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RESEARCH ARTICLE

Study of the resistance of *Arum korolkowii* to high-temperature impacts of different intensity at physiological, biochemical, molecular and genetic levels

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Abstract

An investigation was carried out to study the number of physiological, biochemical, molecular and genetic reactions of the wild plant *Arum korolkowii* to high-temperature effects of different intensities. The seedlings showed different reactions to high hardening and damaging temperatures at the physiological and biochemical levels leading to an increase in the heat resistance of plants, inhibition of growth, and a slight decrease in the hydration of leaf tissues depending on the temperature range and the exposure time. However, the effect of damaging temperatures on plants led only to a short-term increase in their heat resistance, causing a complete stop of growth and a sharp drop in the hydration of leaf tissues.

Keywords: Temperature stress, *Arum korolkowii*, heat shock proteins, gene transcription, resistance, plant survival

Introduction

The ambient temperature is one of the environmental factors that strongly impacts plants' vital activity and productivity. Almost always excess heat negatively affects the process of plant growth and development, often causing their death (Mosa et al. 2017). Any deviations of the ambient temperature from the values optimal for the growth and development of plants cause a wide range of physiological, biochemical and molecular genetic changes in their cells and tissues. It is associated with adaptation or with the appearance of various disorders and/or damage, the development of destructive processes that ultimately lead to the death of plants. Among many reactions of plants in response to an increase in temperature, the most fully studied features of growth, respiration and photosynthesis, as well as the synthesis of stress (shock) proteins, changes in membranes and hormonal system in the plant (Hatfield and Prueger 2015; Niu and Xiang 2018). It is noted that most of these changes are non-specific (general), although specific changes have been recorded, inherent only in plants experiencing high-temperature exposure (Bahuguna and Jagadish 2015; Wan and Jiang 2016). At the same time, the peculiarities of changes in physiological, biochemical, molecular and genetic parameters in plant cells and tissues under high-temperature influences of different intensity remain insufficiently studied. Numerous data indicate that the nature and direction of changes in many of these indicators can vary significantly (both quantitatively and qualitatively) depending on the intensity of the unfavorable factor, which is probably important, if not the main

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importance for the process of stability formation in these conditions.

However, it is only at the optimal temperature that the genetically determined growth, development and production opportunities are fully realized in plants. At temperatures above or below the optimum, these processes slow down or stop, but the processes of adaptation and/or damage are activated. The optimal temperature values are not the same for different types of plants. For instance, wheat, the temperatures ranges from 13 to 22°C depending on the type and place of growth (Hatfield and Prueger 2015).

Violations of cell division processes may be one reason for slowing down plant growth in unfavorable temperature conditions. Thus, with an increase in the temperature acting on plants, the number of dividing cells sharply decreases (et al. 2015), at temperatures above 40°C, the structure of chromosomes is destroyed and cell division completely stops. In addition, the fission effect of high temperatures significantly affects cell growth by stretching. Intracellular turgor pressure is considered to be the driving force of cell stretching. Under the action of high temperatures, the plant may experience a shortage of water and, accordingly, there is a decrease in intracellular turgor pressure, which greatly slows down cell growth. Plant growth is also affected by changes in the content of phytohormones. This can be observed at a significant increase in the content of growth inhibitors (abscisic acid and ethylene) at the initial moment of the action of high temperatures against and at a decrease in the content of growth-activating hormones (auxins, gibberellins, cytokines). This is one of the main reasons for a decrease in plant growth rates. Other equally important causes of growth inhibition include disturbances in energy processes – photosynthesis and respiration. As a rule, the temperature optimum of growth does not coincide with the optimum of photosynthesis and respiration. Respiration is inhibited at much higher temperatures than growth and photosynthesis (see below). Therefore, under conditions of temperatures above 35°C the consumption of organic substances for respiration exceeds their formation during photosynthesis, which can lead not only to growth inhibition but also to a decrease in the dry mass of plants. The growth of plants is also significantly influenced by the spectrum of synthesized proteins. Thus, under the action of high temperatures, the synthesis of some proteins increases, most often stressful, and the synthesis of so-called "household" proteins decreases. All of the above can lead to a decrease in plant productivity and sometimes to their death.

