STUDY OF THE RESISTANCE OF *ARUM KOROLKOWII* HIGH-TEMPERATURE IMPACTS OF DIFFERENT INTENSITY AT THE PHYSIOLOGICAL, BIOCHEMICAL, AND MOLECULAR AND GENETIC LEVEL

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Abstract

Background: Climatic optimum, unfortunately, is often an inaccessible condition for growing plants. The reaction of plants to temperature stress determines the area of growth of each individual species, and also affects the process of acclimatization of plants in a particular region.

The main purpose of the study was the investigation of a number of physiological and biochemical, as well as molecular and genetic reactions of the wild plant *Arum Korolkowii* to high-temperature effects of different intensity.

Methods: The heat resistance of *Arum Korolkowii* was studied by conducting a morphometric, biochemical, and genetic comparative analysis of plants of the same species grown under conditions of temperature stress after exposure to elevated temperatures (30-50 °C), as well as grown under conditions of temperature optimum (22 °C) – the control group.

Results and Conclusions: Our research on *Arum Korolkowii* seedlings showed that at the physiological and biochemical level, the reaction of plants to the effect of high hardening and damaging temperatures differs. 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 and causes a complete stop of growth and a sharp drop in the hydration of leaf tissues.

Keywords: *Arum Korolkowii*, gene transcription, heat shock proteins, plant survival, resistance, temperature stress.

Resumen

Antecedentes: Desafortunadamente, el clima óptimo es a menudo una condición inaccesible para el cultivo de plantas. La reacción de las plantas al estrés por temperatura determina el área de crecimiento de cada especie individual y también afecta el proceso de aclimatación de las plantas en una región particular.

El objetivo principal del estudio fue la investigación de una serie de reacciones fisiológicas, bioquímicas y genéticas moleculares de la planta silvestre *Arum Korolkowii* a los efectos de alta temperatura de diferente intensidad.

Métodos: La resistencia al calor de *Arum Korolkowii* se estudió mediante la realización de un análisis comparativo morfométrico, bioquímico y genético de plantas de la misma especie cultivadas en condiciones de estrés térmico después de la exposición a temperaturas elevadas (30-50 °C), así como cultivadas en condiciones de temperatura óptima (22 °C) – el grupo de control.

Resultados y Conclusiones: Nuestra investigación sobre las plántulas de *Arum Korolkowii* mostró que, a nivel fisiológico y bioquímico, la reacción de las plantas al efecto de las altas temperaturas de endurecimiento y daño difiere. El efecto de las altas temperaturas de templado conduce a un aumento de la resistencia al calor de las plantas, la inhibición del crecimiento y una ligera disminución de la hidratación de los tejidos de las hojas. Por el contrario, el efecto de las temperaturas dañinas en las plantas conduce solo a un aumento a corto plazo de su resistencia al calor y provoca una parada completa del crecimiento y una fuerte caída en la hidratación de los tejidos de las hojas.

Palabras clave: *Arum Korolkowii*, transcripción génica, proteínas de choque térmico, supervivencia vegetal, resistencia, estrés térmico.

The ambient temperature is one of the environmental factors that have the strongest impact on the vital activity of plants and their productivity. Almost always an 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, which is associated either 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 membranes and hormonal system in plant (Hatfield & Prueger 2015; Niu & Xiang 2018). It is noted that most of these changes are nonspecific (general), although specific changes have been recorded, inherent only in plants experiencing high-temperature exposure (Bahuguna & Jagadish 2015; Wan & Jiang 2016). At the same time, the peculiarities of changes in physiological, biochemical and molecular 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 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 productivity 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. For different types of plants, the optimal temperature values are not the same. For instance, for wheat, these temperatures range from 13 °C to 22 °C depending on the type and place of growth (Hatfield & Prueger 2015).

Violations of cell division processes may be one of the reasons for slowing down the growth of plants in unfavorable temperature conditions. Thus, with an increase in the temperature acting on plants, the number of dividing cells sharply decreases (Centomani *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 has a significant effect on 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. The temperature optimum of growth, as a rule, 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 significantly influenced by the spectrum of synthesized proteins as well. 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. It is known that 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 and thereby activate the synthesis of HSP.

Despite the great interest of researchers in the synthesis of 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. f. 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 *A. 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 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 the contribution of HSP to the heat resistance of plants is currently being actively discussed, although many aspects still 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, which leads to the development of ER stress. At the same time, an unfolded protein response (UPR) is activated (Liu & 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.

The ***purpose*** of the current work was to investigate a number of physiological and biochemical as well as molecular and genetic reactions of wild plants *Arum Korolkowii* to high-temperature effects of different intensity.

Materials and methods[Second order headings]

The studies were carried out on *Arum Korolkowii* plants (wild type) grown in laboratory conditions (hydroponic method).

Research Design

The studies were carried out in laboratory conditions using artificial climate chambers. 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 h. 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 – from 15 min. to 96 h. The control was seedlings grown at a temperature of 22 °C, which is the optimum temperature range for the studied species.

The research was carried out on the scientific equipment of the South clinical & Genetic Laboratory at the South Kazakhstan Medical Academy.

Research methodology

Arum Korolkowii heat resistance was evaluated in after two conducted manipulations:

a) heating at 5-minute of the die-cuts 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.

b) exposure to elevated temperatures (30-50˚C) for 1-3 days and regrowth 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 the shift of the 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 min 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 min. 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.

To *analyze the level of gene* transcripts, a leaf sample (50 mg) was fixed in liquid nitrogen. Total RNA for the analysis of gene expression 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 sec.; annealing at 58 °C for 30 sec.; elongation at 72 °C for 30 sec. The specificity of the amplification products was checked by melting PCR fragments.

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

$E = 10^{1/a}$,

(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.

