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THE INFLUENCE OF HEAT SHOCK AND DESICCATION ON BOXWOOD (*BUXUS SEMPERVIRENS* L.) LEAVES' PHOTOSYSTEM II AND ANTIOXIDANT SYSTEMS ACTIVITY

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Abstract: This research aimed to investigate the response of one- and two-year-old box (*Buxus sempervirens* L.) leaves to the action of heat shock (HS) and desiccation. These factors influenced the photosystem II activity of the leaves and the degradation of hydrogen peroxide by leaf extracts. The development of these processes was specific depending on the age of the leaves and season of their collection for analysis. Thus, the studied characteristics could assure the elaboration of new, rapid methods of assessing the resistance of leaves to high temperatures, depending on the period (season) of vegetation and their age. At different seasons of the year, the resistance of box leaves to HS tends to correspond to seasonal temperatures, reaching the highest level in summer, intermediate level in spring and autumn, and the lowest in winter. Regardless of the season, the one-year-old leaves are more resistant and have a higher capacity to recover from HS damage than the two-year-old leaves.

Keywords: photosystem II, leaves, heat shock, resistance, seasonal and age variations, *Buxus sempervirens*

Introduction

Under the influence of high temperatures in plants reactive oxygen species (ROS) [27, 15, 21] and damage to cellular structures are induced [7, 17]. The effectiveness of these processes depends on the genetic specificity [19], the physiological state of the plants [7], and the dose of the stress factor [19, 21]. Given the complexity of the processes involved in the response and adaptation of plants to thermal stress, this poses a serious threat to plants, especially in the light of the threat from global climate warming [11]. Photosynthesis is among the functions of plant cells sensitive to stress caused by extreme temperatures and inhibition before altering other cellular processes [24]. High-temperature response target sites are integrated into photosystem II (PSII) and generate ROS [27, 24]. The high-temperature stress also generates heat shock proteins and secondary metabolites [17].

The resistance of plant leaves to the action of high temperatures depends on thylakoid membrane sensitivity and determines the activity of photochemical reactions of chloroplasts. Several studies have shown that the leading cause of heat inhibition of photosynthetic function is associated with the level of PSII activity [3, 31, 30]. Plants have developed several molecular mechanisms to cope with high temperatures and protect the photosynthetic system from damage. It has been shown that increased ambient temperatures activate several mechanisms of increased damage provoked by heat. They include the splitting-up of proteins from the light-harvesting complex of the PSII [29, 23] and temperature-induced conformational changes in the PSII complex [12]. The conversion of violaxanthin to zeaxanthin assures the stabilization of the PSII in thylakoid

membranes of plant leaves exposed to doses of heat shock that do not exceed their resistance [14, 13].

In the last decades, the mechanisms that determine the thermotolerance of plants have been studied. The specificity of plants' reaction to the action of high temperatures was monitored by recognizing their influence on the dynamics of chlorophyll fluorescence parameters [6, 5]. In the presence of light, the transfer of energy and electrons occurs. These processes induce the formation of the reactive oxygen species ROS, which are responsible for the activation of protein oxidation and the functional modification of PSII activity. In addition, in the presence of light [22] and exposure to high temperatures [1], ROS inhibit *de novo* protein biosynthesis.

To study the specificity of the adaptation of plants to extreme temperatures, we chose the ornamental shrub box (*Buxus sempervirens* L.), which is evergreen, unaffected by soil conditions, and resistant to intense summer and winter temperatures [16, 10]. The box plant possesses leaves of one-, two- and three-year-age, photosynthetically active all year. These suggest that box can serve as a model subject to investigate specificity of adaptation to different seasonal conditions, depending on the age of the leaf. There are several methods to study the effects of stress factors on plants. That said, most of them require substantial time with reduced possibilities to involve a sufficient number of research samples, limiting their widespread use. We overcome these difficulties by means of the chlorophyll fluorescence method, fast, sensitive, and non-destructive [18].

We aimed to study the resistance and ability to restore the state of functional balance to one- and two-year-old box leaves after desiccation or application of heat shock (HS) with different temperatures. The leaves reached their homeostasis state on the 12th day after applying HS at different temperatures (period sufficient to achieve the maximum possible level of restoration of PSII functions after application of HS). We also investigated the activity of degradation of hydrogen peroxide (H_2O_2) of leaf extracts with a different desiccation level.