An increase in ambient temperature causes not only physiological and biochemical but also serious molecular genetic changes, the main of which is the reprogramming of the cell genome, as a result of which the synthesis of some proteins (most often stressful) is induced and/or increases and the synthesis of other proteins decreases (Fig. 1). Currently, it is believed that the synthesis of stress proteins plays an important, possibly key role in the formation of increased heat resistance in plants.

Activation of the expression of genes responsible for the synthesis of heat shock proteins (HSP) is one of the first non-specific reactions of plants to the effects of adverse temperatures. Various signaling molecules (calcium ions, reactive oxygen species (ROS), etc.) and transcription factor (TF) are involved in the activation of HSP gene transcription. In the promoter of HSP genes, there is a conservative sequence-5'-aGAAg-3', called the "heat shock element." Transcription factors of heat shock (HSF) interact with these elements, activating the synthesis of HSP.

Despite researchers' great interest in synthesizing HSP in plants, information about their changes in plant resistance formed under high-temperature influences of different intensity are rather fragmentary. It is known that *Aloe vera* (L.) Burm. with an increase in temperature (from 25 to 45°C) the genes expression level of HSP70, HSP100 and ubiquitin increases (Huerta et al. 2013). In *Arabidopsis thaliana*, at a temperature of 37°C, which does not affect cell viability, the content of HSP101, HSP70 proteins sharply increases and the content of HSP17,6 increases to some extent, and damaging temperatures (above 39°C) led to a decrease or complete inhibition of the synthesis of these proteins. HSP60 synthesis, on the contrary, increased with an increase in temperature exposure from 37 to 50°C (Stepanov 2009).

In animals, this issue is covered much more comprehensively. For instance, it is known that high temperatures lead to the accumulation of signaling molecules (calcium ions in the cytoplasm, lipid signaling molecules, ROS, improperly packaged proteins, etc.) and TF. At the same time, with an increase in ambient temperature in mammalian cells, their content may change (Balogh et al. 2013). Thus, a moderate concentration of signaling molecules under mild stress activates gene expression and synthesis of HSP, which leads to adaptation of the body. Their higher content under "severe stress" also triggers

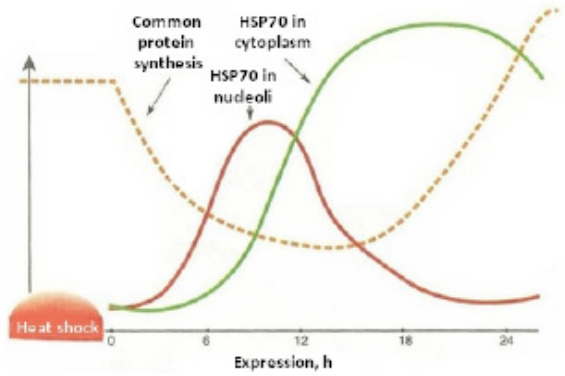

Fig. 1. Changes in protein synthesis in cells of living organisms in response to the action of high temperatures (according to Malyshev 2012)

the expression of HSP genes, but in this case, the synthesis of HSP itself does not always occur. In addition, a certain level of signaling molecules, at which most often there is no formation of HSP, i.e. with deleterious stress, induces processes associated with programmed cell death (PCD).

Consequently, the question of HSP's contribution to plants' heat resistance is currently being actively discussed, although many aspects remain insufficiently studied. The proteins involved in quality control of other newly synthesized proteins include both the above-mentioned HSP and various endoplasmic reticulum (ER) proteins.

