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THE INFLUENCE OF ULTRAVIOLET-B IRRADIATION AND TEMPERATURE ON BEAN SEEDS GERMINATION AND ON KINETIC INDICATORS OF MILLISECOND-DELAYED EMISSION AND EPR SIGNALS

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Abstract. The present research is devoted to studying of effect of ultraviolet (UV-B) rays and temperature on morphological and physiological indexes of seeds and millisecond delayed light emission (ms-DLE) of chlorophyll a in leaves of bean sprouts. It is found that low doses of UV-B rays (1,5-2,2 x 10^4 erg/mm^2) and temperature (40^0C) can have a stimulating effect on the morphological and physiological indicators of bean seeds. It is shown that the intensity of slow (SP) and the fast phase (FP) of millisecond ms-DLE of chlorophyll-a depends on the dose of UV-B and temperature. Found that on irradiation of dry bean seeds in seedling leaves the donor side of PS II is more resistant to UV-B than on the third phase of swelling. Sequential action of UV-B radiation and temperature (40^0C) increases resistance of bean seeds. It is shown that the intensity of the broad EPR signal ($\Delta H \approx 450$ G, $g = 2.32$) that presumably belong to iron oxide nanoparticles, rises with the increase of UV-B radiation. If one type of stress (UV-B) is able to induce resistance to the development of another type of stress (temperature), one can assume the presence of common factor that controls the start of certain protective mechanisms.

Keywords: *bean seeds of leaves, delayed light emission, UV-B rays, temperature broad EPR signals.*

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1. Introduction

Anthropogenic influence on the biosphere has some ecological consequences for the world community, causing the global depletion of the ozone layer and an increase in the level of UV-B radiation. The effect of UV irradiation on organisms, particularly on plants have been investigated widely during recent years. Photobiological significance of UV-B irradiation is increased on the background of stress created by other environmental factors [14]. As a rule all environmental factors have a complex effect on plants. At the same time one factor can intensify or weaken the effect of another one. In particular, the climatic factors can influence the spread of environmental pollutants, for example, at high temperatures the gas emissions are spread over longer distances. At present, there are only a few works devoted to the influence of joint or sequential action of various factors on plants. It has been established that mechanical effects (wind gusts, cuts or touching plants, etc.) are able to increase the resistance of plants to different abiotic and biotic factors (extreme temperatures, salinity, etc.) [9, 17]. High tempering temperatures had the same effect on wheat sprouts [15, 18]. It was found that the effect of UV irradiation favors the maintenance of the life of cucumber plants at high temperatures .In some medicinal herbs (wormwood, basil, fennelflower), pre-treatment with UV irradiation reduced the negative effect of salinization [12].

Today, the focus of the investigators is on the study of the biochemical and molecular genetic aspects of plant resistance to stress temperatures [8, 7]. It is assumed that the activation of antioxidative enzymes, the synthesis of chaperone proteins or the accumulation of low molecular weight amino acids play an important role in increasing the resistance of plants to the consecutive or joint action of adverse environmental factors [4]. Nevertheless, the mechanisms of plant adaptation to the complex action of different kind of adverse factors remain to be little studied [19, 13, 1]. In connection with this fact the further research is needed on the mechanisms of plant cross-adaptation (phenomena when the effect of one factor leads to an increase in resistance to a factor of a different nature).

Increasing the dose of ultraviolet rays leads to a decrease in growth and reproductive characteristics uniquely affecting all the physiological processes in plants. The low doses of UV-B rays can increase the stability of photosynthetic apparatus to stress factors. This is probably due to the stimulation of phenolic compounds and carotenoids [16]. The present research is devoted to studying of effect of ultraviolet (UV-B) rays and temperature on growing and kinetic indicators of ms-DLE of bean sprouts.

2. Materials and methods

Seeds of *Phaseolus vulgaris L.* served as a material for experiments. The water thermostat was used for studying of effect of temperature (30^0-55^0 C) on bean seeds. When measuring the millisecond delayed emission of light (ms-DLE) in the leaves of bean seedlings, a phosphoroscope was used to detect induction curves of delayed fluorescence [11, 4, 5]. The mercury lamp SVD-120 served as the source of UV radiation. Upon irradiation, a quartz tube was hanged on a rotating installation, which provided uniform irradiation. Dry seeds and beans in the III phase of swelling were exposed to radiation. Irradiation was carried out with a dose of $1.5\text{x}10^4$ - $4.5\text{x}10^4$ erg/mm^2. The unirradiated seeds served as a control.

