
AUXILIARY DISCIPLINES

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TEMPERATURE DEPENDENCE OF THE FATTY-ACIDS COMPOSITION OF MITOCHONDRIAL MEMBRANES OF PEA SEEDLINGS BEEN TREATING WITH NITROGEN OXIDE DONOR UNDER WATER DEFICIENCY

Research article

Abstract

Background: Water deficiency and temperature stress modify cell and organelle membranes, affecting their functions and cell metabolism. In this case, a change in the ratio of unsaturated to saturated fatty acids occurs in the membranes, which leads to a change in the selective permeability of the membranes and the activity of the enzymes associated with them. With the combined action of these factors on the plant organism, synergism in the action of these stressors on the metabolism of plant cells may be observed, or the action of one stressor will reduce the effect of action of another. The exogenous use of signaling molecules or their donors, in particular nitrogen oxide donors, induces plant resistance to stress factors. The aim of the study was to study the fatty acid composition of mitochondrial membranes of etiolated pea seedlings under conditions of insufficient moisture at different temperatures (24° and 17°) and treated with a nitric oxide donor-a tetranitrosyl iron complex with thiosulfate ligands (TNIC-thio).

Methods: The functional state of the mitochondria was studied per the level of lipid peroxidation by the spectrofluorimetry, by a fatty acid composition of mitochondrial membranes with the chromatography technique.

Results: Insufficient moisture led to the activation of lipid peroxidation, which caused changes in the content of C₁₈ and C₂₀ fatty acids (FA). The content of linolenic acid in the membranes of mitochondria of seedlings under water deficiency (WD) and a temperature of 24 ° decreased by 21.3%, and in the membranes of mitochondria of seedlings grown under the same conditions, but at 17 ° - by 13%. Changes occurred in the content of C₂₀ FA: the content of 20: 2 ω6 and 20: 1 ω9 decreased almost 2.5 times and 1.5 times, respectively (WD + 24° and WD + 17°). The treatment of pea seeds with 10⁻⁶M TNIC-thio prevented lipid peroxidation, prevented changes in the composition of the FA membranes of mitochondria and prevented the inhibition of growth of pea seedlings in conditions of water shortage.

Conclusion: It is assumed that a decrease in temperature from 24 ° to 17 ° increases the resistance of pea seedlings to insufficient moisture, which is manifested in less significant changes in the fatty acid composition of mitochondrial membranes i.e. observed changes indicate the presence of cross-adaptation. The antistress activity of the drug is apparently due to the fact that it is a NO donor and is determined by its antioxidant activity.

Keywords: nitric oxide donors, water deficiency, lipid peroxidation, mitochondria, fatty acids, cross- adaptation.

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ТЕМПЕРАТУРНАЯ ЗАВИСИМОСТЬ ЖИРНОКИСЛОТНОГО СОСТАВА МЕМБРАН МИТОХОНДРИЙ ПРОРОСТКОВ ГОРОХА ПРИ ОБРАБОТКЕ ДОНОРОМ ОКСИДА АЗОТА В УСЛОВИЯХ ДЕФИЦИТА ВОДЫ

Научная статья

Аннотация

Введение: Дефицит воды и температурный стресс модифицируют клеточные мембраны и мембраны органелл, влияя на их функции и метаболизм клетки. При этом в мембранах происходит изменение соотношения ненасыщенных жирных кислот к насыщенным, что приводит к изменению избирательной проницаемости мембран и активности ассоциированных с ними ферментов. При совместном действии этих факторов на растительный организм может наблюдаться синергизм в действии этих стрессоров на метаболизм растительных клеток, или же действие одного стрессора будет нивелировать действие другого. Экзогенное применение сигнальных молекул или их доноров, в частности доноров NO, индуцирует устойчивость растений к действию стрессовых факторов. Целью исследования было изучение жирнокислотного состава мембран митохондрий этилированных проростков гороха, находящихся в условиях недостаточного увлажнения при различных температурных режимах (24° и 17°) и обработанных донором оксида азота - тетранитрозильным комплексом железа с тиосульфатными лигандами (ТНКЖ-тио).