Material and Methods

In our research, we used one- and two-year-old leaves of box collected from plants growing in the protection area of the Institute of Genetics, Physiology, and Protection of Plants, Chişinău. To study the influence of HS on the box leaves, they were immersed for 5 minutes in distilled water at different temperatures (24–61°C), subsequently determining the photosystem II (PSII) activity. The design of the experiments was completely randomized with six replicates. We simultaneously studied the suppression of photosynthetic processes and the ability to recover the damage provoked by exposure of leaves to HS. For this, we incubated the box leaves in glass crystallizers on filter paper moistened with the recommended solution for *in vitro* culture of isolated roots [32] (excluding vitamins and sucrose), with a relative air humidity of 100%. The leaves were illuminated for 12 days with luminescent lamps at the intensity of photosynthetic active radiation (PAR) 60 μmol m^{-2} s^{-1}, a day length of 16 hours, and a day/night temperature of 26/24°C. We determined the PSII activity dynamics at different periods after exposure of leaves to HS with different temperatures. To assess the effect of temperature on the photosynthetic machinery, the leaves were pre-treated by immersing them for 5 minutes in distilled water at various temperatures between 50 and 61°C, maintained at accuracy ±0.05°C with the help of the ultrathermostat U-10 (Germany). In experiments previously carried out with oak leaves [8] and here repeated with those of box, we demonstrated that at the mentioned duration of exposure to

excessive temperatures, the damage to the leaves mainly depends on the extensive factor (temperature). In these conditions, the influence of the phenomena of extension of deterioration caused by the extensive factor (duration of exposure) is minimized. In earlier mentioned conditions, the leaves from the control variant maintained viability at a constant level for at least 30 days, and the leaves preliminarily exposed to HS, depending on the dose, accrued the maximum quantum yield of PSII, specific (characteristic) for each treatment of HS, during 3–10 days after the application. It is essential to mention that the maintained isolated box leaves viability sharply decreased when under artificial conditions they were illuminated at a light intensity lower than 20 μmol m^{-2} s^{-1} or higher than 80 μmol m^{-2} s^{-1}. The effective quantum yield of PSII $Y = [(F_m' - F_t) / F_m']$ measured with a PAM-2100 chlorophyll modulated fluorimeter (*H. Walz*, Germany), after irradiation of control leaves and those treated with HS at PAR activity equal to 20 μmol m^{-2} s^{-1} for 15 min at 25°C before each measurement. F_m' is the maximum fluorescence yield reached with a saturation pulse when the leaf is irradiated, and F_t represents the fluorescence yield measured at the moment, under equilibrium conditions. The minimum fluorescence (F_o) was excited at 655 nm with a modulation frequency of 600 Hz, and the maximum fluorescence (F_m'), was measured with a modulation frequency of 20 kHz. F_m' was generated by 0.8 s saturated flashes from a built-in miniature halogen lamp. Finally, the quantum efficiency of photosynthesis is determined by assessing the value of the Yield parameter (Y) from the equation: $Y = [(F_m' - F_t) / F_m']$.

In particular experiments, the decrease of the functional state after desiccation of the leaves, taken for analysis in the winter, was determined. The leaves were gradually dehydrated from 73% (initial humidity) to 5% (when the parameter Yield decreased up to zero) by incubating them in a thermostat at 25°C, with the air humidity at 60%. Subsequently, the leaves were rehydrated by their being placed on moistened filter paper and incubated under conditions identical to those used to determine the influence of HS on the effective quantum yield of PSII of control leaves. The percentage of initial humidity (H_i) of the leaves was determined based on the difference between the initial mass (M_i), and that determined after incubating them at 105°C over a period, sufficient to reach constant final weight (M_f) by applying the equation: $H_i = [(M_i - M_f) / M_i] 100\%$. The humidity at intermediate points, at time t (H_t), was determined from equation $H_t = [(M_t - M_f) / M_i] 100\%$, where Mt is the mass of leaves after t hours of desiccation. The influence of dehydration and subsequent rehydration of box leaves on PSII activity was determined using the fluorimetry method described above.

The capacity of extracts from leaves to degrade hydrogen peroxide is recognized by using the oximetry technique. First, 200 mg of leaves were homogenized in 2 mL of buffer solution of 0.2 M Tris-HCl, pH 7.4, and incubated for 30 minutes at 25°C, then centrifuged for 15 minutes at 4000g. For initiation of the enzymatic reaction, 40 μl of extract from leaves and 60 μl of 0.05% hydrogen peroxide were mixed with 1.5 mL of a buffer solution with 0.2 M Tris-HCl, at pH 7.4. The activity of H_2O_2 decomposition, under the influence of extracts from leaves, was calculated to 1 mg of protein, determined according to the method of Bradford (1976). The dynamics of oxygen removal due to enzyme activity in the extracts were determined using the YSI 5300A oximeter (USA). The index determined with the help of the oximeter estimates the total capacity (enzymatic and non-enzymatic) of hydrogen peroxide detoxification, expressed relative to 1 mg of the proteins in the extract. Although catalase and the antioxidant substances extracted from the leaves ensure only part of the ROS detoxification processes their summary activity correlates with the physiological state of the leaves being under favourable conditions or stress.