EPR studies The freshly collected and dried leaves at room temperature (25-27°C) and seeds of up mentioned plants were used. The spectra of investigating plant objects have been registered by EPR spectrometer Varian E4 (USA) at room temperature (293 K), in observing condition, and were shown in indications with respective figures.

3. Results and discussion

The intensity of ms-DLE kinetic indices are changed in dry seeds of bean depending on temperature. After the effect of 30^0–40^0C temperature on dry seeds of bean the intensity of the accelerated phase of the ms-DLE induction curves of chlorophyll - a is increased by 28% and 40% consequently in leaves of bean sprouts and it decrease by 24% at 55^0C. However on the third phase of swelling after the effect of high (45^0-50^0C) temperature it is noted the increase of intensity of SF by 68% and 25%consequently and decrease by 20% at 55^0C temperature. The slow phase is increased by 18% in dry bean sprouts affected by 40^0C temperature. The intensity of the slow phase is decreased when the temperature is increased and it is 20% at 55^0C temperature compared with control. After the effect of (30^0C-55^0C) temperature on dry seeds of bean on the third phase of swelling the intensity of the slow phase is increased by 89% (45^0C), and it decreased by 20% at 55^0C.

After 30 minutes of thermic treatment of bean seeds (dry and on the third phase of swelling) the rapid fluorescence which attributes the state of P680 can conserve 70-80% of ability of electrons to transfer to the Q_B side and at the same time it is less sustainable (it is 50% in dry bead seeds treated by 55^0C temperature) compared with slow phase which attributes the recombination of the reaction center with Y_Z [2] or Mn clusters [3]. The intensity of the slow phase in the seeds exposed to temperature at the third phase of swelling is the highest (80%) in this point (55^0C temperature). High temperature dependence of rapid phase reflecting the processes at the acceptor side of the PS II indicates the protein damage in the reaction center under the influence of increasing temperature. It can be assumed that this is due to protein damage.

After the consequent impact of UV-B irradiation and temperature to dry bean seeds the intensity of the fast phase is higher than in control, while it is slightly decreased during impact of 4,5 x10^4 erq/mm^2 doses. During impact of small doses (1,5-2,2 x10^4 erg/mm^2) the intensity decreased respectively by 25% and 18% in the III phase of swelling, while it increases at other doses (3- 3,7 x10^4 erg/mm^2) (Figure 1).

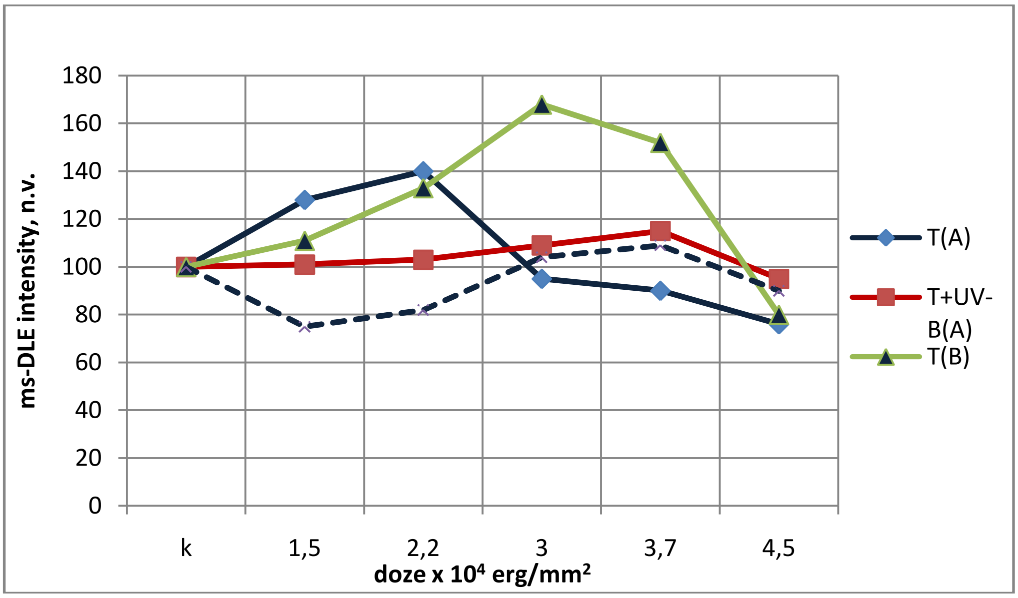

Figure 1. Dependence of rapid phase of ms-DLE induction curve of chlorophyll - a in the leaves of the bean seedling doze of UV irradiation and temperature (40^0 C)
(A -dry, B-irradiation the third phase of swelling).