Методы: Функциональное состояние митохондрий оценивали по интенсивности перекисного окисления липидов спектрофлуориметрическим методом и по изменению жирнокислотного состава мембран митохондрий, который исследовали методом хроматографии.

Результаты: Недостаточное увлажнение приводило к активации ПОЛ, которое вызывало изменения в содержании C₁₈ и C₂₀ жирных кислот (ЖК). Содержание линоленовой кислоты в мембранах митохондрий проростков, находящихся в условиях дефицита воды (ДВ) при температуре 24°, снизилось на 21,3%, а в мембранах митохондрий проростков, проращиваемых в тех же условиях, но при 17° - на 13%. Изменения происходили и в содержании C₂₀ ЖК: содержание 20:2 ω6 и 20:1 ω9 снизилось почти в 2,5 раза и в 1,5 раза соответственно (ДВ+24° и ДВ+17°). Обработка семян гороха 10⁻⁶М ТНКЖ-тио предотвращала ПОЛ, препятствовала изменениям в составе ЖК мембран митохондрий и предупреждала торможение роста проростков гороха в условиях дефицита воды.

Выводы: Предполагается, что снижение температуры с 24° до 17° повышает устойчивость проростков гороха к недостаточному увлажнению, что проявляется в менее значимых изменениях жирнокислотного состава мембран митохондрий т.е. наблюдаемые изменения свидетельствуют о наличии перекрестной адаптации. Антистрессовая активность препарата, по-видимому, обусловлена тем, что он является донором NO и определяется его антиоксидантной активностью.

Ключевые слова: доноры оксида азота, дефицит воды, перекисное окисление липидов, митохондрии, жирные кислоты, перекрестная адаптация.

1. Introduction

The study of the combined effect of several abiotic factors on plants has recently been of great interest, since such a system more fully models the natural habitat of a plant than the effect of any one factor. At the same time, most studies note deterioration in the condition of plants when the second stress factor is added, but in several studies, the authors found that the introduction of the second factor mitigates metabolic disorders caused by the action of another factor. For example, elevated CO₂ and ozone concentrations can have a more favorable effect on plants compared with each of the factors applied individually [16]. Experiments on pea seedlings have shown that the combined effect of water scarcity and a decrease in temperature (a situation that occurs in nature, for example, in the spring in cold conditions after a snowless winter) led to a disruption in the bioenergetic characteristics of mitochondria. These characteristics were gradually restored during the recovery period. However, the complete restoration of respiratory activity of mitochondria was observed only under the combined effect of water deficiency and low temperature on pea seedlings [22]. Water deficiency modifies cell and organelle membranes, affecting their functions and cell metabolism and thereby on plant growth and development [18], [28].

The temperature regime is no less important for the growth and development of plants. Changes in temperature affect plant cell membranes and organelle membranes, in particular, mitochondrial membranes, changing the membrane fluidity and permeability due to changes in the composition of lipids and / or interactions between lipids and membrane-associated proteins [13], [37]. It is known that in many regions, the early period of plant growth occurs with constant temperature fluctuations during the day. So, quite often there are sharp drops in temperature at night or in the pre-morning hours. Moreover, in the spring, with a short-term decrease in temperature and water availability is insufficient (after a snowless winter), two major environmental factors are observed to act together on the seedlings: lack of moisture and a decrease in temperature. In this regard, the study of rearrangements of plant cell metabolism under conditions of insufficient moisture at various temperature conditions is of particular interest.

Energy metabolism plays an important role in the adaptive reactions of the body [14] and mitochondria are directly involved in the body's response to changes in the environment [17]. However, under stressful conditions, the mitochondrial electron transport chain is a source of excessive generation of reactive oxygen species (ROS) [3]. An increase in the generation of ROS by mitochondria under stress can lead to activation of lipid peroxidation (LPO) and mitochondrial swelling [35]. The consequence of the "peroxide" swelling of mitochondria (or the formation of large pores in the outer membrane) is the release of apoptogenic proteins from the intermembrane space into the cytoplasm and the activation of the apoptosis by mitochondrial pathway.

