalases in these oak species can be used as an indicator of recovery processes, reflecting the metabolic transformations involving these enzymes. An increase in the activity of these enzymes in the cells of the oak species studied suggests an efficient adaptive response to thermal stress and the species' ability to detoxify reactive oxygen species. Conversely, a decrease in activity might indicate either a reduction in thermal stress or a diminished need for an antioxidant response, or a deficiency in the antioxidant capacity, signaling potential oxidative damage at the cellular level. Thus, our study contributes to the understanding of adaptive mechanisms to thermal stress under various ecological conditions, highlighting the role of both antioxidant substances and oxidases and catalases in these adaptive processes.

When evaluating plant resistance, studies have reported that the activity of antioxidant enzymes increases under thermal stress to neutralize the reactive oxygen species generated (DAS & al. 2014; SEWELAM & al. 2016). These findings suggest that adaptation to thermal stress involves finely tuned regulation of both enzymatic and non-enzymatic activities to mitigate the negative effects of reactive oxygen species and maintain cellular homeostasis. Additionally, it has been observed that low activity of these enzymes may indicate two distinct scenarios:

1. **Low Stress levels:** If the thermal stress is reduced or the plant has effectively adapted and stabilized the reactive oxygen species, the antioxidant enzymatic activity decreases as the demand for neutralization diminishes.
2. **Inability to respond:** When enzymatic activity remains low despite the presence of thermal stress, it could indicate an insufficiency or “fatigue” in the plant's antioxidant response.

Building on previous discussions, it has been observed that pedunculate oak trees from center and southern regions exhibit an enhanced adaptive response to thermal stress, mirroring the reactions noted in sessile oak trees from the same areas. The comprehensive assessment of all antioxidant substances in downy oak revealed a decline in their activity in the leaves of trees from northern regions following a prolonged recovery period, particularly after five days. A similar trend was noted for catalase activity in trees from the central region. These findings suggest that cellular homeostasis in downy oak is reestablished after extended recovery periods following thermal stress. Therefore, both enzymatic and non-enzymatic adaptations allow oak species to maintain a balance between the production of reactive oxygen species and their detoxification capacity, thereby optimizing their function and survival under stress conditions.

## Conclusions

This study assessed the total activity of antioxidant substances, oxidases, and catalases in the leaves of three oak species (*Quercus robur*, *Q. petraea*, and *Q. pubescens*) subjected to thermal shock, followed by a five-day recovery period. The findings revealed distinct variations as well as common responses in antioxidant activity, influenced by the ecological origin of the oak stands in the Republic of Moldova.

Leaves of pedunculate oak and downy oak from center and southern regions, as well as sessile oak from the center region, exhibited consistently high total antioxidant activity throughout the recovery period. Additionally, increased oxidase activity was observed in pedunculate oak and sessile oak from the center and southern regions, and in downy oak from the center region. Moreover, high catalase activity was evident in the leaves of sessile oak from the central region. These specific antioxidant responses in particular ecological zones suggest an enhanced adaptive capacity of these trees to thermal shock.

Conversely, pedunculate oak and sessile oak trees from the northern region showed a decrease in total antioxidant and oxidase activity during the recovery period, indicating an increase in leaf thermotolerance. Similar responses were observed across all types of antioxidant substances in pubescent oak from the northern region, indicating an enhancement of leaf thermotolerance after extended recovery periods.

Overall, the results indicate that leaf thermotolerance in the investigated oak species varies and exhibits specific changes depending on environmental conditions. These adaptations reflect distinct responses of oak stands to thermal stress, highlighting regional variations in the physiological responses of oak species.

## References

ALLEN C. D., MACALADY A. K., CHENCHOUNI H., BACHELET D., MCDOWELL N., VENNETIER M. & COBB N. 2010. A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. *Forest Ecology and Management*. **259**: 660-684. https://doi.org/10.1016/j.foreco.2009.09.001

ASADA K. 2006. Production and scavenging of reactive oxygen species in chloroplasts and their functions. *Plant Physiology*. **141**: 391-396. https://doi.org/10.1104/pp.106.082040

