AUXILIARY DISCIPLINES

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FUNCTIONAL STATE OF MITOCHONDRIA IN VARIOUS PEA SEEDLING CULTIVARS DIFFERING IN RESISTANCE TO WATER DEFICIENCY AND INFLUENCE OF IRON TETRANITROSYL COMPLEX WITH THIOSULFATE LIGANDS ON IT

Research article

Abstract

Different cultivars of plants have various ability to tolerate adverse environmental effects without a sharp decrease in growth processes and yields. The present study was carried out to study the effect of water deficiency (WD) on the morphological, biochemical and bioenergetic characteristics of two cultivars of pea seedlings differing in resistance to WD. At the same time, the effect of nitric oxide donor a tetranitrosyl iron complex with thiosulfate ligands (TNIC-thio) on the resistance of pea seedlings to insufficient moisture was studied.

The functional state of the mitochondria was studied per rate respiration of mitochondria, by the level of lipid peroxidation by the spectrofluorimetry, and a fatty acid composition of mitochondrial membranes with the chromatography technique, by the study of mitochondrial morphology with the method of atomic force microscopy.

Water deficiency (WD) led to the activation of lipid peroxidation, which caused swelling of mitochondria and changes in the content of C_{18} and C_{20} fatty acids (FA) in the membranes of these organelles. Wherein there was a decrease in the content of linolenic acid by 15% in the mitochondrial membranes of the less resistant cultivar, while in the resistant cultivar it decreased by only 6%. Note that in the membranes of mitochondria more resistant to water deficiency there were 3,23 times more unsaturated C_{20} FA. The water scarcity was accompanied by an increase in the content of these fatty acids. Differences in the FA composition of mitochondrial membranes affected the bioenergetic characteristics of mitochondria. WD caused a decrease in the maximum oxidation rates of NAD-dependent substrates by mitochondria of seedlings. At the same time, the rate of oxidation of NAD-dependent substrates and the efficiency of oxidative phosphorylation in the more resistant cultivar were higher. Treatment of pea seeds with 10^{-8}M TNIC-thio restored the bioenergetic characteristics of mitochondria in pea seedlings of both cultivars. However, the efficiency of oxidative phosphorylation in the respiratory chain of mitochondria of the water-deficiency more resistant cultivar was 15,7% higher

The increased content of unsaturated VLCFAs in the mitochondrial membranes of pea seedlings contributes to maintaining the bioenergetic characteristics of mitochondria, and, consequently, the energy metabolism of the cell under stress conditions.

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

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ФУНКЦИОНАЛЬНОЕ СОСТОЯНИЕ МИТОХОНДРИЙ РАЗЛИЧНЫХ СОРТОВ ПРОРОСТКОВ ГОРОХА, РАЗЛИЧАЮЩИХСЯ ПО УСТОЙЧИВОСТИ К ДЕФИЦИТУ ВОДЫ И ВЛИЯНИЕ НА НЕГО ТЕТРАНИТРОЗИЛЬНОГО КОМПЛЕКСА ЖЕЛЕЗА С ТИОСУЛЬФАТНЫМИ ЛИГАНДАМИ

Научная статья

Аннотация

Разные сорта растений обладают различной способностью переносить неблагоприятные воздействия внешней среды без резкого снижения ростовых процессов и урожайности. Настоящее исследование проведено с целью изучения влияния водного дефицита на морфологические, биохимические и биоэнергетические показатели двух сортов проростков гороха, различающихся устойчивостью к водному дефициту. Параллельно изучали влияние донора оксида азота тетранитрозильного комплекса железа с тиосульфатными лигандами (ТНКЖ-тио) на устойчивость проростков гороха к недостаточному увлажнению.

Функциональное состояние митохондрий оценивали по скорости потребления кислорода митохондриями, по интенсивности перекисного окисления липидов, регистрируемого спектрофлуориметрическим методом, по изучению жирнокислотного состава мембран митохондрий, методом хроматографии и по исследованию морфологии митохондрий методом атомно-силовой микроскопии.