It can be assumed that drugs that can prevent lipid peroxidation due to excessive generation of reactive oxygen species by mitochondria will help increase plant resistance to stressful conditions.

The use of these physiologically active substances makes it possible to prevent lodging of grain crops, accelerate seed germination and fruit maturation, contributes to increased productivity and quality of grown products, increases resistance to pathogens and parasites [10], [21], [32]. The adaptogenic effect of plant growth regulators (PGRs) is probably due to a decrease in ROS generation by mitochondria and prevention of LPO. At the same time, the problem of finding new biologically active substances that increase the resistance of plants to the effects of stress factors is quite relevant. In our

studies, we turned our attention to nitric oxide donors. This choice of research object is due to the fact that nitric oxide is a signaling molecule involved in a wide variety of physiological processes in plants, including germination, development, flowering and aging [8]. At the same time, various studies have shown the presence of a positive correlation between an increase in the NO content in various organs and tissues of plants under stress (during drought, salinization, increased content of heavy metals and extreme temperatures) and adaptation of plants to changing environmental conditions [30]. The adaptogenic effect of nitric oxide is probably determined by the ability of NO to inhibit free radical oxidation reactions catalyzed by redox active iron ions due to the binding of free iron ions in nitrosyl complexes [1]. In addition, nitric oxide can trigger the expression of antioxidant genes or activate antioxidant enzymes, for example, by post-translational modifications [24]. Of particular interest is the study of the effect of nitric oxide donors on the resistance of plants to water deficiency, which negatively affects the growth and development of plants, thereby limiting the productivity of crops [27]. The treatment of plants with exogenous NO donors, regulating resistance to abiotic stress, possibly contributes to preventing a decrease in crop yields under stressful conditions [15], [29], [34].

As a nitric oxide donor, we used sodium μ 2-dithiosulfate-tetranitrosyldiferrate tetrahydrate [$\text{Na}_2 [\text{Fe}_2 (\text{S}_2\text{O}_3)_2 (\text{NO})_4]_2 \times 4\text{H}_2\text{O}$] (crystalline TNIC-thio, or iron-thiosulfate complex). The advantage of this donor, which belongs to the class of nitrosyl iron complexes with thiosulfate ligands, over other nitric oxide donors is due to the fact that it emits nitric monoxide at physiological pH values without any (thermal, redox, or photo) activation, and upon its decomposition no toxic substances are formed, ensuring environmental safety [26].

The aim of our study was to study the effect of water deficiency in various temperature conditions and TNIC-thio on the functional state of pea seedlings mitochondria. The effect of water deficiency on the pea seedlings mitochondria was studied in two temperature regimes: at 17 ° and at 24 °. It can be assumed that with minor changes in the strength of one of the factors, the combined effect of two abiotic factors (water scarcity and a slight decrease in temperature) will allow plants to more successfully adapt to extreme conditions. In addition, the choice of mitochondria as the objects of study is determined by the fact that the implementation of anti-stress programs requires large energy expenditures [14].

2. Methods

The study was carried out on mitochondria isolated from pea seedlings (*P. sativum*, cv. Flora-2)

2.1. Tetranitrosyl complex of iron with thiosulfate

Crystalline water-soluble nitric oxide donor μ 2-dithiosulfate-tetranitrosyldiferrate tetrahydrate (iron complex with thiosulfate) [$\text{Na}_2 [\text{Fe}_2 (\text{S}_2\text{O}_3)_2 (\text{NO})_4]_2 \times 4\text{H}_2\text{O}$] (TNIC-thio) was synthesized at the Institute for Problems of Chemical Physics of the Russian Academy of Sciences. The generation of NO and the formation of mononitrosyl intermediate and the particle [Fe (S₂O₃)] - from TNIC-thio is only begun 40 minutes after the dissolution of the complex [11].