The data obtained were processed statistically, determining the mean value and the standard deviation of the mean [4, 26].

Results

The data included in Figure 1 suggest that initially (curve 1), the quantum yield of PSII of the leaves in the control variant was relatively low, reaching only 0.3 units. The low value of the PSII activity was because the leaves were under winter stress conditions, determined by the combined influence of negative temperatures and partial desiccation caused by frost. We emphasize that the initial humidity of the two-year-old leaves, taken for analysis in winter, was equal to 73%, and in the spring, their water content increased to 77%. There is information [25] that the processes of sugar biosynthesis use phosphates and reduce phosphorylation efficiency due to slowing down electron transport. The reduction of the water content of the leaves during artificial desiccation from 73% to 53%, then to 5%, was accompanied by decreasing quantum yield of PSII from 0.30 to 0.11 and 0, respectively (curve 1). In the first interval, the relative change of the PSII activity and H_2O_2 degradation, calculated to one percent of desiccation, were practically equal (2.4 and 2.6, respectively). In the next interval (interval two), these rates were characterized by values that differ, 2.6 and 1.3, respectively. Thus, the efficacy of PSII decreased uniformly throughout the range of reducing water content in leaves (from 73% to 5%). Compared to the second interval of leaf dehydration, H_2O_2 degradation in the first interval of the dehydration period was more substantial. The combined influence of several processes on reducing the quantum yield of the PSII increased with the level of leaves dehydration. In parallel, the rise in activity of H_2O_2 degradation enzymes with desiccation slowed down due to decreasing the activity of degradation of the proteins implicated in these processes. As the result of gradual leaf desiccation from 73% to 5%, the quantum yield of PSII decreased from 0.3 to 0. We initiated rehydration by placing the leaves on wet filter paper and incubating them in a thermostat with a relative air humidity of 80–90%. After three days of exposure to these conditions, the water content of the leaves increased from 5% to 68% (Figure 1, curves 2 and 4). Although after the completion of rehydration, the water content in leaves was lower than the initial level (compare 68% and 73%), the final restoration efficacy of PSII was at a higher level (compare 0.3 up to 0.76). The activity of H_2O_2 decomposition under the influence of leaf extracts was higher (compared to 0.0087 and 0.0124 U/mg). It follows that the conditions of incubation of leaves for hydration were those for restoring their viability. The presented data confirm those in the literature about the negative influence of ROS on plant viability during desiccation [9]. In general, we can conclude that both the dynamics of PSII activity and the activity of enzymes and substances with reducing properties that catalyze the degradation of H_2O_2 can serve as indicators of changes in the functional state of box leaves subjected to dehydration and rehydration.

The data shown in Figure 2 demonstrate that the activity of the processes determining the decomposition of H_2O_2 in extracts from the leaves taken for analysis in different seasons of the year was specific. The relatively low activity of these enzymes was characteristic of the box leaves taken for study in the winter and summer, significantly higher in the leaves in the spring and autumn. We tested the above conclusion in research with box leaves of 1 and 2 years of age, taken for analysis in the middle of seasons (winter, spring, summer, and autumn). We consider these indices the summary activity of metabolic processes that ensure the maintenance of the viability of leaves and whole plants in each season of the year. In the winter, the metabolic processes and

their indirect influence on ROS formation depend on maintaining the leaves' vitality. In other seasons, the biochemical processes necessary for adaptation and functioning of the leaves under specific season conditions combine with supplying the whole plant with organic substances synthesized in the leaves due to photosynthesis. These biochemical processes require activation of photosynthesis, associated with increased ROS formation and the induction of the functions necessary for their degradation. Interestingly, the data in Figure 2 suggest that in extracts from one-year-old leaves, regardless of the season, the activity of reductive substances and enzymes that catalyze the degradation of H_2O_2 was significantly higher compared to that in the solution of extracts from the two-year-old leaves. ROS degradation enzymes' higher activities suggest that metabolic processes in one-year-old leaves were also higher.