Недостаточное увлажнение приводило к активации ПОЛ, что вызывало набухание митохондрий и изменение содержания C_{18} и C_{20} жирных кислот (ЖК) в мембранах этих органелл. При этом дефицит воды (ДВ) вызывал снижение содержания линоленовой кислоты на 15% в мембранах митохондрий мало устойчивого к ДВ сорта, в то время как у устойчивого сорта - всего на 6%. При этом в мембранах митохондрий, устойчивых к дефициту воды сорта, ненасыщенных C_{20} ЖК было в 3,23 раза больше. ДВ сопровождался увеличением содержания этих жирных кислот. Различия в составе ЖК митохондриальных мембран влияли на биоэнергетические характеристики митохондрий. Недостаточное увлажнение сопровождалось снижением максимальных скоростей окисления НАД-зависимых субстратов. При этом скорости окисления НАД-зависимых субстратов и эффективность окислительного фосфорилирования у устойчивого сорта были выше. Обработка семян гороха 10^{-8}М ТНКЖ-тио восстановила биоэнергетические характеристики митохондрий у проростков гороха обоих сортов. Однако эффективность окислительного фосфорилирования в дыхательной цепи митохондрий устойчивого к ДВ сорта была на 15,7% выше.

Можно предположить, что повышенное содержание ненасыщенных ЖКОДЦ в мембранах митохондрий проростков гороха способствует поддержанию биоэнергетических характеристик митохондрий, а, следовательно, и энергетического метаболизма клетки в условиях стресса.

Ключевые слова: дефицит воды, перекисное окисление липидов, митохондрии, жирные кислоты с очень длинной цепью, доноры оксида азота.

1. Introduction

One of the most effective ways to increase the resistance of plants to stress, and consequently to increase crop yields, is the use of biologically active compounds in crop production. Such compounds include natural and synthetic antioxidants, as well as compounds that affect gene expression or the activity of antioxidant enzymes. These compounds also include nitric oxide donors [1], [2]. Nitric oxide can trigger the expression of antioxidant genes or activate antioxidant enzymes, for example, through post-translational modifications [3]. The antistress properties of NO can be determined by the fact that it is able to bind free iron ions in the composition of nitrosyl complexes. This possibly leads to inhibition of free radical oxidation reactions, which are catalyzed by redox active iron ions [4]. Therefore, NO can act as an antioxidant, although synergism in the action of NO and H_2O_2 is also possible [1]. In our studies, we paid attention to iron nitrosyl complexes with thiol-containing ligands. These compounds serve as natural carriers of NO; in addition, they protect cells from the dangerous effects of NO [4]. The advantage of nitrosyl iron complexes with thiol-containing ligands over other nitric oxide donors is due to the fact, that they release nitrogen monoxide at physiological pH values without any (thermo-, redox- or photo-) activation. Moreover, the decomposition of these complexes does not produce toxic substances [5].

It is known that the main target for nitric oxide are clusters of iron-sulfur proteins Fe-S. First of all, these are iron-sulfur proteins of mitochondria. These organelles play one of the main roles in plant adaptation to stress factors. At the same time, the amount and degree of saturation of fatty acids that make up the lipid bilayer of mitochondrial membranes changes, which, apparently, is a signal of the action of a stress factor [6], [7]. Changes in the lipid component of membranes probably have an effect on protein-lipid interactions, and therefore on the activity of proteins-enzymes of the mitochondrial respiratory chain. Perhaps this to some extent provides protection of these organelles and cells from oxidative stress. However, with the prolonged action of a stress factor or with a strong stress effect, the antioxidant-prooxidant equilibrium shifts towards an increase in the content of reactive oxygen species in the cell. At the same time, mitochondria and chloroplasts are one of the main sources of ROS [8], [9]. Excessive generation of ROS leads to activation of lipid peroxidation, mitochondrial dysfunction and, consequently, disruption of the energy metabolism of the cell. It can be assumed, that drugs that reduce the generation of ROS by mitochondria will increase the resistance of the cell and the whole organism to stress. Probably, this property may have a complex of iron with thiosulfate ligands. In terms of resistance to stress, there are plant cultivars that are highly resistant and have low resistance to various stress factors of the external environment.