2.2. Pea seeds germination

Pea (*Pisum sativum* L., cv. Flora 2) seeds were washed with soapy water and 0.01% KMnO₄. Control seeds were then soaked in water, experimental seeds – in 10⁻⁶M TNIC-thio for 1 h. Thereafter, seeds were kept into covered trays on moistened filter paper in darkness at a temperature of 24° for 2 days. Then, half the seedlings of the control group were divided into 4 parts: one part of the seedlings (water deficiency+ 17°; WD+ 17°) and TNIC-thio treated seedlings (water deficiency +17°, TNIC-thio; WD+ 17°, TNIC-thio) were transferred on dry filter paper, where they were at 17 °. Another group of seedlings (WD+ 24°) and TNIC-thio treated seedlings (WD+ 24°, TNIC-thio) were under the same conditions at a temperature of 24 °. Another half of the control plants were retained in closed trays on wet filter paper, where they were kept at 24 ° for 6 days. After one day of water deficiency, seedlings of all four WD groups were transferred to wet filter paper, where they were kept at 17 ° and 24 ° for the next three days. . On the sixth day, mitochondria were isolated from seedling epicotyls.

2.3. Isolation of mitochondria

Isolation of mitochondria from 6-day-old epicotyl of pea seedlings (*P. sativum*) performed by the method [9] in our modification. The first centrifugation at 25000g for 5 min., the second at 9 at 3000g for 3 min. The precipitate was separated and the supernatant centrifuged at 11000 g for 10 min to mitochondria sedimentation. The sediment was re-suspended in 2-3 ml of the solution that contained: 0.4 M sucrose, 20 mM KH₂PO₄ (pH 7.4), 0.1 % FA-free BSA and mitochondria were precipitated by centrifugation at 11000 g for 10 min.

2.4. Lipid peroxidation (LPO) activity

LPO activity was assessed by fluorescent method [20]. Lipids were extracted with a mixture of chloroform and methanol (2:1). Lipids of mitochondrial membranes (3–5 mg of protein) were extracted in the glass homogenizer for 1 min at 10°C. The registration of fluorescence was performed in ten-millimeter quartz cuvettes on the spectrofluorimeter FluoroMax-HoribaYvon GmbH (Germany). The fluorescence excitation wavelength was 360 nm, the emission was 420–470 nm. The results were expressed in arbitrary units of fluorescence per mg protein.

2.5. Fatty acid methyl esters (FAMES)

FAMES were produced by acidic methanolysis of mitochondrial membrane lipids [19], [36].

2.6. FAMES quantification

FAME quantification was performed using a Kristall 2000M chromatograph (Russia) with flame-ionization detector and quartz capillary column DB-1 (60 m×0.32 mm, phase film thickness of 0.25 μm, firm J&W Scientific», USA). FAME analysis was performed at the programmed temperature increase from 120 to 270°C at the rate of 4°C/min. The temperature of injector and detector – 270°C; the helium carrier gas rate was 2.0 mL/min, dividing the flow at the entrance to the column – 1:40. Each sample contained 2 μL of the hexane peak extract. The FAME content in the samples was calculated as the ratio of the peak area of a corresponding acid to the sum of peak areas of all found FAMES [23]. The standard deviation of the average values of peak areas obtained in three measurements did not exceed 5% (relative). Mathematical processing of the result was carried out with Microsoft Excel and Sigma Plot 10.

2.7. FAMES identification

The identification of FAMES in the samples was performed on the basis of mass spectra obtained after separation of the FAMES under conditions similar to gas chromatographic analysis using a Hewlett Packard- 6890 instrument (USA). Mass spectra were obtained in the electron impact blow regime at an ionizing voltage of 70eV and a scanning speed of 1C per decade of masses in the region of 40-400 Dalton.

2.8. Statistical processing

Statistical processing of experimental data was carried out by determining the arithmetic mean and their standard errors. Significance of differences between variants with value $P \leq 0.05$.

The following reagents were used:

BSA (Bovine serum albumin) (V-fraction), Sucrose (Sigma-Aldrich, USA), KCl, 1,4-dithio-dl-teritol (Fluka, Germany), potassium carbonate, methanol, chloroform (Merck, Germany), hexane (Panreac, Spain), acetyl chloride (Acros, Belgium), Tris, EDTA (Ethylenediaminetetraacetic acid), Hepes (4-(2-Hydroxyethyl)piperazine- 1-ethanesulfonic acid) (Biochemica Ultra, for molecular biology) (MB Biomedicals, Germany).