In the case of sequential exposure of UV irradiation and temperature to dry seeds to the intensity of the ms-DLE slow phase changes insignificantly (it decreases by 19% at high doses of UV irradiation 4,5 x10^4 erg/mm^2). It changes by 27% in bean seeds exposure to the effect of low doses of UV irradiation (1,5-2,2x10^4 erg/mm^2) and temperature at the third phase of the swelling of bean seeds and increases up to control level under the influence of other doses followed by the decreasing by 20% under effect of high doses (4,5 x10^4 erq/mm^2) (Figure 2).

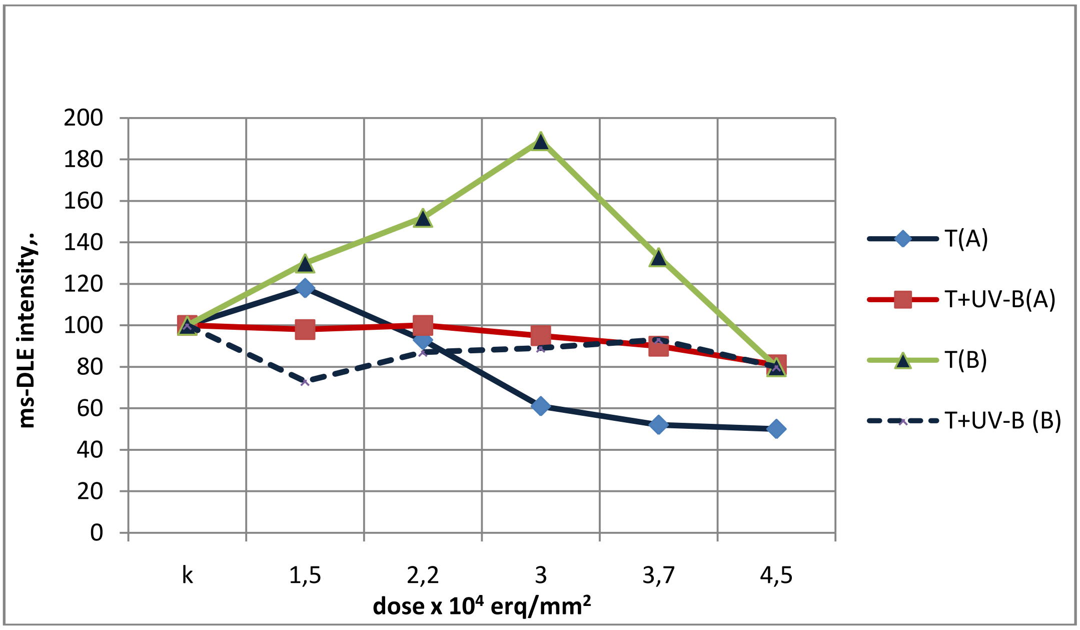

Figure 2. Dependence of slow phase of ms-DLE induction curve of chlorophyll - a in the leaves of the bean seedlings on doze of UV irradiation and temperature (40^0C) (A -dry, B-irradiated on the third phase of swelling).

Thus, it was revealed that after the influence to bean seeds by the high doses (3-4,5 x10^4 erq/mm2) of the UV – B irradiation at the III phase of the swelling the intensity of the ms-DLE phases and SL levels is close to that in the control, which can be explained with the fact that these doses are the cause of defense effect in such seeds. We can conclude that the certain doses (2,2- 4,5 x 10^4 erq/mm^2) of the UV-B irradiation increase the resistance of bean seeds to the influence of temperature (40^0C), and this effect is more expressive in dry seeds than in swollen ones.

At the consistent impact with UV-B irradiation and temperature (40^0C) on dry bean seeds the FP, SP and SS levels in the induction curves of the ms- are less differ from control. The impact of temperature (40^0C) followed by the impact of UV-B irradiation on bean seeds causes some changes to the induction curves of the ms- DLE in the chlorophyll– a of the bean seedlings. However, the impact of temperature and UV-B irradiation in this sequence on the seeds in the III phase of swelling causes the decrease in FP and SP intensity of the ms-DLE compared with the control and increase in the stationary level.