To assess the specificity of the processes of inhibition and restoration of PSII activity after exposure of box leaves to HS at different temperatures, we determined their relative efficiency of the quantum yield of PSII with that of controls (Figure 3). After HS's application, the quantum yield of PSII decreased, reaching the minimum value specific for each exposure temperature. Subsequently, this level gradually increased. The higher HS's temperature, the deeper dropped the value of the quantum yield of the PSII, and later after exposure to HS, it reached the minimum. Also, the higher the temperature of exposure to HS, the more pronounced the tendency to slow down the recovery and reach the final level of recovery (practically constant) of the quantum yield of PSII. After exposure to HS with relatively moderate temperatures (between 50° and 54°C), the achieving of minimal values of the quantum yield of PSII manifested on the first day after exposure to HS; the recovery was completed on days 3-5 after its application. As a rule, in these variants, the relative values of the quantum yield of PSII after recovery exceeded the value characteristic for control leaves. After the exposure of leaves to HS with temperatures between 56° and 58°C (the temperatures of exposure to HS being relatively high), the period necessary for reaching the minimum values of the quantum yield of PSII was longer and accompanied by incomplete restoration of PSII activity. Exposure of leaves to HS at temperatures higher than 58°C has led to an irreversible decrease in the quantum yield of PSII up to zero. Exposure to temperatures higher than 58°C provoked irreparable damage to leaves, and the PSII has lost the capacity to restore. These data suggest that the application of HS for 5 minutes with temperatures below 54°C caused the reversible decrease of the functional activity of PSII. HS at higher temperatures (between 54° and 58°C) provoked severe damage, and recovery of the PSII activity was only partial. After HS a temperature of 8°C and higher exceeded the zone of PSII tolerance. Based on the evaluation of the dynamics of the relative quantum yield of PSII after applying HS with different temperatures for 5 minutes on the box leaves, the distribution by their response was divided into three zones: 1 – tolerance zone (HS with temperatures lower than 54°C); 2 – the area of partially reversible damage (HS with temperatures between 54 and 58°C); 3 – irreversible damage area (HS with temperatures exceeding 58°C). After exposing the leaves to HS with temperatures compatible with the leaf's viability, the PSII efficacy restoration manifested over 5–10 days. After recovery, the level of quantum yield of PSII remains virtually constant for at least 30 days. Due to this, the summary response of the box leaves to action of HS at different temperatures can be characterized by determining the parameters quantum yield of PSII in days 10-12 after its application. In general terms, the response of box leaves to HS at different temperatures was similar to that obtained with oak leaves [8].