In this regard, the aims of our work was to study the effect of water deficiency and TNIC-thio on the functional state of etiolated pea seedlings mitochondria of the more resistant cultivar Nemchinovsky 100 to water deficiency and the less resistant cultivar Flora 2.

2. Methods

The study was carried out on etiolated pea seedlings mitochondria of a more resistant to water deficiency cv. Nemchinovsky 100 (*P.sativum*, cv. Nemchinovsky 100) and a less resistant cv. Flora 2 (*P.sativum*, cv. Flora-2).

Tetranitrosil complex of iron with thiosulfate. Crystalline water-soluble nitric oxide donor μ2-dithiosulfate-tetranitosyldiferrate tetrahydrate (iron complex with thiosulfate) $[Na_2 [Fe_2 (S_2O_3)_2(NO)4]_2 \times 4H_2O$ (TNIC-thio) was synthesized at the Institute for Problems of Chemical Physics of the Russian Academy of Sciences [5], [10].

Pea seeds germination**.** Pea (*Pisum sativum* L., cv. Flora 2) seeds or pea (*P.sativum*, cv. Nemchinovsky 100) were washed with soapy water and 0,01% $KMnO_4$. Control seeds were then soaked in water, experimental seeds – in 10^{-8}M TNIC-thio for 1 h. Thereafter, seeds were transferred into covered trays on moistened filter paper in darkness for 2 days. Then, half of the control (water deficiency; WD) and TNIC-thio treated seedlings (water deficiency + TNIC-thio; WD+ TNIC-thio) were transferred in the open trays on dry filter paper. Another half of the control plants were retained in closed trays on wet filter paper, where they were kept for 5 days. After one day of water deficiency, seedlings were transferred to cover trays on wet filter paper, where they were kept for the next two days. On the fifth day, mitochondria were isolated from seedling epicotyls

Isolation of mitochondria. Isolation of mitochondria from 5-day-old epicotyl of pea seedlings (*P. sativum*) performed by the method [11] in our modification. The epicotyls having a length of 1,5 to 4,5 cm (25-30 g) were placed into a homogenizer cup, poured with an isolation medium in a ratio of 1:2, and then were rapidly disintegrated with scissors and homogenized with the aid of a press. The isolation medium comprised: 0,4 M sucrose, 5 mM EDTA, and 20 mM KH_2PO_4 (pH 8,0), 10 mM KCl, 2 mM 1, 4-Dithio-di-theiritol, and 0,1% fatty acids-free (FA free) BSA. The homogenate was centrifuged at 25000g for 5 min. The precipitate was re-suspended in 8 ml of a rinsing medium comprising of: 0,4 M sucrose, 20 mM KH_2PO_4, 0,1% FA free BSA (pH 7.4) and centrifuged at 3000g for 3 min. The supernatant was centrifuged for 10 min at 11000 g for mitochondria sedimentation. The sediment was re-suspended in 2-3 ml of the solution that contained: 0,4 M sucrose, 20 mM KH_2PO_4 (pH 7,4), 0,1 % FA-free BSA and mitochondria were precipitated by centrifugation at 11000 g for 10 min.

Rate of Mitochondria Respiration. Respiration in mitochondria was recorded polarographically (an LP-7 polarograph, Czech) using a Clark oxygen electrode. The incubation medium contained 0,4 M sucrose, 20 mM Hepes–Tris (pH 7,2), 5 mM KH_2PO_4, 4 mM $MgCl_2$, and 0,1% BSA (28°C). The rate of respiration was expressed in ng.atom O_2/(mg protein min).

Lipid peroxidation (LPO) activity. LPO activity was assessed by fluorescent method [12]. 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.

Fatty acid methyl esters (FAMEs). FAMEs were produced by acidic methanolysis of mitochondrial membrane lipids [13], [14]. The mitochondrial suspension (200 μL) was placed in a special hermetically closed tube, 5 mL of methanol was added, and the sample was placed in the freezer for 1 h. Thereafter, 600 μL of acetyl chloride was added, and the sample was heated for 1 h with stirring. FAMEs were extracted with hexane, and solutions obtained were analyzed.

FAMEs quantification. FAME quantification was performed using a Kristall 2000M chromatograph (Russia) with flame-ionization detector and quarts 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 [15]. 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.