3. Results

To search for concentrations of the drug (TNLC-thio) that effectively inhibit lipid peroxidation in mitochondrial membranes, a model “aging” of mitochondria was used. This model consisted of 20 minute incubation of the pea seedlings mitochondria in a hypotonic salt medium. This incubation caused a 3-fold increase the intensity of lipid peroxidation. The introduction of TNIC-thio into the incubation medium of mitochondria led to the prevention of LPO activation. This effect depended on the concentration of the drug in the mitochondrial incubation medium. The most effective concentrations were 10^{-6} , 10^{-9} , and 10^{-11} M [2]. A decrease in the intensity of lipid peroxidation with the drug could indicate the presence of anti-stress properties at its. Verification of this assumption was carried out on a model of water deficiency. Under conditions of water deficiency, activation of lipid peroxidation was observed in the membranes of seedlings mitochondria. The intensity of LPO was determined by the fluorescence intensity of the end products of lipid peroxidation (Schiff bases). In this case, the fluorescence intensity of lipid peroxidation products increased 2-fold in the pea seedlings mitochondrial membranes at 24 ° and 1.4 times in the mitochondrial membranes of seedlings at 17° (fig.1).

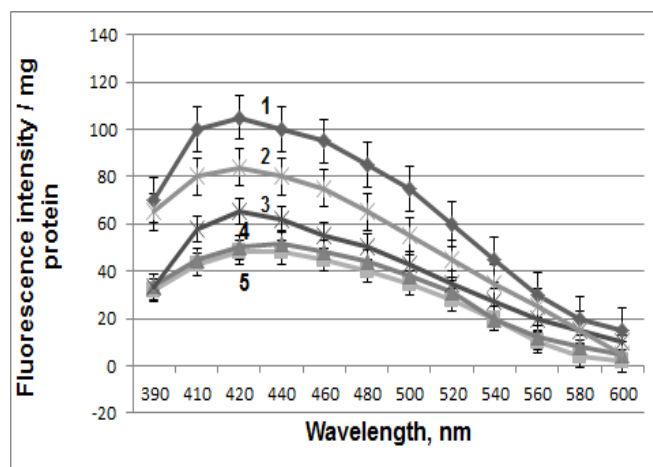


Figure 1 – Fluorescence spectra of lipid peroxidation products (Schiff's bases) in the mitochondrial membranes of pea seedlings exposed to water deficiency (WD) and treatment with 10^{-6} M TNIC-thio. 1 – WD+ 24 °; 2 – WD+ 17 °; 3- WD+ 24 °, 10^{-6} M TNIC-thio; 4 – WD+ 17 °, 10^{-6} M TNIC-thio; 5 - pea seedlings of the control group

Soaking the seeds in a 10^{-6} M solution of TNIC-thio prevented the activation of LPO in both cases: the fluorescence intensity of LPO products was reduced to almost the control level. Changes in the physicochemical characteristics of mitochondrial membranes were accompanied by changes in the fatty acid composition of membranes of these organelles. Significant changes were observed in the content of C_{18} fatty acids. The content of linolenic acid in the membranes of

mitochondria seedlings being at a temperature of 24 ° decreased by 21.3%, while in the membranes of mitochondria seedlings germinated at 17 ° - by 13% (Fig. 2). So, $\sum C_{18}$ polyunsaturated FA / $\sum C_{18}$ monounsaturated FA under conditions of insufficient moisture at a temperature of 24° decreased from 18.32 ± 0.25 to 15.71 ± 0.21 . At a temperature of 17 °, the decrease occurred to 17.41 ± 0.22 . The changes were also observed in the content of C₂₀ fatty acids. The contents of 20: 2 ω6 and 20: 1 ω9 decreased almost by 2.5 times and 1.5 times, respectively (WD + 24°). In this case, $\sum C_{20}$ polyunsaturated FA / $\sum C_{20}$ monounsaturated FA decreased from 1.02 ± 0.08 to 0.77. Germination of seeds at a temperature of 17 ° in conditions of water scarcity led to an increase in the content of 20: 2 ω6 FA by 2.9 times. In these conditions, the content of 20: 1 ω9 remained unchanged, and $\sum C_{20}$ polyunsaturated FA / $\sum C_{20}$ monounsaturated FA increased by 2 times. These data are probably indicative of “conjugate stability”, which due to a moderate decrease in germination temperature to 17 °. Treatment of pea seeds 10⁻⁶M TNIC-thio prevented changes in the fatty acid composition of mitochondrial membranes.

