



## Organophosphorus Plant Growth Regulators Provides High Activity Complex I Mitochondrial Respiratory Chain *Pisum sativum L* Seedlings in Conditions Insufficient Moisture

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### Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

### Article Information

DOI: 10.9734/ARRB/2015/12378

Editor(s):

(1) George Perry, Dean and Professor of Biology, University of Texas at San Antonio, USA.

Reviewers:

(1) Anonymous, University Carthage, Tunisia.

(2) Naser Arabzadeh, Faculty of Agriculture and Natural Resources Research Center of Kerman- IRAN.

(3) Anonymous, National Institute of Agricultural Sciences, Cuba.

(4) Anonymous, AIMST University, Malaysia.

Complete Peer review History: <http://www.sciencedomain.org/review-history.php?iid=653&id=32&aid=6031>

Original Research Article

Received 28<sup>th</sup> June 2014  
Accepted 20<sup>th</sup> August 2014  
Published 10<sup>th</sup> September 2014

### ABSTRACT

**Aims:** Water deficiency causes an enhanced generation of reactive oxygen species (ROS) by mitochondria, leading to oxidative stress. It is known that plant growth regulators (PGRs) increase the resistance of plants to stresses and, evidently, to a water stress. Such PGRs are melaphen – a melamine salt of bis (oxymethyl) phosphinic acid and a pyraphen salt of bis (oxymethyl) phosphinic acid 2,4,6- triaminopyrimidine. The aim of this work was to elucidate the effects of insufficient watering and treatment of seeds of pea (*Pisum sativum L*) with PGRs melaphen or pyraphen on the fatty acid composition of the lipid fraction membranes and bioenergetic functions of mitochondrial in etiolated 6-day pea seedlings.

**Place and Duration of Investigation:** Emanuel Institute of Biochemical Physics Russian

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Academy of Sciences, Moscow, Russia and Timiryazev Institute of Plant Physiology Russian Academy of Sciences, Moscow, Russia, between April 2010 and October 2011.

**Methodology:** Functional state of mitochondria was researched by rate of mitochondria respiration, by the level of lipid peroxidation and study of fatty acid composition of mitochondrial membranes by chromatography.

**Results:** A water deficiency has caused changes in the fatty acid (FA) composition which manifested themselves in increasing the content of saturated FAs and decreasing the content of unsaturated FAs containing 18 and 20 carbon atoms. The treatment of the seeds with melaphen ( $2 \times 10^{-12}$  M) has prevented the transformation of pool of fatty acids in lipid fraction of mitochondrial membranes. Pyraphen ( $10^{-14}$  M) has prevented the changes only in the content of FAs containing 20 carbon atoms. Changes in the FA compositions of membrane lipids were correlated with changes in the maximum rates of oxidation of NAD-dependent substrates ( $r = 0.765$  for  $C_{18}$ ).

**Conclusion:** It is suggested that tolerance to water stress is determined by the antioxidant system protecting the unsaturated  $C_{18}$  and  $C_{20}$  fatty acids from changes caused by oxidative stress, i.e., of activation of free radical processes.

**Keywords:** *Pisum sativum*; mitochondria; plant growth regulators (PGRs); insufficient watering, lipid peroxidation; fatty acid composition; respiratory chain.

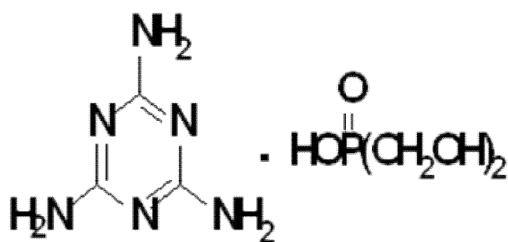
## ABBREVIATIONS

*IW* – insufficient watering; *Mph*-melaphen; *Pyr* -pyraphen; *FA*- fatty acid.