The analysis of the results showed that the numerical value of the SP/SS ratio is increased depending on temperature in samples subject to temperature (30^0-40^0C) influence and it is decreased insignificantly at the high temperature (55^0C). The numerical value of the SP/SS ratio was not changed (Figure 3).

The figure 3 shows that the numerical value of the ratio of SL/SS in the dry bean seedlings after influence of temperature (40^0C) followed by the effect of UV-B irradiation is changed less within the doses of 1,5-3x10^4 erg/mm^2. The numerical value is increased slightly after the impact of the 3,7-4,5 x 10^4 erg/mm^2 doses. At the III phase of swelling of the bean seeds the numerical value increases slightly after the impact of 2,2 x 10^4 erq/mm^2 doses and the SP/SL ratio is decreased 2 times under the impact of the higher doses. The FP/SS ratio is high in dry irradiated seedlings. The numerical vaule of this ratio is less than control in 2 and 2,5 times in bean seedling irradiated in the III phase of the swelling.

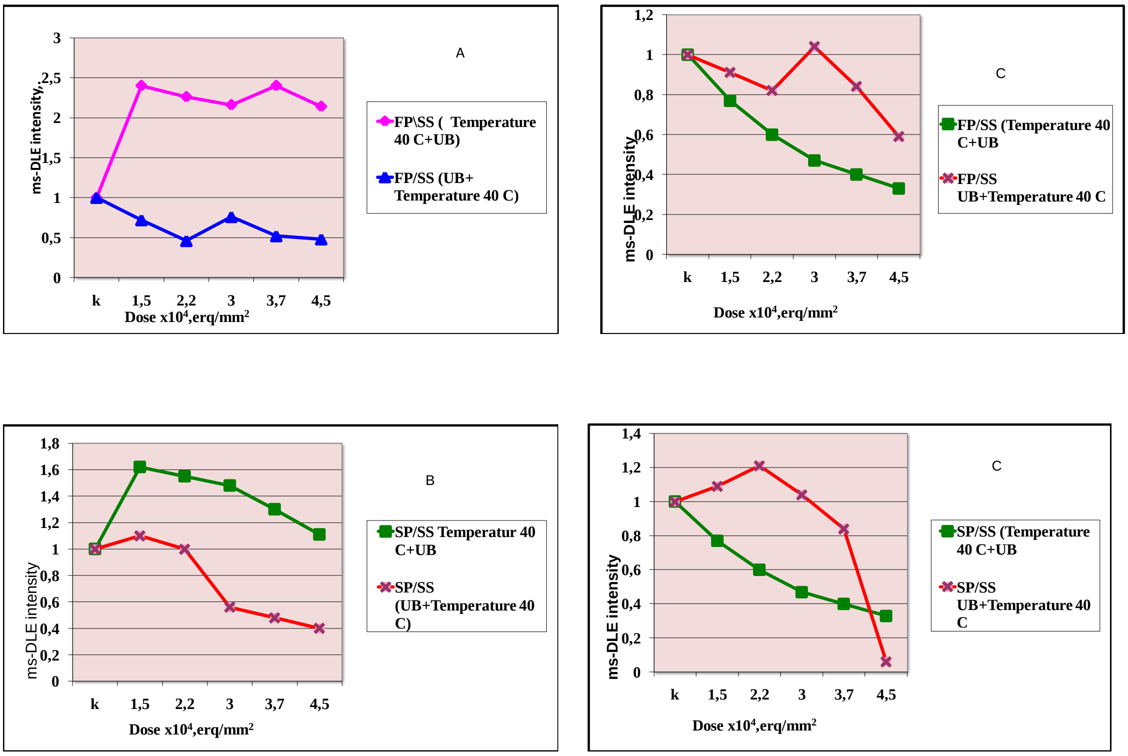

Figure 3. The ratio of phases of the ms-DLE induction curves of the chlorophylle-a to the intensity of stationary levels (A, B- dry C,D - III phase of swelling) in the leaves of the bean seedlings

Now we'll discuss the changes of the FP/SS, SP/SS ratios after the sequenced impact of UV-B irradiation + temperature (40^{0}C) to bean seeds in dry state and in the III phase of the swelling (Figure 7 B).

It is shown from the results of the figure 7 B, depending the impact of increasing doses of the UV-B irradiation the numerical value of the FP/SS and the SP/SS ratios is higher than in control (in dry irradiated seeds). We noted this rate in the bean seedlings in the III phase of swelling and the mentioned numerical value is decreased slightly after impact of the 4,5 x 10^4 erq/mm^2 dose.