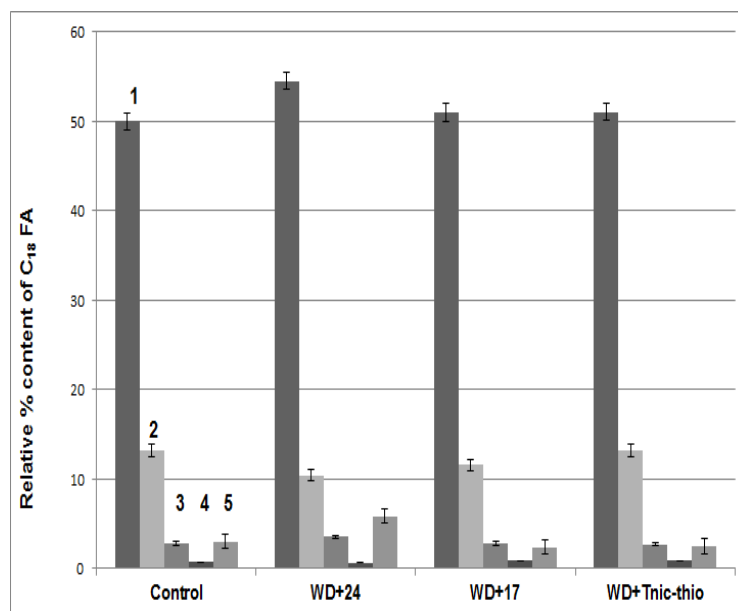


Figure 2 – The effect of water deficit + various temperature conditions (WD + 24° and WD+17°) and TNIC-thio on the relative % content of C₁₈ FAs in the membranes of pea seedlings mitochondria. X-axis: the studied groups of seedlings; Y-axis: relative % content of C₁₈ fatty acids; 1-18: 2 ω6; 2-18: 3 ω3; 3-18: 1 ω9; 4-18: 1 ω7; 5-18: 0.

Changes in the fatty acid composition of mitochondrial membranes, which affected their functional state, were probably, reflected in physiological parameters: on the growth of seedlings. It is known that pea seedlings are very sensitive to water deficiency. Water scarcity inhibited growth processes, which is consistent with literature data [31]. However, the length of the roots of pea seedlings at 17° in water deficit conditions was 2 times longer than that of the roots of seedlings subjected to water deficiency at 24°, which may indicate an increase in the resistance of pea seedlings to water deficiency under decreased temperature from 24 ° to 17 ° (fig. 3).

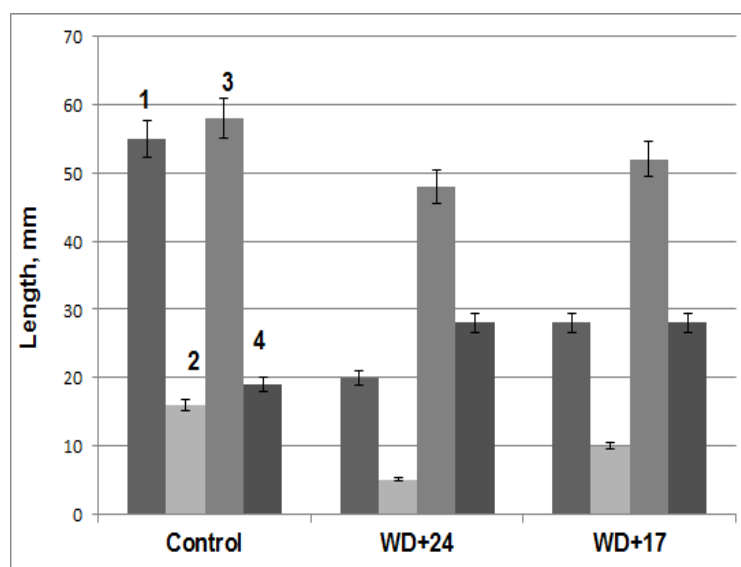


Figure 3 – The effect of water deficiency and TNIC-thio on the length of shoots and roots of 6-day-old pea seedlings; 1- shoot; 2-root; 3-shoot + 10⁻⁶ M TNIC-thio; 4 - root + 10⁻⁶ M TNIC-thio.