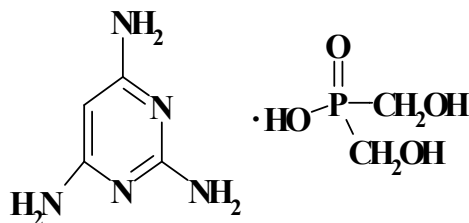
## 1. INTRODUCTION

Water is an essential factor that maintains the vital functions of plants. All physiological processes proceed with the participation of water; therefore, water is one of the vitally important ecological factors that affect the growth and development of plant organisms and the propagation of plants on the Earth. Water participates in all metabolic processes by maintaining the structure of cytoplasm and the stability of its colloidal components, and also by supporting a certain conformation of proteins. At present, has been accumulated the numerous data demonstrating that even weak water deficit affected plant metabolism and thus their growth and development [1,2]. In conditions of water deficit in plants delayed biosynthesis of organic compounds, and the hydrolytic reactions are enhanced, in result disturbed the growth processes [3]. Metabolism of plants survived even short-term strong drought could not be recovered [3]. Water deficit modifies cell membranes, affecting their function and metabolism of the cells [4]. The energy metabolism plays a significant role in adaptive response of the organism. Mitochondria play a key role in the energy, redox and metabolic processes in cell [5,6]. However, under stress conditions these organelles are a major source of reactive oxygen species (ROS) [7,8]. Interaction ROS with poly-unsaturated fatty acids which are in the composition of the mitochondrial

membranes lipids, in particular, like with linoleic and linolenic acids, leads to the activation of lipid peroxidation (LPO). Appearance of hydrophilic products oxidation as a result of lipid peroxidation alters the structure of the lipid bilayer membranes in the hydrophobic areas and leads to mitochondrial dysfunction. As known from the literature, regulators of plant growth and development (PGRs) improve plant's tolerance to biotic and abiotic stresses, in particular to deficit of water. Their application gives the opportunity to regulate the most important processes in the plant body, fully realize the potential of the variety inherent in the nature of the genome and selection [9,10]. These biologically active substances must be safe for human health and the environment. Such preparation is melaphen – a melamine salt of bis(oxyethyl)phosphinic acid, which was synthesized at the Arbuzov Institute of Organic and Physical Chemistry of the Kazan Scientific Center, Russian Academy of Sciences ( $LD_{50}$  -2000 mg/kg for mice; has not been identified and DNA -damaging activity in any of the studied concentrations of melaphen and this drug showed no mutagenicity in the Ames test in experimental variants with and without metabolic activation it) [11]. To compare of the protective properties of melaphen with preparations having similar structure we used derivative of pyrimidine derivative - pyraphen (salt of bis-(oxyethyl)phosphinic acid, 2,4,6-triaminopyrimidine) ( $LD_{50}$  -for this drug we was not determined).



Melaphen



Pyraphen

It should be noted that the Effectiveness of the preparations will depend on their concentration, since the plant growth regulators, like other the biological active substances exhibit their activity in the dependence from dose [12,13]. Therefore, for the plant growth regulators, in particular for melaphen or pyraphen, it is necessary to select the most effective concentrations that will provide the increasing the stability of plant organisms to stresses. Since stress lead to increasing of the generation of ROS by mitochondria, we suggested that the possible protective properties of melaphen as a plant growth regulator, probably, may be associated with decreasing of the excessive production of ROS and, consequently, with decreasing the intensity of lipid peroxidation processes in biological membranes, mainly in the membranes of mitochondria, influencing the functional state of mitochondria. It is well known that water deficiency decreases the functional activity both of chloroplasts and of mitochondria [14]. Therefore it was of interest to find out it is possible whether the change of bioenergetic characteristics of mitochondria under conditions of water scarcity and seed treatment with melaphen. Investigation of the protective properties of the preparation was carried out using a  $2 \times 10^{-12}$  M melaphen, i.e., in the concentration in which this plant growth regulator reduced the intensity LPO to the control level [15].