It is revealed from the results that the low radiation doses (1,5-2,2x10^{4}erq/mm^2) protects the bean seeds in dry and swelling (III phase) state from damages. It can be assumed that this is due to the increase in number and activity of antioxidants the regulators of free radical reactions during stimulating impact of temperature and the mentioned doses of the UV irradiation on bean seeds.

Registration of broad EPR signal in plant samples (leaves and seeds of plants from control and radioactive (contaminated areas.) EPR spectra of leaves of Elaeagnus L. (elaeagnus), having dried at room temperature was shown in figure 4, which were collected from radioactive contaminated areas, registered at room temperature 297 K.

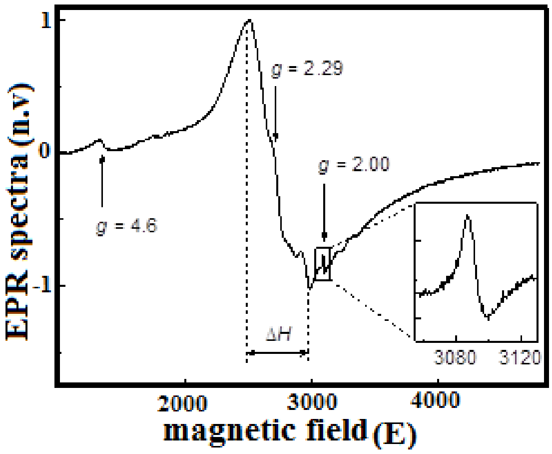

Figure 4. EPR spectra of leaves of Elaeagnus L. having dried at room temperature, collected from radioactive contaminated areas (PED 140 mR/hour). The registration condition: UHF power 10 mW, magnetic field HF-modulation amplitude 0,5 mTl, field center 2800 mTl, field scanning 4000 mTl, temperature297 K.

It is seen from the figure, the main contribution, includes aboard EPR signal to this spectra, in which the maximum position of the low-field component changed from g=2,29 and signal of half-width 320 mTl. Apart of this signal, in this spectrum one discloses a narrow intensive signal of free radicals and a weak signal of trivalent iron complexes at g=4,6. A broad EPR signal was registered in leaves of Elaeagnus L., which were collected in various times of year (Fig.5). It is seen from the figure that during the spectra registration at room temperature every time one observes a characteristic broad EPR signal, in which the maximum position of the low –field component changed from g=2,38 and signal of half-width 320 mTl.

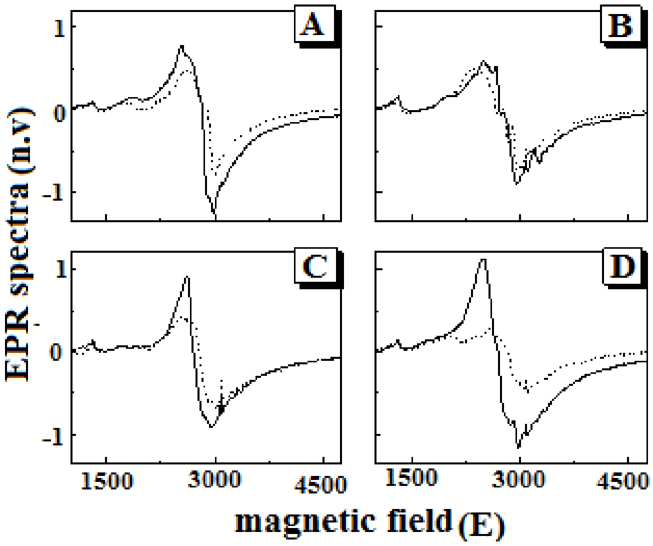

Figure 5. EPR spectra of the dried at room temperature leaves Elaeagnus angustifolia collected at different times of the year: A-May.; B-June; C – July; D – October. With dashed lines shows EPR spectra of leaves samples of the plants growing on control sites; solid line-EPR spectra of the leaves of plants growing in a contaminated area (140 ± 20 mR/hr) (13)