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.

Atomic force microscopy (AFM). Samples of mitochondria for atomic force microscopy (AFM) were prepared on a polished silicone substrate. Before air drying, the mitochondria on the silicone substrate were fixed with 2% glutaraldehyde for 2 min. followed by rinsing with water. The study was carried out on the device SOLVER P47 SMENA at a frequency of 150 kHz in the semicontact mode. The NSG11 cantilever with a radius of curvature of 10 nm was used. Some geometric parameters of the image of mitochondria were determined using “Image Analysis”. The cross-section was made at an altitude of 30 nm. The volume of the image of mitochondria corresponded to the product of the cross-sectional area of the image of the mitochondria and the average height of this image in the section area.

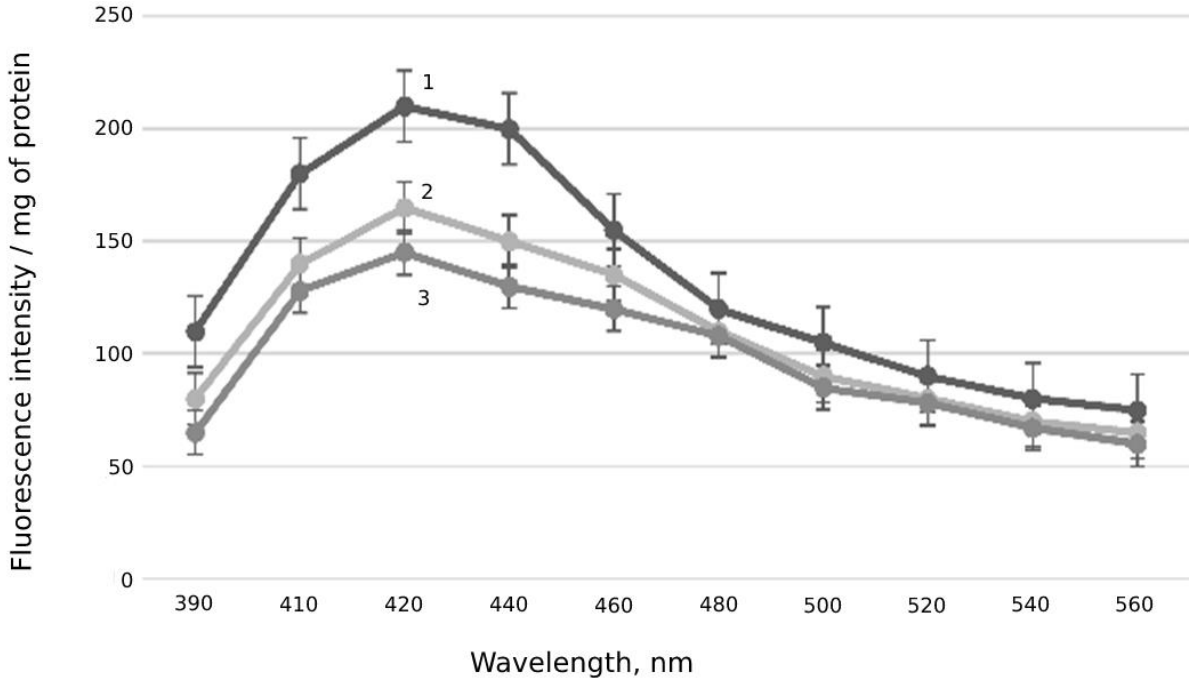
Statistical processing. Statistical processing of experimental data was carried out by determining the arithmetic mean and their standard errors. Significance of differences between variants with value $P \leq 0,05$.

The following reagents were used: BSA (Bovine serum albumin) (V-fraction), Sucrose, HEPES(4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid), malate, glutamate (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 (Biochemica Ultra, for molecular biology) (MB Biomedicals, Germany).