## 2. MATERIALS AND METHODS

### 2.1 Plant Material

The study was carried out on mitochondria isolated from pea seedlings (*P. sativum*, culture Flora-2) obtained in standard conditions and in the conditions of insufficient humidifying and on mitochondria isolated from sugar beet root storage (*Beta vulgaris* L.), obtained in standard conditions.

### 2.2 Pea Seeds Germination

Pea (*Pisum sativum* L., cv. Flora-2) seeds were washed with soapy water and 0.01% KMnO<sub>4</sub>. Control seeds were then soaked in water, experimental seeds – in  $2 \times 10^{-12}$  M melaphen for 1 h. Thereafter, seeds were transferred into covered trays on moistened filter paper in darkness for 2 days. After 2 days, half of control and half of melaphen treated seeds were transferred in the open trays on dry filter paper (water deficit treatment). Another half of control plants were retained in closed trays on wet filter paper, where they were kept for 6 days. After two days of water deficit treatment, seeds were transferred to covered trays on wet filter paper, where they were kept for next two 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*) or from sugar beet root storage (*Beta vulgaris* L., cv. Verhnyachenskaya 31) performed by the method [15] in our modification. The epicotyls having a length of 1.5 to 5 cm (20-25 g) or 30 g of sugar beet root storage 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, 5mM EDTA, and 20 mM KH<sub>2</sub>PO<sub>4</sub> (pH 8.0), 10mMKCl, 2mM 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 comprised: 0.4 M sucrose, 20mM KH<sub>2</sub>PO<sub>4</sub>, 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 solution contained: 0.4 M sucrose, 20 mM

$\text{KH}_2\text{PO}_4$  (pH 7.4), 0.1 % FA-free BSA and mitochondria were precipitated by centrifugation at 11000 g for 10 min.

## 2.4 Rate of Mitochondria Respiration

Respiration in mitochondria was recorded polarographically (an LP-7 polarograph, Czech Republic) using a Clark oxygen electrode. The incubation medium contained 0.4 M sucrose, 20 mM Hepes–Tris (pH 7.2), 5 mM  $\text{KH}_2\text{PO}_4$ , 4mM  $\text{MgCl}_2$ , and 0.1% BSA (28°C). Other additives: 10 mM malate, 10 mM glutamate, 125 $\mu\text{M}$  ADP, 10 $^{-6}$  M FCCP (carbonyl cyanide-ptrifluoromethoxyphenylhydrazone). The rate of respiration was expressed in ng-atom O/mg protein x min.

## 2.5 Fatty Acid Methyl Esters (FAMES)

FAMES were produced by acidic methanolysis of mitochondrial membrane lipids [16,17]. Mitochondrial suspension (200 $\mu\text{L}$ ) was placed in a special hermetically closed tube, 5mL of methanol was added, and the sample was placed in the freezer for 1h. Thereafter, 600 $\mu\text{L}$  of acetyl chloride was added, and the sample was boiled for 1h with stirring. FAMES were additionally purified by TLC on glass plates with silica gel KSK (Russia). FAMES were extracted with hexane, and solutions obtained were analyzed.

## 2.6 FAME Identification

FAME identification was performed by chromat mass spectrometry (GCMS) using a Hewlett-Packard-6890 spectrophotometer with a HP-5972 mass-selective detector and by the retention times [18]. FAME were separated in the HP-5MS capillary column (30 m  $\times$  0.25 mm, phase film thickness of 0.25 $\mu\text{m}$ ) at programmed temperature increase from 60 to 285°C at the rate of 5°C/min. Evaporator temperature is 250°C, detector temperature is 280°C. Mass spectra were obtained in the regime of electron impact ionization at 70 eV and the scan rate of 1 s/10 mass in the scan mass range of 40–450 a.u.m.