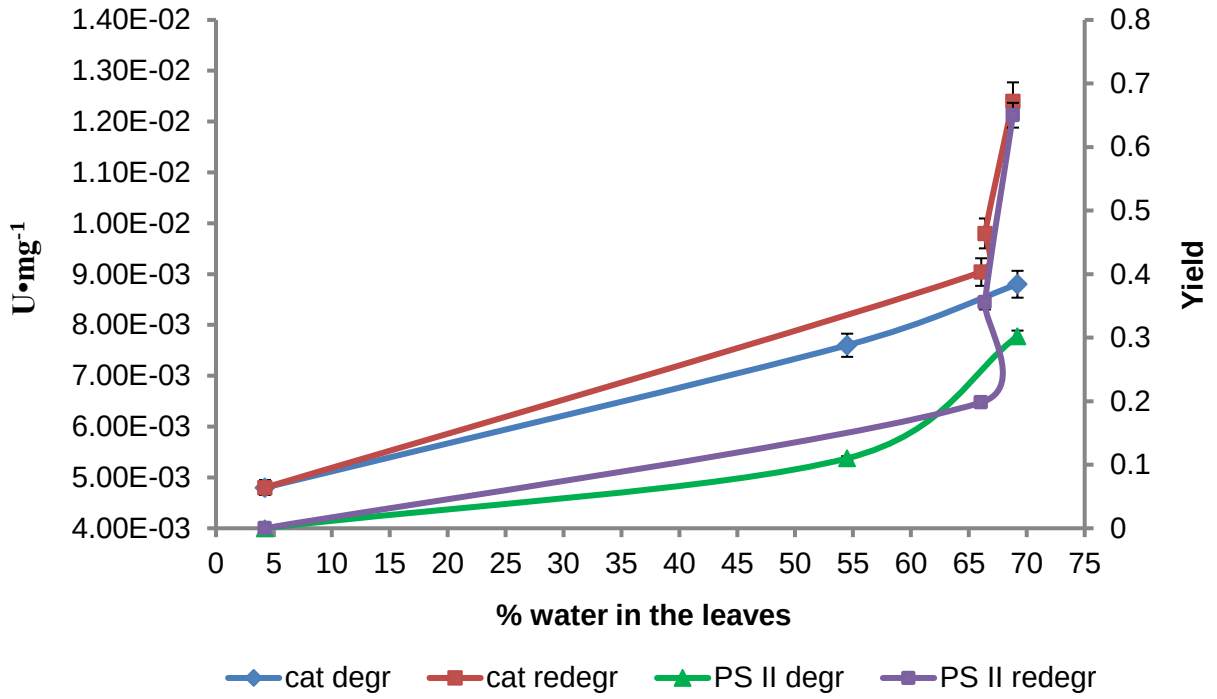

Fig. 1: The Yield dynamic of box leaves PSII (curves 1 and 2 - right ordinate), and activity of the H_2O_2 degradation catalyzed by the extracts from leaves, expressed as the catalase U of activity per 1 mg of proteins (curves 3 and 4 – left ordinate), during dehydration (curves 1 and 3), and subsequent rehydration (curves 2 and 4)

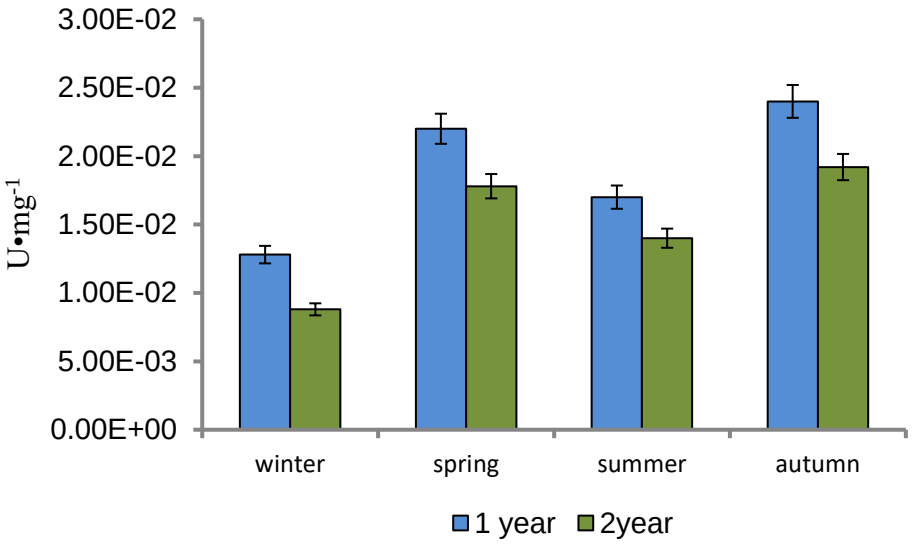

Fig. 2: The activity of hydrogen peroxide-degrading compounds (expressed as the catalase U of activity per 1 mg of proteins) in extracts from the one- and two-year-old leaves of box, taken for analysis in the winter, spring, summer, and autumn. The expression of the activity is in unities of catalase activity per 1 mg of protein in extracts

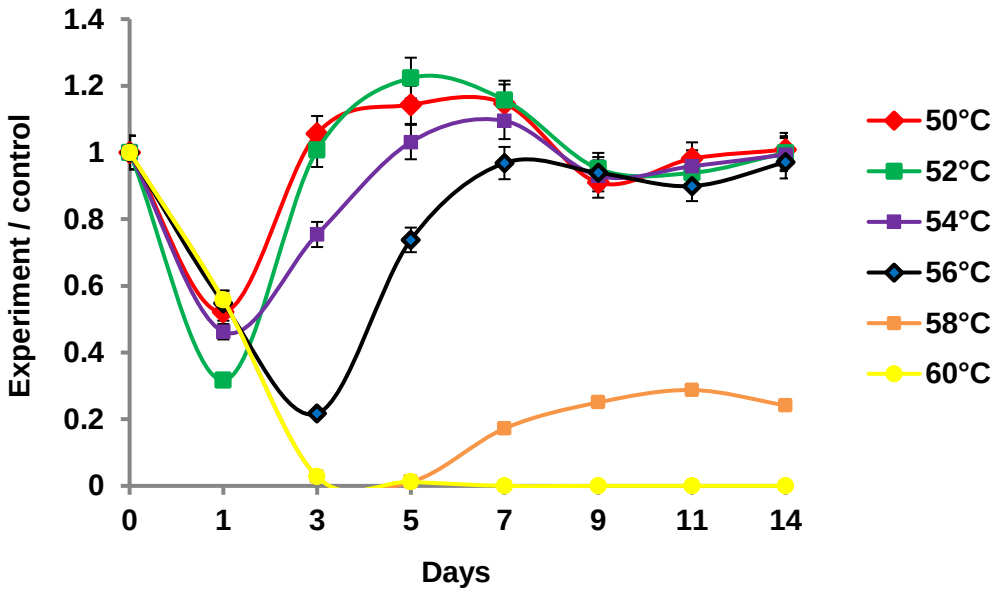

Fig. 3: The ratio of quantum yield of PSII of box leaves taken for analysis in the summer and exposed for 5 minutes to HS with different temperatures in experimental variants to that of the quantum yield of PSII of leaves of the control variant