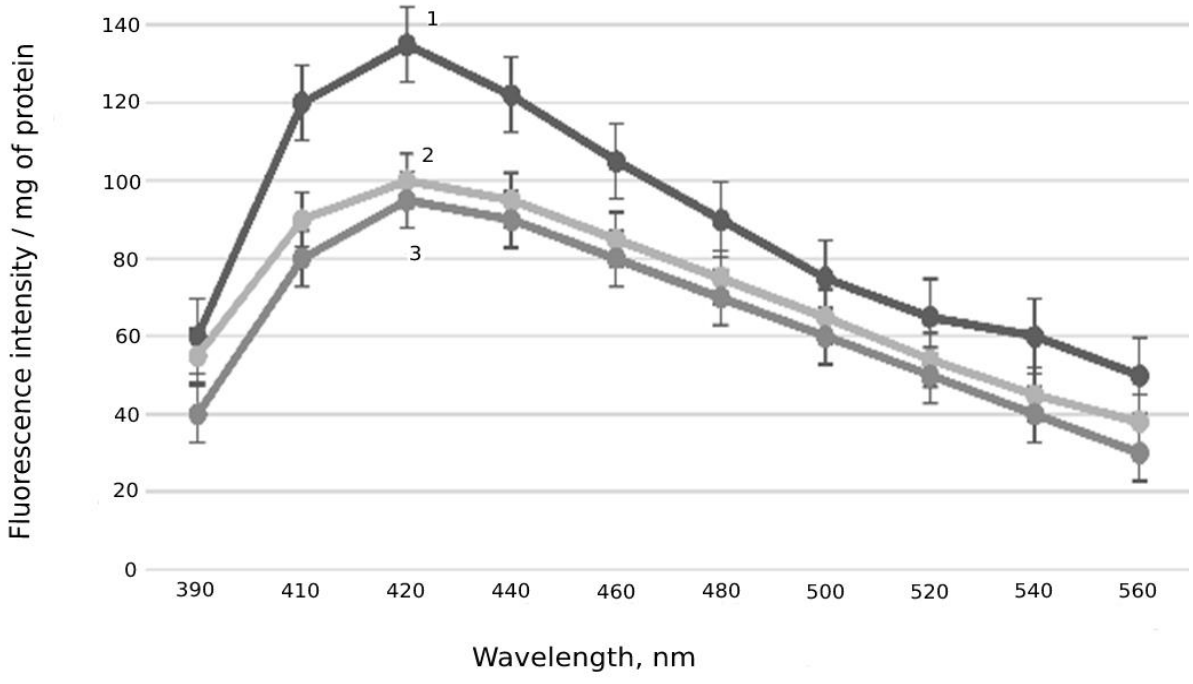
3. Results and discussion

Water scarcity, which causes osmotic stress, is one of the main environmental factors limiting the expansion of the range and the increase in the productivity of agricultural crops. Like all stress effects, water deficiency (WD), probably, increases the generation of ROS by mitochondria and, consequently, activates LPO in the membranes of these organelles [8]. Indeed, WD was accompanied by LPO activation in mitochondrial membranes of the studied cultivars of pea seedlings. At the same time, the fluorescence intensity of LPO products in pea seedling mitochondrial membranes differing in resistance to water deficiency increased 1,4 times (Fig. 1a, 1b). However, by absolute magnitude, the fluorescence intensity of the final LPO products (Schiff bases) in the mitochondrial membranes of seedlings of the Flora 2 (cultivar less resistant to WD) was higher than that of the

more resistant cultivar Nemchinovsky 100, which is probably explained by differences in the fatty acid composition of mitochondrial membranes.



A



B

Fig. 1 – Fluorescence spectra of lipid peroxidation products in mitochondrial membranes of pea seedlings:
A **–** cultivar Flora-2; *B* **–** cultivar Nemchinovsky 100; *1* – water deficit; *2* – water deficit + TNIC-thio; *3* – control

It is known, that lipid peroxidation leads to a violation of the osmotic balance between the matrix and the intermembrane space of mitochondria. As a result, there is swelling of the mitochondria [16]. It could be assumed that mitochondria of pea seedling differing in resistance to water deficiency would also differ in morphological characteristics. In this regard, was performed by examining the morphology of mitochondria by atomic force microscopy (AFM). AFM images of the mitochondria of the control groups of both cultivars were almost the same: 0,2±0,03μm^3 (Flora 2) and 0,15±0,03μm^3 (Nemchinovsky 100) (fig.2).