Under unfavorable conditions, improperly synthesized proteins can aggregate and appear in the ER cavity, leading to ER stress development. At the same time, an unfolded protein response (UPR) is activated (Liu and Howell 2010; Deng et al. 2013). A protein quality control system functions in plant cells, which important components are cytoplasmic chaperones and ER proteins. The functioning of this system probably ensures the resistance of cells and plants as a whole to the action of extremely high temperatures. Failures in the operation of this system can provoke the launch of processes related to the PCD. However, how the activity of this system changes under high-temperature influences of different intensity is practically not studied. Therefore, studies of physiological and biochemical as well as molecular and genetic indicators characterizing the response of plants to high-temperature impacts of different intensity, carried out on the same object under strictly controlled environmental conditions, will allow us to better understand the mechanisms by which plants acquire increased stability and are able to tolerate adverse conditions without harmful consequences. Therefore, an investigation was carried out to study the reactions of wild plants *Arum korolkowii* to high-temperature effects of different intensity at a number of physiological, biochemical, molecular and genetic levels.

Materials and methods

Experimentation

A wild plant species, *Arum korolkowii* was selected to study the effect of temperature in artificial climate chambers under laboratory conditions in hydroponic nutrient medium. Plants were grown for 7 days on a nutrient solution containing 3.15 mM NH_4NO_3, 1.55 mM KH_2PO_4, 1.55 mM $MgSO_4$, 24 microns H_3BO_3, 21 microns iron citrate ($FeC_6H_5O_7$), 10 microns $MnSO_4$, 3.1 microns $CuSO_4$, 2.55 microns $(NH_4)_2MoO_4$, 1.55 microns $ZnSO_4$, and 5 mM $Ca(OH)_2$, pH 6.4, at an air temperature of 22°C, its relative humidity of 60–70%, headlight illumination of 180 mmol/(m^2*s), photoperiod of 14 hours. Then the seedlings were subjected to temperature influences of different intensity (experiment). The temperature range varied from 30 to 50°C while maintaining the general conditions unchanged. Duration of high-temperature exposure was from 15 minutes to 96 hours. The control was seedlings grown at a temperature of 22°C, which is the optimum temperature range for the studied species. The experiments were conducted on the scientific equipment of the South Clinical & Genetic Laboratory at the South Kazakhstan Medical Academy.

Research procedure

The heat resistance of *Arum korolkowii* was evaluated after the following manipulations:

- Heating the die-cuts for 5 minutes from the sheet in a water thermostat with a sequential increase in temperature with an interval of 0.4°C (Aleksandrov 1963). As a criterion of stability, the death temperature of 50% (LT50) of leaf palisade cells was used, determined using a light microscope ("Micros", Austria) (40x lens) for the destruction of chloroplasts and cytoplasm coagulation and,
- Exposure to elevated temperatures (30–50°C) for 1–3 days and re-growth at a temperature of 22°C. The criteria of stability were the degree of damage to the leaves of seedlings and the survival rate of plants.

The protein content was determined according to Bradford (1976), which is based on shift of absorption spectrum towards the values of 595 nm of the bright blue Coomassie dye when it binds to the protein, directly proportional to the concentration of the latter. To prepare the Bradford reagent, 100 mg of bright blue Coomassie was dissolved in 50 mL of ethanol 96% solution, 10 mL of phosphoric acid 85% solution was added to this liquor and brought to a volume of a 1 L by bidistillate. To determine the content of soluble proteins in the sample, plants with a 0.3 g weight were homogenized in 3 mL 0.1 M K and Na phosphate buffer, the resulting homogenate was centrifuged for 20 minutes at 15,000 rpm. After that, 100 mL of the supernatant were taken and 5 mL of Bradford reagent was added. The samples were left at 23°C for 10 minutes. The optical density was measured on a PD-303S APEL JAPAN spectrophotometer at a wavelength of 595 nm. As a control, 5 mL of Bradford reagent was used with the addition of 100 mL of 0.1 M K and Na phosphate buffer. The protein content was determined by a calibration curve constructed using bovine serum albumin.