## 2.7 FAME Quantification

FAME quantification was performed using a Kristall 2000M chromatograph (Russia) with flame-ionization detector and quartz capillary column SPB-1 (50 m  $\times$  0.32 mm, phase film thickness of 0.25  $\mu\text{m}$ ). FAME analysis was performed at programmed temperature increase

from 120 to 270°C at the rate of 4°C/min. Temperature of injector and detector – 270°C; the helium carrier gas rate was 1.5mL/min. Each sample contained 2 $\mu\text{L}$  of the hexane extract. The FAME content in samples was calculated as the ratio of peak area of a corresponding acid to the sum of peak areas of all found FAMES.

## 2.8 Lipid Peroxidation (LPO) Activity

LPO activity was assessed by fluorescent method [19]. Lipids were extracted by the 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. Thereafter, equal volume of distilled water was added to the homogenate, and after rapid mixing the homogenate was transferred into 12mL centrifuge tubes. Samples were centrifuged at 600 g for 5 min. The aliquot (3 mL) of the chloroform (lower) layer was taken, 0.3mL of methanol was added, and fluorescence was recorded in 10mm quartz cuvette with a spectrofluorometer (FluoroMaxHoribaYvon, Germany). Background fluorescence was recorded using a mixture of 3mL chloroform and 0.3 mL methanol. The excitation wavelength was 360 nm; the emission wavelength was 420–470 nm. The results were expressed in arbitrary units per mg protein.

## 2.9 Unsaturation Index

Unsaturation index was calculated as a total percentage of unsaturated fatty acids with a certain number of atoms multiplied by the number of double bonds and divided by 100. For example, for fatty acids with 18 carbon atoms, the unsaturation index is equal to  $18:1\omega_9 + 18:1\omega_7 + 18:2\omega_6 \times 2 + 18:3\omega_3 \times 3/100$ .

## 2.10 The Unsaturation Coefficient

Unsaturation coefficient is the ratio of a total percentage of unsaturated fatty acids with a certain number of atoms to the percentage of saturated fatty acids with the same number of atoms. For example, for FAs with 18 carbon atoms, the unsaturation coefficient is  $(18:2\omega_6 + 18:3\omega_3 + 18:1\omega_9 + 18:1\omega_7)/C18:0$ .

## 2.11 Statistics

Tables and figures present means and their standard deviations. The number of experiments was 10 in Fig. 1 and Fig. 2, 120 in Fig. 4, 8 in Table 1 and 10 in Table 2. The correlations

between coefficients of C<sub>18</sub> unsaturation and the rates of NAD-dependent substrate oxidation were calculated using Statistica v6 (software for Windows).

## 2.12 Reagents

The following reagents were used: potassium carbonate, methanol, chloroform (Merck, Germany), hexane (Panreac, Spain), acetyl chloride (Acros, Belgium), sucrose, Tris, EDTA, FCCP, malate, glutamate, succinate, ADP, FA-free BSA (Sigma, United States), Hepes (MB Biomedicals, Germany).

## 3. RESULTS AND DISCUSSION

According to the literature the various stresses lead to the activation of lipid peroxidation (LPO) [20-23]. Under stress conditions, the main sources of ROS are mitochondria and chloroplasts. Since we studied the role of mitochondria in the sustainability of pea seedlings in condition of insufficient watering, we needed to develop a model simulating stress, to find conditions under which mitochondria will be increased the generation of ROS, and, therefore, will be activated LPO. We solved this problem by having developed a model of "aging" (the mitochondria isolated from sugar-beet storage roots was incubated in a hypotonic medium at room temperature). The incubation of mitochondria in a hypotonic solution of sucrose caused a weak swelling of mitochondria and growth of the ROS generation that resulted in a 3 to 4-fold increase in the intensity of fluorescence of LPO products [14], which is consistent with the data Earnshaw [24]. To enhance this effect, we introduced into the incubation medium an inorganic phosphate, resulting in occur the swelling of mitochondria and activating ROS [25-27]. Having developed a model simulating stresses, we could proceed to a search for concentrations of melaphen or pyraphen that would effectively decrease the LPO intensity in mitochondrial membranes. The introduction of melaphen or pyraphen into the incubation medium of mitochondria caused the reduction of LPO intensity, which shows dependence from dose. Melaphen decreased the intensity of fluorescence of LPO products in membranes of "aged" mitochondria in concentrations of  $2 \times 10^{-7}$ ;  $2 \times 10^{-12}$  and  $2 \times 10^{-18}$  -  $2 \times 10^{-22}$  M. Pyraphen (the pyrimidine derivative) decreased the content of LPO products in mitochondrial membranes to the control values in concentrations of  $10^{-5}$ ;  $10^{-7}$ ; and  $10^{-14}$  -  $10^{-18}$  M (Fig. 1).