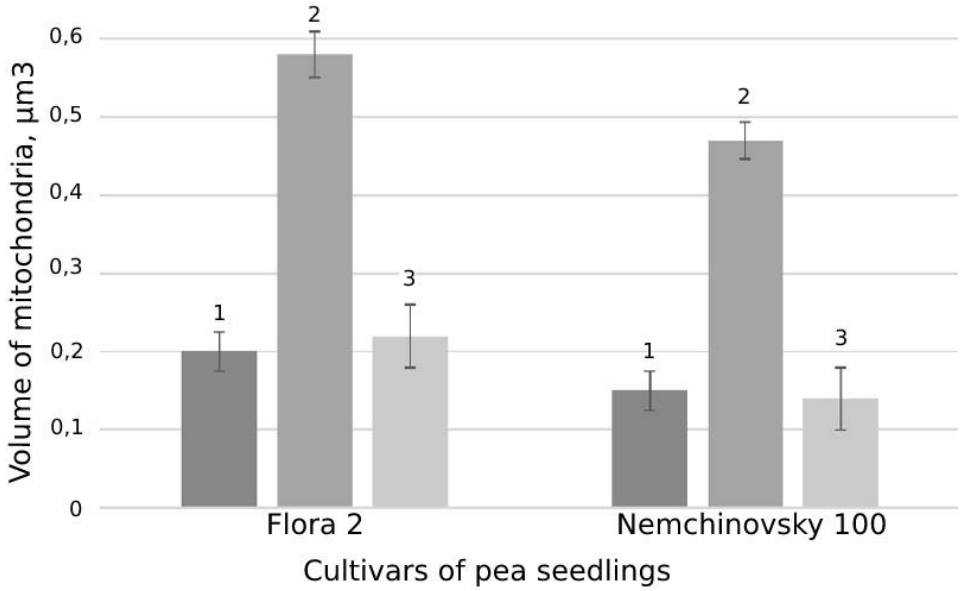


Fig. 2 – Effect of water deficiency and 10^{-8} M TNIC-thio on the volume of mitochondria: *1* – control; *2* – water deficiency; *3* – water deficiency+ 10^{-8} M TNIC-thio.

Water deficiency, activating lipid peroxidation, caused swelling of mitochondria. At the same time, mitochondria of large sizes were observed. The size of mitochondria of the cv. Flora 2 (0,58±0,03 µm^3) exceeded that of cv. Nemchinovsky 100 mitochondria (0,47±0,03 µm^3). Treatment of pea seeds with 10^{-8}M TNIC-thio prevented swelling of mitochondria, and their sizes did not differ from those of the control group: 0,22 ± 0,05 µm^3 (Flora 2) and 0,14 ± 0,05 µm^3 (Nemchinovsky 100). Thus, the mitochondria of the less resistant cultivar of pea seedlings cv.Flora 2 in conditions of water scarcity had larger sizes than the mitochondria of the more resistant cultivar to water scarcity cv. Nemchinovsky 100, i.e. they swelled more. However, the amplitude of mitochondrial swelling of both cultivars of pea seedlings did not differ much. It is possible that the observed differences are due to different fatty acid composition of mitochondrial membranes.

A study of the fatty acid composition (FA) of the cultivars Flora 2 and Nemchinovsky 100 showed differences in these indicators: the relative percentage of C_{18} unsaturated fatty acids in the mitochondrial membranes of the cv. Flora 2, which is less resistant to water deficiency, was 67,65 ± 0,36%, while in the more resistant cv.Nemchinovsky 100 had 57,91±0,46%. (Table 1). At the same time, the content of 18:3 ω3 and 18:1 ω9 in the pea seedlings mitochondrial membranes of cv. Nemchinovsky 100 was 2 times less than that of the Flora 2 cultivar.