A leaf sample (50 mg) was fixed in liquid nitrogen to analyze the level of gene transcripts. Total RNA for gene expression analysis was isolated from biological samples obtained every 2 hours using the Furtado et al. (2014) technique. The integrity of the isolated ribonucleic acid (RNA) was tested by electrophoresis in 1% agarose gel. The amount of RNA was determined spectrophotometrically on a NanoDrop 2000 spectrophotometer (Thermo Scientific, USA), by the ratio of wavelengths 260:280. The first cDNA chain was synthesized using Promega reagents and protocol (GoTaq ® 2-Step RT-qPCR System, A6010) in a volume of 20 µL. The resulting complementary DNA (cDNA) was diluted

to the final concentration in a solution of 12.5 ng/μL. The quantity and quality of the isolated cDNA were determined spectrophotometrically on the NanoDrop 2000/200 device. The level of plant gene expression was assessed using real-time PCR amplifiers on QuantStudio5 (appliedbiosystems, by Thermo Fisher Scientific) and DNA Technology DT322, using an amplification kit with intercalating dye SYBR Green (Eurogen, Russia). The sequences of primers were taken from literary sources by Irina A. Nilova (Dissertation for PhD (Biological Sciences), Petrozavodsk, 2019, so that only cDNA reacts, the list of primers is given in Table 1. The synthesis of primers was carried out on the DNA/RNA Synthesizer H8 "Germany". The 25 mL PCR mixture contained 100 ng cDNA, 1 pcM of direct and reverse primers, 5 mL of reaction mixture and 16 mL of deionized water free of nucleases. PCR protocol: cDNA denaturation 5 min at 95° C; 40 cycles: denaturation at 95°C 30 s; annealing at 58°C for 30 seconds.; elongation at 72°C for 30 seconds. The specificity of the amplification products was checked by melting PCR fragments.

The efficiency of PCR (E) was calculated according to the formula (1):

$$E = 10^{1/a}, \quad (1)$$

where *a* is a coefficient of the equation of dependence of the threshold cycle (Ct) on the logarithm of the initial concentration of DNA matrices. PCR for the reference and target genes proceeded with similar efficiency, approximately equal to 2.

Data processing

Each experiment was repeated not less than 3 times. The biological repetition within each variant of the experiment is 3 to 6 times, analyzing growth indicators for 50 times. The normality of the distribution was checked using the Shapiro-Wilk test. In the case of a normal distribution of general data, parametric tests were used, the data were presented in the form of arithmetic averages and their standard errors, the reliability of differences was assessed using the Student's tests (at $p \leq 0.05$). If the distribution was different from normal (in the case of gene expression data), nonparametric tests (Wilcoxon-Mann-Whitney and Kruskal-Wallis) were used.

Results and discussion

Heat resistance of leaf cells

During the course of the study, the nature of changes in the heat resistance of *Arum korolkowii* leaves under constant prolonged exposure to plants at temperatures in the range of 30-50°C was investigated. The heat resistance of *Arum korolkowii* remains unchanged in case exposed plants to temperatures of 30 to 50°C for 10 minutes. as the plants did not show significant differences. Here and further, the initial level is the value of the indicator recorded in weekly seedlings of *Arum korolkowii* grown at an air temperature of 22°C.

At the next stage, current experiment was continued with an increase in time of exposure to heat shock to 7 days (Fig. 2). Under the influence of a temperature of 30°C, an increase in heat resistance was noted after 3 hours, and its maximum was recorded after 2-3 days. At the same time, the maximum value of heat resistance had reached, after 1 day, at 40 to 45°C. A further increase in temperature at 50°C