As mentioned above, the processes that affect the kinetics of PSII recovery after exposing the leaves to HS at different temperatures largely depend on ROS detoxification activity, the contents of which increase with increasing the activity of metabolic processes implicated in recovering damages. We studied the summary influence of the reductive substances and the activity changes of enzymes that determine the H_2O_2 decomposition in the extracts from leaves exposed to HS at various temperatures (Figure 4), with temperatures between 50° and 54°C (zone of tolerance) depending on the HS temperature. The data in Figs. 4 and 3 demonstrate that after HS was applied, the activity of H_2O_2 decomposition was high over a long time. The activation of metabolism and, consequently, the induction of ROS formation have been long-lasting. Activation of metabolism after exposition to HS was caused by maintaining the repair processes and, later, by restoring the leaves' viability. The HS with temperatures that caused damages that were only partially recoverable (HS temperatures between 54° and 58°C) was associated with decreasing the decomposition of H_2O_2 processes.

The width of the temperature zones of HS that caused the rapid decrease of the quantum yield of PSII is approximately the same, but the temperatures that constitute each zone differ. If we consider the range of the HS temperatures that determined the decrease of the relative quantum yield of PSII from 0.2 up to 0.8, we can mention that the width of this zone practically did not exceed 2°C (Figure 5). Due to specific adaptation to seasonal conditions of the leaves collected in summer, this zone is shifted by at least 4°C in the region with higher temperatures than similar areas, characteristic of the leaves collected for analysis in the winter. The temperature zones of HS that cause the partially reversible recovery of damage to the leaves taken for study in the spring and autumn practically overlap and occupy intermediate positions between zones that characterize the winter and summer leaves. The data presented in Figures 3, 4, and 5 indicate the complementarity between the quantum yield of PSII and the activity of decomposition H_2O_2 processes. In general, the parameters mentioned characterize the level of damage and recovery

after exposure of box leaves to HS at different temperatures. The processes of restoring of PSII efficacy correlated with the specific activity of reductive substances and enzymes participating in ROS degradation.

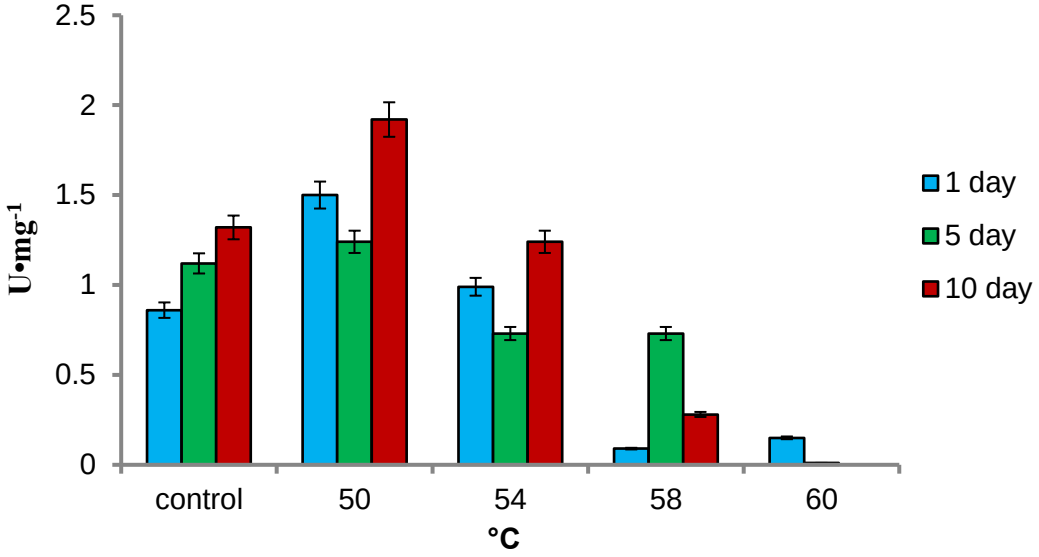

Fig. 4: The peroxide-degrading activity of extracts (expressed as the catalase U of activity per 1 mg of proteins) from two-year-old leaves, taken for analysis in summer, in control and experimental variants, exposed 5 minutes to HS with temperatures 50º, 54º, 58º and 60ºC, and analyzed on day 1, 5, and 10 after the application of HS. The activity peroxide-degradation represented as the unities of catalase activity per 1 mg of protein in extracts