Table 1 – Effect of water deficiency and TNIC-thio on the fatty acid composition of the total lipid fraction of mitochondrial membranes of pea seedling cultivars resistant and unstable to water deficiency

FAs	Flora 2			Nemchinovskaya 100		
	Control, rel.%	WD, rel.%	WD+TNIC-thio, rel.%	Control, rel.%	WD, rel.%	WD+TNIC-thio, rel.%
12:0	0,36±0,15	0,96±0,10	0,40±0,12	0,35±0,12	1,01±0,15	0,45±0,20
14:0	0,66±0,20	1,58±0,12	0,67±0,08	0,67±0,30	2,00±0,21	1,50±0,10
16:1ω7	0,94±0,12	1,90±0,15	0,93±0,15	0,90±0,12	1,90±0,24	0,52±0,10
16:0	18,00±0,50	22,40±0,80	18,87±1,00	16,16±0,5	14,50±0,03	15,25±0,03
17:0	0,50±0,07	0,90±0,10	1,00±0,08	2,14±0,07	4,80±0,30	1,50±0,16
18:2 ω6	50,00±0,21	43,77±0,40	50,00±0,38	50,00±0,21	42,90±0,43	49,00±0,09
18:3 ω3	12,20±0,02	10,40±0,01	12,53±0,02	5,06±0,02	4,80±0,01	8,91±0,13
18:1 ω9	4,78±0,11	3,49±0,09	2,67±9,10	2,25±0,11	1,80±0,11	3,83±0,21
18:1 ω7	0,67±0,02	0,64±0,03	0,61±0,02	0,60±0,12	0,75±0,13	0,78±0,02
18:0	4,16±0,21	5,83±0,18	2,48±0,25	3,00±0,21	5,83±0,20	3,20±0,22
20:3 ω6	1,17±0,11	0,50±0,14	1,17±0,18	2,76±0,11	3,80±0,32	1,17±0,15
20:2 ω6	0,82±0,23	1,48±0,11	3,69±0,32	4,90±0,23	5,42±0,22	5,09±0,21
20:1 ω9	2,29±0,31	1,54±0,25	2,41±0,21	6,37±0,61	7,30±0,26	7,42±0,28
20:1 ω7	1,26±0,04	1,10±0,03	1,25±0,02	3,84±0,18	–	–
20:0	1,36±0,02	1,90±0,01	0,51±0,04	1,00±0,01	3,10±0,41	1,38±0,18
22:0	0,83±0,42	1,61±0,12	0,1±0,05	–	–	–

Water deficiency caused a decrease in the content of these fatty acids by 1,1 and 1,2 times, respectively. However, the treatment of pea seeds with TNIC-thio led to different results. If in the membranes of pea seedlings mitochondria of a unstable cultivar to water deficiency, the content of unsaturated C_{18} FAs returned to the initial level or was slightly lower then it, then in the membranes of pea seedlings mitochondria of a resistant cultivar to water deficiency - their content increased in comparison with the initial level and exceeded these rates by 1,76 times. That may have contributed to an increase in the resistance of pea seedlings to water deficiency. Indeed, according to the literature, linolenic acid has the ability to modulate the expression of genes involved in the response to abiotic stress conditions, especially those mediated by the transmission of ROS signals [17],

[18]. It should be noted that the content of C_{20} unsaturated fatty acids in the mitochondrial membranes of the Nemchinovsky 100 was 3,23 times higher than that of the Flora 2. VLCFAs (very long chain fatty acids i.e., FAs containing more than 18 carbon atoms) are known to be important molecules that play a critical physiological, structural role in plants and may be involved in the organization of membrane domains [19]. It has been shown that when adapting to adverse environmental factors, the content of VLCFAs in organs and tissues increases significantly [20]. So content of VLCFAs $C_{20–24}$, including di- and trine-unsaturated, in the lipids of the roots of 6-week tobacco (*Nicotiana tabacum* L.) before low-temperature hardening (8°C, 6 days) was 18,6% of the total FA, after hardening their content increased to 24,7% [21]. According to Zhukov A.V. [20], VLCFAs are probably important for cell membranes due to their greater than usual length and ability to be located simultaneously in both layers of the bilayer membrane, thereby holding it together in the most critical situations, which, in particular, and occurs under stress. Indeed, water deficiency caused an increase in the content of unsaturated VLCFAs in the mitochondrial membranes of seedlings of more resistant to water deficiency cultivar, which may indicate a high adaptive capacity of the Nemchinovsky 100 cultivar (Table 1). It should be noted that under these conditions, in the less resistant cultivar to these conditions, there was a slight decrease in the content of unsaturated VLCFAs in mitochondrial membranes was observed. Treatment of pea seeds with nitric oxide donor TNIC-thio, which has antioxidant properties [21], restored the initial content of C_{20} FAs. Considering that FAs with long and very long chains play a role in increasing plant resistance to stress [19], it can be assumed that, precisely, the content of unsaturated C_{20} FAs in pea seedlings mitochondrial membranes determines the resistance of seedlings to water deficiency. It is possible that the adaptogenic effect of TNIC-thio in both cases is associated with an increase in the content of C_{18} and C_{20} unsaturated fatty acids in the lipid phase of mitochondrial membranes.