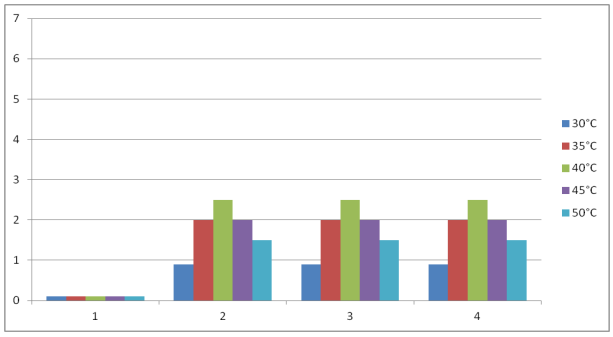

Fig. 2. Study of the high temperatures effect on the dynamics of *Arum Korolkowii* heat resistance, heat shock by heating up to 7 days.* (*Note: 1–Control without exposure to temperature heating, 2-4 test samples)

Table 1. Characteristics of primers for real-time PCR

Gene	Access No. inNCBI	Primer	Primer sequence 5'→3'
TaBI-1	GU564292. 1	upstreamdownstream	CCAGCGGATGGGGCTACGACTGCGAGCATTGTCAGCATCCCG
TaBAX.2	FJ747648. 1	upstreamdownstream	AGAGGTTTGGGCTGCTGATGGGTCCTGTCACGAGGATACTTGGGTC
TaMCAII	GU130248. 1	upstreamdownstream	TCCTTCCTCAAAGAGACCGTTCGCTCCTCAATGTCATCCTTCCCAG
TaBiP	KC894715.1	upstreamdownstream	GCTATTGCCTATGGTTTGGACCTTGCCGTGCTTCTTCT
TaIRE1	CX536022.1	upstreamdownstream	GAAGAAGCCAGGAGATAAAAGCGGTTGATGTGATA
TaHSP90	DQ270237. 1	upstreamdownstream	TCCGACCTCGTCAACAACCACACCGAACTGCCCAATCA
TaHSP70	AF005993.1	upstreamdownstream	AGGAGGAGATTGAGAAGATGGTGCGTCGTCCTTGACCGTGTTGC
TaHSP19	AM422845.1	upstreamdownstream	CCCCGTTCGGTAAGTCCTCGCCAGCATCTGCCGCATCGTC
TaHSP16.9	EU649679. 1	upstreamdownstream	TCCTACCTGCGGTCCGATACAGGCGTCTCCTTCCAGTCCA
Actin	AB181991	upstreamdownstream	GGGACCTCACGGATAATCTAATGAACCTCCACTGAGAACAACATTAC

did not cause an additional increase in heat resistance, on the contrary, a decrease has been observed. Thus, from the results obtained, following temperatures from 30 to 35°C indicated a similar effect on the heat resistance of plants, causing its increase. According to the "zonal" hypothesis (Drozdov et al. 1977, 1984; Titov 1989; Titov et al. 2006), these temperatures should be attributed to the zone of heat hardening. However, the nature of reaction of plants significantly depended on the intensity of the temperature acting on them in its quantitative sense.

The nature of plants response to the effect of 40°C was different. In present case, a peak of stability was recorded. However, the stability at the same time increased only during the first day of the experiment, and it began decreasing at the second day. With an increase in air temperature from 45 to 50°C, an increase in the heat resistance of leaf cells was not observed, and it began to decrease. From the data obtained, it can be concluded that the increase in plants' heat resistance peaks up to a certain degree depending on the species, after which a decrease in heat resistance or adaptation to temperature occurs. Thus, the present study has shown that the nature of changes in the heat resistance of the leaves of *Arum korolkowii* varies both quantitatively and qualitatively. Depending on both the range (zone) to which the temperature acting on the plants belongs, and on the intensity of high-temperature exposure (in the case when plants experience the effect of temperatures related to the same temperature zone).