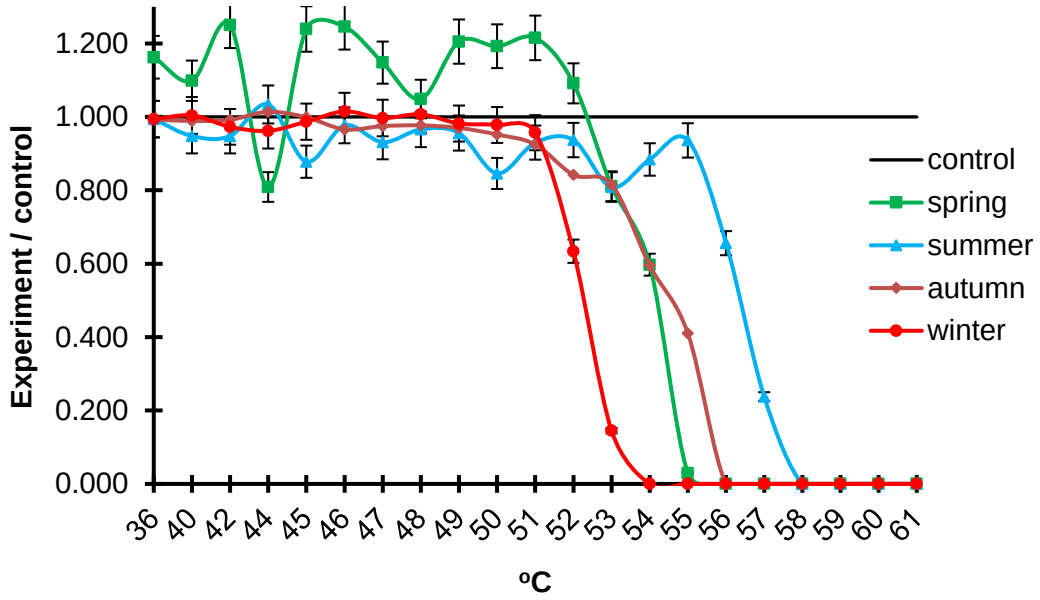

Fig. 5: The ratio of the quantum yield of PSII of the experimental variants to that of the control variant of the box leaves taken for analysis in the winter, spring, summer, and autumn, exposed to HS, for 5 minutes with different temperatures, and analyzed on day 12 after the exposure to HS

Discussion

The data presented above allowed us to propose two accelerated methods for determining the damage caused to box leaves by drought or high temperatures. They suppose desiccation or exposing the leaves to HS. Following the first method, gradual desiccation of the leaves is carried out until the quantum yield of PSII reaches the value of 0.2. According to the second method, the fixed time of leaves exposure to HS with a wide range of temperatures is supposed and appreciating in parallel with the temperature level that completely suppresses the activity of PSII. These methods allow dividing the HS temperatures into zones with different consequences for the efficiency of the quantum yield of PSII and the specific temperature values at the fixed period of exposition to HS. The season and age of leaves also influence these zones' characteristics. The seasonal variability of tolerance of leaves to HS and its influence on reducing the quantum yield of PSII depend on the variation of cell membrane fluidity and the specificity of light-harvesting and electron transfer structures in thylakoids [11, 17]. As seasonal conditions and the age of the leaves influence these processes, it becomes clear that the leaf's response to HS depends on the acclimation of PSII to seasonal temperatures and the ability of leaves to recover the damage caused by HS. In conditions with high temperatures, the functional activity of PSII depends on slowing down the processes of electron transport in the photosynthetically-excited leaf area as a result of the dissociation of the light-harvesting complex and partially reversible inactivation of PSII [34]. Also, the available information suggests that induced ROS after plant exposure to HS provokes damage leading to a diminution of the effectiveness of PSII [33, 17].