Differences in the FA composition of mitochondrial membranes, apparently, should have been reflected in the bioenergetic characteristics of mitochondria. Intact mitochondria of both cultivars differ only in oxidation rates of NAD-dependent substrates in the presence of ADP (Table 2). Nevertheless, the efficiency of oxidative phosphorylation in the cultivar Nemchinovsky 100 was higher by 7,5%.

Table 2 – Effect of water deficiency and TNIC-thio on the oxidation rate of NAD-dependent substrates by pea seedling mitochondria, number of repetitions – 8

Group	V_2, ng O_2 atom/mg protein × min	V_3, ng O_2 atom/mg protein × min	V_4, ng O_2 atom/mg protein × min	V_3/V_4, ng O_2 atom/mg protein × min	FCCP, ng O_2 atom/mg protein × min
Control (Flora 2)	24,80±1,15	72,50±2,10	31,79±1,50	2,28±0.01	74,10±2,80
WD	14,00±1,10	35,60±2,50	22,97±1,00	1,55±0,02.	42,10±2,20
WD+10^{-8}M TNIC-thio	25,74±1,30	75,50±2,60	31,20±1,25	2,42± 0.01	76,40±3,10
Control (Nemchinovsky 100)	25,24±1,11	82,55±2,10	33,69±1,61	2,45±0.01	78,85±1,95
WD	37,38±1,10	50,00±2,10	27,47±1,23	1,82±0,01	55,70±1,12
WD+10^{-8}M TNIC-thio	41,76±1,00	82,60±2,91	29,50±1,34	2,80±0.02	77,62±2,00

Note: Incubation medium: 0,4 M sucrose, 20 mM HEPES-Tris buffer (pH 7,2), 5 mM KH_2PO_4, 4 mM $MgCl_2$, 10 mM malate, 10 mM glutamate. Additional additives: 200 μM ADP, 10^{-6} M FCCP (carbonyl cyanide-p-trifluoromethoxyphenyl-hydrazone). V_2 – substrate oxidation rates, V_3 – substrate oxidation rates in the presence of ADP; V_4 – oxidation rates at rest (substrate oxidation rates when ADP is exhausted)

Under conditions of water deficiency, the differences in the bioenergetic characteristics of both cultivars mitochondria increased. WD led to a decrease in the oxidation rates of NAD-dependent substrates in the state of 3 by mitochondria of both cultivars. However, the oxidation rate of NAD-dependent substrates in the presence of ADP by mitochondria of the Nemchinovsky 100 cultivar exceeded this rate in Flora 2 cultivar mitochondria of the by 40%, and in terms of oxidative phosphorylation efficiency – by 17,4%. Treatment of pea seeds with 10^{-8}M TNIC-thio restored the bioenergetic characteristics of both cultivars pea seedlings mitochondria. However, the efficiency of oxidative phosphorylation in the respiratory chain of mitochondria of the more resistant to water-deficiency cultivar was 15,7% higher (Table 2). These data probably confirm the role of the FAs composition of mitochondria in preserving bioenergetic characteristics under stress conditions.

4. Conclusion

It can be assumed, that the increased content of unsaturated VLCFAs in the mitochondrial membranes of pea seedlings contributes to maintaining the bioenergetic characteristics of mitochondria, and, consequently, the energy metabolism of the cell, which probably underlies the increase in the body's resistance to stress.

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Conflict of Interest

None declared.

Конфликт интересов

Не указан.

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