Further studies were aimed at assessing the survival of plants and the degree of damage to leaves after exposure to temperatures 30, 35, 40, 45, and 50°C. The first of these temperatures, as follows from the data obtained by us, is characterized by a weak and well-pronounced hardening effect, and the second is damaging. The obtained results showed that the survival rate is 100% among plants exposed to temperatures of 30°C and 35°C for 1-3 days and then transferred to re-growth under normal conditions (22°C for 7 days). Upon that, the 1st and 2nd leaves had no visible signs of damage. In contrast, after 7 days of re-growth at an optimal temperature after exposure to a temperature of 40°C for a day, plant survival slightly decreased (up to 95%), and the degree of leaf damage was 50% in the first leaf and 25% in the second leaf (Table 2, Fig. 3). In the case of an increase in the exposure of plants to 2 days, the survival rate decreased to 80%, the degree of damage to the 1st leaf reached 65%, and the second leaf 45%. The effect of temperature from 45 to 50°C for 3 days decreased plant survival by more than 50% and to even more significant damage to the first and second leaves. Temperatures of 30°C and 35°C do not affect the survival of *Arum korolkowii* plants and do not cause visible signs of damage to them, while temperatures of 40-45°C (and above) not only leads to a decrease in the heat resistance of leaf cells, but also to damage to plants, and after 3 days of exposure – to their death.

Dynamics of the content of HSP gene transcripts in leaves

In the last 20-30 years, numerous studies have proved that the synthesis of HSP is a pre-requisite for plants to acquire high heat resistance and the final stage of cellular response to high-temperature effects (Usman 2014). The present study has confirmed that the dynamics of the expression of the HSP70 gene encoding cytoplasmic HSP with a molecular weight of 70 kDa is generally similar when

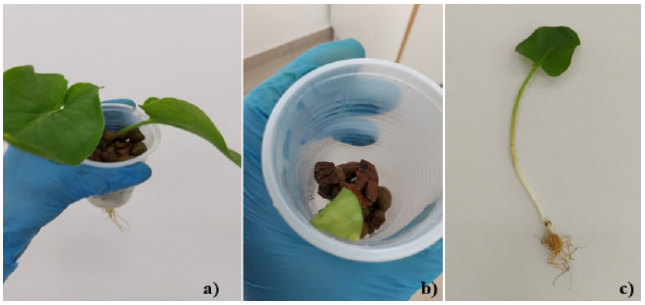

Fig. 3. Appearance of *Arum korolkowii* plants a week after exposure to temperature: a= Control sample (22°C); b = One day at 45°C and c= Three days at 45°C

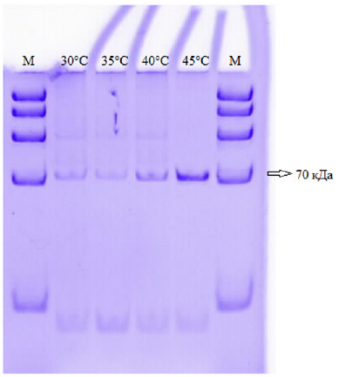

Fig. 4. Electrophoregram in 8% polyacrylamide gel* (*Note: M is a molecular weight marker)

Table 2. The effect of high temperature (45°C) on plant survival and the degree of damage to the leaves of *Arum korolkowii*

Expression of 40°C and 45°C,hours	Survival,%		1st leaf damage,%		2nd leaf damage,%	
	40°	45°	40°	45°	40°	45°
24	100	80	95	65	90	45
48	90	65	850	50	80	25
72	75	35	70	10	65	5

plants are exposed to temperatures of 30, 35, 40, and 45°C. Statistically significant increase in the mRNA content of this gene was observed after 15 min. from the beginning of thermal exposure (Fig. 4), but after reaching the maximum level after 1 h, its decrease occurred (Fig. 5). Statistically significant differences between temperature variants within each exposure were also noted. In general, at a temperature of 40°C mRNA content of the HSP70 gene in leaf cells was significantly higher after 30 min., 1 and 6 h, and significantly lower after 24 and 72 hours than at temperatures of 30 and 35°C.