BOECKX T., WEBSTER R., WINTERS A. L., WEBB K. J., GAY A. & KINGSTON-SMITH A. H. 2015. Polyphenol oxidase-mediated protection against oxidative stress is not associated with enhanced photosynthetic efficiency. *Annals of Botany*. **116**(4): 529-40. https://doi.org/10.1093/aob/mcv081

CHEN I. C., HILL J. K., OHLEMÜLLER R., ROY D. B. & THOMAS C. D. 2011. Rapid range shifts of species associated with high levels of climate warming. *Science*. **333**(6045): 1024-1026. https://doi.org/10.1126/science.1206432

COUMOU D. & ROBINSON A. 2013. Historic and future increase in the global land area affected by monthly heat extremes. *Environmental Research Letters*. **8**: 034018. https://doi.org/10.1088/1748-9326/8/3/034018

COUMOU D., ROBINSON A. & RAHMSTORF S. 2013. Global increase in record-breaking monthly-mean temperatures. *Climatic Change*. **118**: 771-782. https://doi.org/10.1007/s10584-012-0668-1

CUZA P. 2001. Sugestii privind conservarea diversităţii biologice a pădurilor din Republica Moldova. *Analele ştiinţifice ale universităţii de stat din Moldova. Seria „Ştiinţe chimico-biologice*": 181-186.

DAS K. & ROYCHOUDHURY A. 2014. Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. *Frontiers in Environmental Science*. **2**: 53. https://doi.org/10.3389/fenvs.2014.00053

DASCALIUC A., CUZA P. & FLORENŢĂ G. 2018. Potenţialul redox al extractelor din mugurii arborilor stejarului pedunculat, gorunului și stejarului pufos în funcție de anotimp. *Revista pădurilor*. **133**(4): 21-36.

DASCALIUC A., CUZA P. & GOCIU D. 2005. Starea şi perspectivele de ameliorare a pădurilor de stejar pufos (*Quercus pubescens* Willd.) din Republica Moldova. *Analele ştiinţifice ale universităţii de stat din Moldova. Seria „Ştiinţe chimico-biologice*": 405-413.

DINAKAR C., VISHWAKARMA A., RAGHAVENDRA A. S. & PADMASREE K. 2016. Alternative oxidase pathway optimizes photosynthesis during osmotic and temperature stress by regulating cellular ROS, malate valve and antioxidative systems. *Frontiers in Environmental Science*. **7**: 68. https://doi.org/10.3389/fpls.2016.00068

DULIÈRE V., ZHANG Y. & SALATHÉ E. P. 2013. Changes in twentieth-century extreme temperature and precipitation over the Western United States based on observations and regional climate model simulations. *Journal of Climate*. **26**: 8556-8575. https://doi.org/10.1175/JCLI-D-12-00818.1

FINNEGAN P. M., SOOLE K. L. & UMBACH A. L. 2004. *Alternative mitochondrial electron transport proteins in plants*, p. 163-230. In: DAY D. A., MILLAR A. H. & WHELAN J. (eds.). *Plant mitochondria: from genome to function*. Kluwer Academic Publishers, Dordrecht, The Netherlands.

GASTON K. J. 1994. *Rarity*. London: Chapman and Hall, 205 pp. https://doi.org/10.1007/978-94-011-0701-3

GENTILESCA T., CAMARERO J. J., COLANGELO M. & NOLE A. 2017. Drought-induced oak decline in the western Mediterranean region: an overview on current evidences, mechanisms and management options to improve forest resilience. *iForest - Biogeosciences and Forestry*. **10**(5): 796-806. https://doi.org/10.3832/ifor2317-010

GILL S. S. & TUTEJA N. 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry*. **48**(12): 909-30. https://doi.org/10.1016/j.plaphy.2010.08.016

GIURGIU V. 1972. *Metode ale statisticii matematice aplicate în silvicultură*. Bucureşti: Edit. Ceres, 567 pp.

HALLIWELL B. 2006. Oxidative stress and neurodegeneration: where are we now? *Journal of Neurochemistry*. **97**: 1634-1658. https://doi.org/10.1111/j.1471-4159.2006.03907.x

HANSEN J., SATO M. & RUEDY R. 2012. Perception of climate change. *Proceedings of the National Academy of Sciences*. **109**(37): 14726-14727. https://doi.org/10.1073/pnas.1205276109

HODGSON D., MCDONALD J. L. & HOSKEN D. J. 2015. What do you mean, resilient? *Trends in Ecology & Evolution*. **30**(9): 503-506. https://doi.org/10.1016/j.tree.2015.06.010

LARCHER W. 2004. *Ecofisiologia vegetal*. São Carlos: RiMa, 550 pp.