Results Processing

Each experiment was repeated not less than 3 times. The biological repetition within each variant of the experiment is 3-6 times, analyzing growth indicators – 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

Heat resistance of leaf cells

In 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 30-50 °C for 10 min. So, *Arum Korolkowii* plants had not 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 the time of exposure to heat shock to 7 days (Figure 2). Under the influence of a temperature of 30 °C, an increase in heat resistance was noted after 3 h, and its maximum was recorded after 2-3 days.

At the same time, the maximum value of heat resistance was reached after 1 day at 40-45 °C. A further increase in temperature at 50 °C did not cause an additional increase in heat resistance, on the contrary, a decrease has been observed.

Thus, from the results obtained, it follows that temperatures from 30 to 35°C have 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 the 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 our 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 decreased at the second day. With increasing 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 heat resistance of plants has a peak up to a certain degree depending on the species, after which there is a decrease in heat resistance or adaptation to temperature occurs.

Thus, the conducted studies have 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).

Our further studies were aimed at assessing the survival of plants and the degree of damage to leaves after exposure to temperatures of 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 and 35 °C for 1-3 days and then transferred to regrowth under normal conditions (22 °C for 7 days). Upon that, the first and second leaves had no visible signs of damage.

In contrast, after 7 days of regrowth 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, Figure 3). In the case of an increase in the exposure of plants to two days, the survival rate decreased to 80 %, the degree of damage to the first leaf reached 65 %, and the second leaf – 45 %. The effect of temperature from 45 °C to 50 °C for three days led to a decrease in plant survival by more than 50 % and to even more significant damage to the first and second leaves.

Temperatures of 30 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. Temperatures of 30 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 three 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 prerequisite for plants to acquire high heat resistance and the final stage of cellular response to high-temperature effects (Usman 2014).

We have 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 plants are exposed to temperatures of 30; 35; 40; and 45 °C: a statistically significant increase in the mRNA content of this gene was observed after 15 min from the beginning of thermal exposure (Figure 4), but after reaching the maximum level after 1 h, its decrease occurred (Figure 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 h than at temperatures of 30 and 35°C.

Statistically significant differences between the temperature variants at $p<0.05$ within each exposure. 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 h (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 & 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 (appliedbiosystems, 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 the formation of heat resistance of plants. The specificity of the amplification products was checked by melting PCR fragments (Figure 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 & 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 cell –UPR (Wang & 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 ℃ 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, HSP (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).

Discussion

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 the protective mechanisms that it has. 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 was occurred plant 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).

As follows from the purpose of our work, the research 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 (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).

We have shown that at the physiological and biochemical level, the reaction of plants to the effect of high hardening and damaging temperatures differs. 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; causes a complete stop of growth and a sharp drop in the hydration of leaf tissues.

The effect of temperatures on the seedlings of *Arum Korolkowii*, from the range of 30-40 °C provides the increase in heat resistance, while their growth and leaves water content decrease under the further increase of heat resistance. Temperatures from 40 to 45°C at the initial moment of their action also cause a slight increase in the heat resistance of seedlings, however, with a longer exposure (for an hour or more), its rapid decrease occurs, and temperatures of 45 °C and above lead to further damage and death of seedlings.

During the initial period of action on *Arum Korolkowii* seedlings at temperatures of 30; 35 and 40 °C (causing “mild”, “medium” and “hard” stress, respectively), transcripts of the genes of heat shock proteins *HSP70 and HSP90* accumulate in their leaves, encoding chaperone proteins.

Taking into account all the results obtained, it follows that at 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.

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Tables

Table 1. Characteristics of primers for real-time PCR

Gene	NCBI Access No.	Primer	Primer sequence, 5' → 3'
TaBI-1	GU564292. 1	upstream	CCAGCGGATGGGGCTACGACT
		downstream	GCGAGCATTGTCAGCATCCCG
TaBAX.2	FJ747648. 1	upstream	AGAGGTTTGGGCTGCTGATGGGT
		downstream	CCTGTCACGAGGATACTTGGGTC
TaMCAII	GU130248. 1	upstream	TCCTTCCTCAAAGAGACCGTTCG
		downstream	CTCCTCAATGTCATCCTTCCCAG
TaBiP	KC894715.1	upstream	GCTATTGCCTATGGTTTGGACCT
		downstream	TGCCGTGCTTCTTCT
TaIRE1	CX536022.1	upstream	GAAGAAGCCAGGAGATAA
		downstream	AAGCGGTTGATGTGATA
TaHSP90	DQ270237. 1	upstream	TCCGACCTCGTCAACAACC
		downstream	ACACCGAACTGCCCAATCA
TaHSP70	AF005993.1	upstream	AGGAGGAGATTGAGAAGATGGTGC
		downstream	GTCGTCCTTGACCGTGTTGC
TaHSP19	AM422845.1	upstream	CCCCGTTCGGTAAGTCCTCG
		downstream	CCAGCATCTGCCGCATCGTC
TaHSP16.9	EU649679. 1	upstream	TCCTACCTGCGGTCCGATAC
		downstream	AGGCGTCTCCTTCCAGTCCA
Actin	AB181991	upstream	GGGACCTCACGGATAATCTAATG
		downstream	AACCTCCACTGAGAACAACATTAC

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

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

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	0.7^{ns}	1.4	1.7	30.1
IRE1	0.9^{ns}	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	0.2^{ns}	1.0	7.2

Figure legends

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

Figure 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.

Figure 3. Appearance of *Arum Korolkowii* plants a week after exposure to temperature: a – control sample (22 °C); b – one day at 45 °C; c – three days at 45 °C.

Figure 4. Electrophoregram in 8% polyacrylamide gel*

*Note: M is a molecular weight marker.

Figure 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.

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