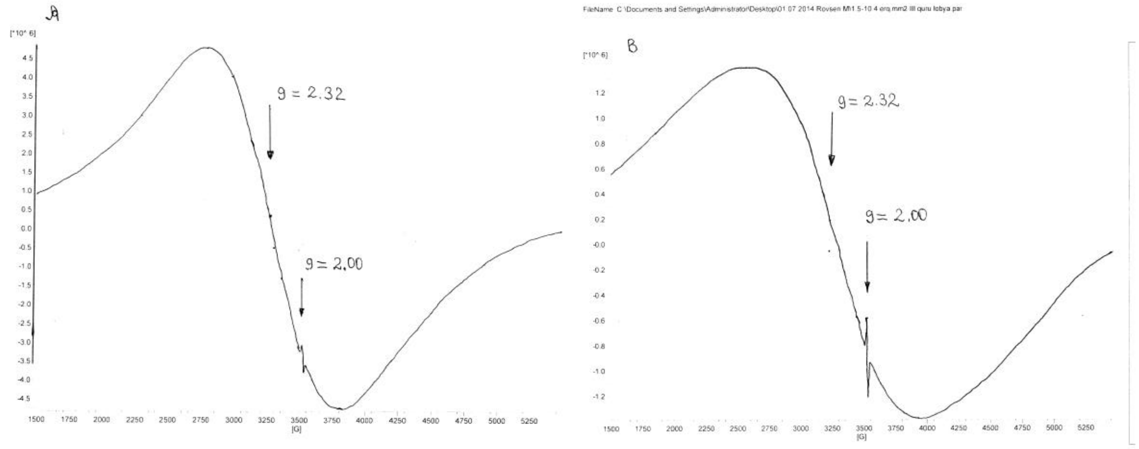

Figure 6. Control (A) and EPR spectra characterizing the magnetic nanoparticles of the bean seedlings exposed to UV – B irradiation in dry state. The registration condition: UHF power 10 mW, magnetic field HF-modulation amplitude 0,5 mTl, field center 2800 mTl, field scanning 4000 mTl, temperature297 K.

As figure 6 shows the intensity of both signals is changed in seedlings got from the irradiated bean seeds. The increase of the EPR spectra (g~2,00 ΔH=10G) characterizing the free radical testify to the creation of defects in the seedlings and increase in the wide EPR spectra (g~ 2,32 ΔH=450G) leads to increase in the number of nanophased magnetic nanoparticles. It is assumed that UV –B rays cause changes in certain level which leads to structural changes in the following phases of development of seed germination. This leads to an increase in the number of Fe3 + Fe2 + ions and as a result of an increase in the intensity of the Fenton reaction, the number of reactive oxygen species increases. Together with the increase in antioxidant heat shock proteins due to biomineralization, the Fe ions are changed into nanoscale Fe_2O_3 particles as a response to stress. That is why we noted in experiments the typical increase in EPR spectra.

It is no accident that the results obtained by us in experiments (increasing the resistance of seeds against UB-B radiation due to the temperature effect) are consistent with the results obtained by Khalilov [6].The comparative analysis of the EPR spectra obtained from the oleaster leaves collected in the clean and radioactively contaminated areas in the different seasons showed that the difference between spectra is low in June and July. However the difference in EPR spectra of the leaves collected in October is significantly high. The results obtained by us experimentally are very close to results obtained in nature.

It is known that in the III phase of swelling in the cells the oxidative stress can occur. At this time, the process of reconstruction of the membrane structure is completed and the metabolism is sharply activated. The level of generation of active forms of oxygen (AFO) by the mitochondria, the endoplasmic reticulum and other cellular systems may exceed the capabilities of the antioxidant apparatus. An extreme high level of AFO can cause damage to the embryonic axis and, as a result, sprouts with a morphological defect are formed. It is clear that this happens as a result of a violation in the cells of the embryonic axis.

Based on the results obtained and the theory of cross – cultural adaptation, it can be assumed that endurance is “formed” against the influence of temperature in the process of adaptation to UV rays. In plant organisms sensitive to UV-B irradiations proteins belonging to heat shock proteins (Hsps) or stress proteins group and having molecular weight of 25 and 75 kD are synthesized. It is assumed that the reason of the increase of resistance of plants to joint and consequent impact of unfauvarouble factors is increase of activity of antioxidant enzymes, synthesis of chaperon proteins or low molecular weight amino acids [4,10] and the same time due to biomineralization, the Fe ions are changed into nanoscale Fe_2O_3 particles as a response to stress.

According to results obtained we can assume that the resistance of bean seeds to high temperature (40^0C) increases during impact of certain doses (2,2- 4,5 x 10^4 erq/mm^2) of UV-B irradiation and this effect is more observed in dry irradiated seeds. Increasing resistance to one type of stress under the action of other stress causes the bean seeds to adapt. If we take into account the fact that one stress increases the resistance to another stress, it can be assumed that there is a general factor controlling certain protective mechanisms.

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