MENENDEZ R., GONZALEZ A., HILL J. K., BRASCHLER B. & WILLIS S. 2006. Species richness changes lag behind climate change. *Proceedings of the Royal Society*. *B*: *Biological Sciences*. **273**(1593): 1465-1470. https://doi.org/10.1098/rspb.2006.3484

NETSHIMBUPFE M. H., BERNER J., KOOY F. V., OLADIMEJI O. & GOUWS C. 2023. Influence of Drought and heat stress on mineral content, antioxidant activity and bioactive compound accumulation in four african *Amaranthus* species. *Plants* (*Basel*). **12**(4): 953. https://doi.org/10.3390/plants12040953

ONO M., ISONO K., SAKATA Y. & TAJI T. 2021. Catalase2 plays a crucial role in long-term heat tolerance of *Arabidopsis thaliana*. *Biochemical and Biophysical Research Communications*. **534**: 747-751. https://doi.org/10.1016/j.bbrc.2020.11.006

PINTÓ-MARIJUAN M., JOFFRE R. & DE AGAZIO M. 2013. Antioxidant and photoprotective responses to elevated $CO_2$ and heat stress during holm oak regeneration by resprouting, evaluated with NIRS (near-infrared reflectance spectroscopy). *Plant Biology*. **15**: 5-17. https://doi.org/10.1111/j.1438-8677.2011.00538.x

ROMAGNOLI M., MORONI S., RECANATESI F., SALVATI R. & MUGNOZZA G. S. 2018. Climate factors and oak decline based on tree-ring analysis. A case study of peri-urban forest in the Mediterranean area. *Urban Forestry & Urban Greening*. **34**(2): 17-28. https://doi.org/10.1016/j.ufug.2018.05.010

RUELLAND E. & ZACHOWSKI A. 2010. How plants sense temperature. *Environmental and Experimental Botany*. **69**(3): 225-232. https://doi.org/10.1016/j.envexpbot.2010.05.011

SAHA B., BOROVSKII G. & PANDA S. K. 2016. Alternative oxidase and plant stress tolerance. *Plant Signaling & Behavior*. **11**(12): e1256530. https://doi.org/10.1080/15592324.2016.1256530

SEWELAM N., KAZAN K. & SCHENK P. M. 2016. Global plant stress signaling: reactive oxygen species at the cross-road. *Frontiers in Plant Science*. **7**: 187. https://doi.org/10.3389/fpls.2016.00187

SOLIMAN W. S., FUJIMORI M., TASE K. & SUGIYAMA S. I. 2011. Oxidative stress and physiological damage under prolonged heat stress in $C_3$ grass *Lolium perenne*. *Grassland Science*. **57**(2): 101-106. https://doi.org/10.1111/j.1744-697X.2011.00214.x

THOMAS C. D., CAMERON A., GREEN R. E., BAKKENES M. & BEAUMONT L. J. 2004. Extinction risk from climate change. *Nature*. **427**: 145-148. https://doi.org/10.1038/nature02121

WANG H., GAO Z., CHEN X., LI E., LI Y., ZHANG C. & HOU X. 2023. BcWRKY22 activates BcCAT2 to enhance catalase (CAT) activity and reduce hydrogen peroxide ($H_2O_2$) accumulation, promoting thermotolerance in non-heading chinese cabbage (*Brassica campestris* ssp. *chinensis*). *Antioxidants*. **12**(99): 1710. https://doi.org/10.3390/antiox12091710

YAO Y., LUO Y., HUANG J. & ZHAO Z. 2013. Comparison of monthly temperature extremes simulated by CMIP3 and CMIP5 models. *Journal of Climate.* **26**: 7692-7707. https://doi.org/10.1175/JCLI-D-12-00560.1

**How to cite this article**:

CUZA P. 2024. Evaluation of antioxidant activity in oak leaves from the Republic of Moldova exposed to heat stress. *J. Plant Develop*. **31**: 37-52. https://doi.org/10.47743/jpd.2024.31.1.948