Statistically significant differences between the temperature variants at $p<0.05$ within each exposure were also observed. The data obtained by us are consistent with the literature data indicating that in *Arum korolkowii*, the expression of HSP70 most often increases during the initial period of high temperature action and then decreases after 5 hours (Xue et al. 2014). The HSP70 protein is one of the most important chaperone proteins involved in protecting the cell from high temperature influences. Therefore, the expression of this gene is usually higher in resistant plant varieties than in sensitive ones (Usman et al. 2015). Depending on the situation in the cells, the HSP70 protein performs various functions. In some cases, HSP70 binds ATP-dependent to hydrophobic sites of partially denatured proteins and prevents their aggregation (Mayer and Bukau 2005). In other cases, it is involved in the transfer of damaged proteins to proteasomes and lysosomes for refolding or degradation.

The level of plant gene expression was also assessed using real-time polymerase chain reaction (PCR) on the QuantStudio5 device (applied biosystems, by Thermo Fisher Scientific) and DNA Technology DT322. We found that not only cytoplasmic HSP70, but also mitochondrial mtHsp70, and chloroplast TaHSC70 participate in protection from the adverse effects of high temperatures in *Arum korolkowii*. In addition, the BiP protein, a representative of the HSP70 family localized in ER, participates in plants' heat resistance formation. The specificity of the amplification products was checked by melting PCR fragments (Fig. 6).

The effect of high temperatures on plants can lead to the accumulation of a large number of improperly packaged proteins in the ER and cytosol, which causes the development of ER stress and even cell death (Wan and Jiang 2016). Therefore, strengthening control over the quality of protein packaging is an important component of the formation of plants heat resistance. In response to ER stress, a protective mechanism is activated in the plant

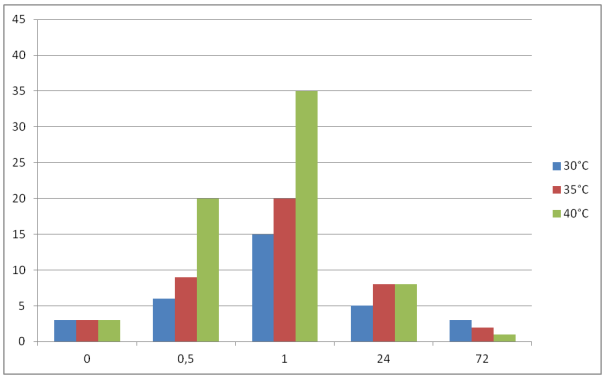

Fig. 5. The effect of high temperatures on the dynamics of the level of HSP70 gene transcripts in the leaves of *Arum korolkowii** (*Note: 0-45 Transcript level in units, 0-72 measurement in hours)

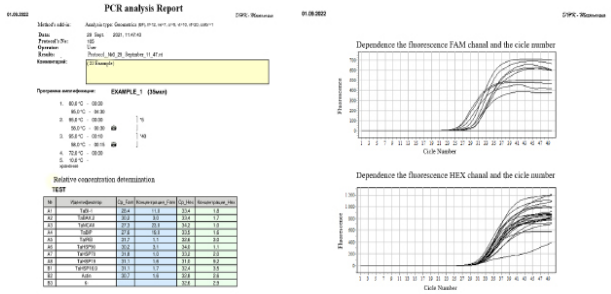

Fig. 6. Plant gene expression level has also been evaluated using polymerase chain reaction (PCR) in real time on the DNA Technology DT322 device

Table 3. Molecular genetic reactions of *Arum korolkowii* plants at hardening (30°C) and damaging (45°C) temperatures

Indicator	Temperature and exposition time			
	30°C, 72 h	35°C, 72 h	40°C, 24 h	45°C, 1 h
Gene expression, rel. un.				
HSP70	1,7	10,7	1,5	34,4
BiP	0,1	45,5	12,3	74,1
HSP90	1,5	10,2	1,0	10,0
HSP16,9	1,6	44,1	2,7	76,8
HSP19	0,7ns	1,4	1,7	30,1
IRE1	0,9ns	7,0	3,4	0,3
BI-1	1,9	2,1	1,9	14,4
BAX.2	1,8	8,3	1,2	30,9
MCAII	1,8	0,2ns	1,0	7,2