Treatment of pea seeds by TNIC-thio in conditions of water deficiency stimulated root growth by 2.8 and 5.7 times (17° and 24°, respectively). At the same time, the roots of the seedlings were 1.7 times longer than in the control, which is of great importance for increasing the stability of plants in water scarcity.

4. Conclusion

The changes in the fatty acid composition of the mitochondrial membranes of pea seedlings revealed in our experiments are comparable with the results on the effect of water deficiency on the fatty acid composition of maize mitochondrial membrane lipids and lipids from apricot leaves [7], [25]. However, with a moderate decrease temperature to 17 ° C, the decrease in the content of C₁₈ unsaturated FAs was insignificant, while the content of C₂₀ unsaturated FAs even increased compared to the control. Perhaps this is due to some increase in the resistance of pea seedlings to the studied stressors. The role of a very long chain FA (VLCFAs) (containing more than 18 carbon atoms) in the physiology of plant resistance has been little studied, but in some cases their participation in the plant response to adverse effects has been shown. In general, numerous abiotic stressors (salinization, hypothermia, hypoxia, heavy metals) increased the content of VLCFAs in certain plant species. Biotic stresses also led to an increase in the content of VLCFAs. Thus, the induced resistance of *Arabidopsis* to bacterial pathogens is probably associated with an increase in the endogenous level of VLCFAs [33]. In addition, it was found that in soleros adapted to osmotic stress, the vegetative organs contain 4–64% VLCFAs [4].

It can be assumed that changes in the fatty acid composition of the membranes of pea seedlings mitochondria under conditions of water deficiency with a decrease in temperature from 24 to 17 ° are due to the cross- adaptation of the seedlings to the combined action of these two stressors. Previously, the phenomenon of cross- adaptation has been demonstrated with the sequential action of stressors, for example, heat shock and water deficiency, lowering temperature and resistance to infections [5], [6]. Nevertheless, in the work of Generozova et al (2019) it was shown that the combined effect of water deficiency and low temperature has a less damaging effect on the functional state of mitochondria of pea seedlings and growth than separately applied stress factors. In this case, a more rapid and complete restoration of seedling growth and bioenergy characteristics of mitochondria had observed in the recovery period. In our work, we have shown that with minor changes in the strength of one of the factors (a slight decrease in temperature), the simultaneous combined effect of this factor with severe dehydration had a protective effect on the functional state of the mitochondria, which is clearly demonstrated by minor changes in the fatty acid composition of the lipids of the mitochondrial membranes.

The adaptogenic properties of sodium μ 2-dithiosulfato-tetranitosyldiferrate tetrahydrate (TNIC-thio) are apparently due to the fact that it is a NO donor and is determined by its antioxidant activity. Indeed, an experimental comparison of the antioxidant properties of TNIC-thio with the effectiveness of NO dissolved in water under the same conditions showed that the antioxidant activity of the drug is determined by the action of NO released during the hydrolysis of TNIC-thio [12]. The result of inhibition of LPO by the drug is maintenance of pool of unsaturated fatty acids with 18 and 20 carbon atoms at the initial (control) level, which prevents changes in the functional state of mitochondria due to water deficiency. Preservation of the functional activity of mitochondria ensures the maintenance of energy metabolism of the cell, which is of great importance in increasing the body's resistance to stress factors. Based on the data obtained, it can be concluded that TNIC-thio is a promising drug for use as a regulator of plant growth and development.

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Conflict of Interest

None declared.

Конфликт интересов

Не указан.

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