The distribution of box leaves in zones with qualitative-different responses to HS temperatures is determined by the temperature level (the intensity factor, variable) and the duration of exposition (the extensive factor, constant in our investigations). We intend to study the influence of HS duration on temperature ranges in the future mentioned zones. Previously we demonstrated that the period of exposition to HS qualitatively influenced the leakage of electrolytes from the box leaves [20]. Therefore, we suppose that the regularity elucidated in the presented research will also characterize the response of the box to different durations of HS exposure. The data presented here suggest that the thermotolerance of box leaves in the summer was significantly higher than those collected in winter and other seasons due to adaptation. Regardless of the season, the one-year-old box leaves were more resistant to HS than those that are two years old. They were more tolerant by leaf resistance in the winter (Figure 1) and by the capacity to acclimatize at high temperatures, fully manifested in the summer (Figure 4). In general, the dynamic of quantum yield of PSII, combined with enzymes catalyzing the degradation of H_2O_2, represents essential parameters that characterize the change of the thylakoids of the chloroplasts' functional state depending on the age of the leaf age and the season. The value of the relative quantum yield of PSII depends on the equilibrium between processes that promote HS damages and the benefits from their repair. The integrity of the light-transferring complex, the capacity for its reactivation, and the enzymatic and non-enzymatic quenching of the ROS, especially H_2O_2, are the main factors determining the restoration of the functional state of PSII. The balance between these two groups of processes determined PSII quantum efficiency. After box leaves' exposure to HS with moderate temperatures, the activity of PSII was established at a constant level on days 5-10 after applying HS. As shown by the data in Figure 5, this equilibrium level sharply decreases when the HS temperatures exceed individual values that mark the transition in zone II of HS temperatures (the area of limited tolerance). The temperatures that make up this zone are specific to the leaves taken

for analysis at different seasons. The size of this zone is about 4^{o} C (see Figure 5). With increasing HS temperature (the extensity factor), homeostasis restoration was increasingly delayed and established at a lower level. The severity of damage influenced this level, and ROS's high content partially accelerated the inhibition of functional recovery of the PSII.

In general, the results presented confirm the data obtained by us previously with oak leaves [8, 5]. They demonstrate that extending HS duration (extension factor) only quantitatively affects the ability of leaves to recover from HS damage. The data presented above suggest that after HS application, the physiological state of the leaves depends on the nature of the injuries, their severity increasing with increasing HS temperature. From a practical point of view, it is essential to appreciate the arrangement and amplitude of the temperature range in zone II (the area of limited tolerance), which would occur upon exposure to HS under natural conditions (after a long-term exposition). The temperature range of this zone is specific for different species of plants and related genotypes. By specifying these parameters, we can appreciate the consequences of the threat of global warming for vegetation because, by prediction, the average global temperature will increase by 0.3°C every ten years until 2100 [28]. In the light of the data presented, it is necessary to elucidate the PFII reaction of box leaves to shock, depending on the age of the leaf, in different seasons of the year with negative temperatures.

Conclusions

1. The resistance of box leaves to the action of HS, or desiccation, can be assessed in an accelerated manner using the following procedures:

a) The exposure of leaves to HS carried out during a fixed time, with different temperatures, and appreciating the dynamics of the change of the efficiency of PSII, or the activity of hydrogen peroxide degradation processes;

b) The gradual desiccation of leaves and determining the dynamics of the change of the efficiency of PSII or the hydrogen peroxide degradation processes;

2. At different seasons of the year, the resistance of box leaves to HS tends to correspond to seasonal temperatures, reaching the highest level in summer, intermediate in spring and autumn, and lowest in winter.

3. Regardless of the season, one-year-old box leaves are more resistant and have a higher capacity to recover from HS damage than do two-year-old leaves.

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INFLUENȚA ȘOCULUI TERMIC ȘI A DESHIDRATĂRII ASUPRA FOTOSISTEMULUI II ȘI A ACTIVITĂȚII SISTEMELOR ANTIOXIDANTE ALE FRUNZELOR DE CIMIŞIR (*BUXUS SEMPERVIRENS* L.)

(Rezumat)

Cercetătorii au avut ca scop să investigheze răspunsul (reacția) frunzelor de cimișir (*Buxus sempervirens* L.) cu vârsta de unul și doi ani la acțiunea șocului termic (ȘT) și a deshidratării. Acești factori au influențat activitatea fotosistemului II în frunze și degradarea peroxidului de hidrogen în extractele din frunze. Desfășurarea proceselor respective a fost specifice în funcție de vârsta frunzelor și anotimpul colectării lor pentru analize. Procesele studiate ar putea asigura elaborarea unor metode noi, rapide, de apreciere a rezistenţei frunzelor la temperaturi ridicate, în funcţie de perioada (sezonul) de vegetaţie şi de vârsta acestora. În diferite anotimpuri ale anului, rezistența frunzelor de cimișir la acțiunea ȘT tinde să corespundă temperaturilor sezoniere, atingând cel mai ridicat nivel vara, nivel intermediar primăvara și toamna, iar cel mai scăzut iarna. Indiferent de anotimp, frunzele de un an sunt mai rezistente și au o capacitate mai ridicată de recuperare a deteriorărilor provocate de ȘT în comparație cu frunzele de doi ani.

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