cell –UPR (Wang and Zhang 2016). The main regulator of UPR is the BiP protein (Kørner et al. 2015). Activation of protective mechanisms depends not only on the intensity, but also on the duration of high-temperature exposure. So, in our case, a number of mechanisms of heat resistance of plants, *e.g.,* associated with the accumulation of transcripts of the HSP70 and HSP90 genes, are activated during the initial period (from 15 min to an hour) of the action of high temperatures. Therefore, it can be assumed that an increase in the heat resistance of plants by 1.9°C to short-term heating, registered at the initial moment of the damaging temperature of 45°C (1 h), is largely ensured by activating the synthesis of HSP, i.e. an increase of *HSP70, HSP90, HSP16,9, HSP19* genes expression (Table 3).

Upon that, HSPs (in particular, HSP70, HSP90) makes a much smaller contribution to the comparable increase in thermal stability (1.6°C), noted at 35 °C, since its achievement at this temperature requires a much longer effect (72 h). Thus, we have shown that during the initial period of the damaging temperature (45°C), the activity of most of the protective mechanisms studied by us is higher than under the action of hardening temperatures (30 and 35°C). Obviously, the higher the temperature, the more damage it causes, but it is also important to strengthen the resistance from plants, which lies in the fact that in such a situation the body mobilizes all its protective mechanisms. Nevertheless, with an increase in temperature (or the duration of its exposure), there is an increasing "tension" or, putting it differently, the intensity of stress increases.

Based on the results obtained, it can be said that "mild", "medium" and "hard" stress is characterized by the accumulation of transcripts of the genes *HSP70* and *HSP90*. In addition, with "medium" and "hard" stress, the accumulation of transcripts of the *BiP*, *HSP16, 9 HSP19* and genes also occurs, and with "soft" stress, the changes in the content of transcripts of these genes are least apparent. It should be noted that the response of plants also depends on the duration of high-temperature exposure. However, their accumulation alone is not enough to preserve the viability of plants under the prolonged action of damaging temperature, as evidenced by a sharp decrease in the heat resistance of seedlings after an hour of warming up plants at 45°C and then their death.

The increase in air temperature observed in recent years in many regions of the world due to global climate change is considered one of the important reasons for the decline in plant yields (Morgunov et al. 2018). The present work that has been focused on studying the dynamics of heat resistance of *Arum korolkowii* seedlings under the action of high temperatures of varying intensity, as well as on a number of physiological, biochemical and molecular genetic reactions is highly relevant with respect to accumulation of transcripts of a number of genes encoding proteins involved in the mechanisms of formation of heat resistance, as well as coding proteins that prevent the development of PCD or participating in PCD. The study has demonstrated that, the reaction of plants to the effect of high hardening and damaging temperatures differs at the physiological and biochemical levels. In particular, the effect of high tempering temperatures leads to an increase in the heat resistance of plants, inhibition of growth, and a slight decrease in the hydration of leaf tissues. On the contrary, the effect of damaging temperatures on plants leads only to a short-term increase in their heat resistance (30-40°C), causes a complete stop of growth and a sharp drop in the hydration of leaf tissues. Taking into account the present findings that follows the sub-damaging temperatures ("mild" and "medium" stresses), the protective mechanisms of plants are comprehensively able to cope with structural and functional disorders appearing in their cells, plants in this case successfully adapt, and their heat resistance increases. At damaging temperatures (hard stress), the protective mechanisms can no longer cope with the numerous violations and/or damages that occur, the resistance of plants drops sharply and they eventually die.

Authors' contribution

(BG, GP); Designing of the experiments (AY, GA, AltB, AssB); Contribution of experimental materials (AY, GA, ZY, RA); Execution of field/lab experiments and data collection (AY, GA, AltB, ZY, AssB); Analysis of data and interpretation (AY, GA, BG, GO); Preparation of the manuscript (GA, AssB, ZY).

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