Investigation of the protective properties of the preparations was carried out at a model of insufficient watering using a  $2 \times 10^{-12}$  M melaphen and  $10^{-14}$  M pyraphen in concentrations, in which these plant growth regulators decreased the LPO intensity to the control values. As objects of study used mitochondria isolated from 6 day-old etiolated pea seedlings. Choice of pea seedlings as a research object due to the fact that the seeds of peas quickly sprout and it is possible to investigate in the adaptive model. In addition, pea is very sensitive to water stress [28] and from the seedlings (6 day -old) can get enough material for isolation of mitochondria. The insufficient watering led to LPO activation in the mitochondrial membranes of pea seedlings. In this case, the fluorescence intensity of LPO products increased 3 times (Fig. 2). These data are consistent with the published data on the effect of a water stress on the activation of free radical oxidation in membranes of wheat seedlings [29,30].

It is probable that the LPO activation may lead to changes in the fatty acid composition of mitochondrial membranes. Therefore, in the subsequent experiments we studied the effect of insufficient watering and PGRs on the fatty acid composition of the overall lipid fraction of mitochondrial membranes. Water deficit led to the increase in the relative content of saturated and a decrease in the content of unsaturated fatty acids in mitochondrial membranes of pea seedlings. The relative content of linoleic acid was reduced by 11%, and of linolenic acid – by 19%. The content of stearic acid increased by 41%, which resulted the decrease in the total content of C<sub>18</sub> unsaturated FAs relative to the content of stearic acid from  $16.61 \pm 0.30$  to  $10.59 \pm 0.20$  (Table 1).

Changing the content of fatty acids with 18 carbon atoms due to dehydration are also observed in membrane lipids of potato, suspension of cells, and membrane lipids of the leaves of *Arabidopsis thaliana* and apricot [31-34]. In all these cases, insufficient watering was accompanied by a decrease in the contents of linoleic and linolenic acids and an increase in the content of stearic acid in mitochondrial membranes. Substantial changes occurred also in the relative content of fatty acids with 20 carbon atoms. The pool of 20:2  $\omega$ 6 has decreased by 2.7 times, 20:1  $\omega$ 9 – 1.4 times, and 20:1  $\omega$ 7 – 1.3 times. At the same time, the content of eicosanoic acid (20:0) increased more

than twofold. As a result, the ratio (20:1 $\omega$ 7 + 20:1 $\omega$ 9 + 20:2 $\omega$ 6)/C20:0 in lipids of mitochondrial membranes decreased from 3.65 $\pm$ 0.03 to 1.20 $\pm$ 0.16. The unsaturation index of fatty acids with 20 carbon atoms decreased from 0.0531 $\pm$ 0.0010 to 0.0317 $\pm$ 0.0010. These changes may entail changing and lipid-protein interactions and, consequently, the activity of membrane-bound enzymes, in particular, of the enzymes of the mitochondrial respiratory chain. Indeed, insufficient watering resulted in decreasing the maximum oxidation rates of NAD-dependent substrates. Rates of oxidation of NAD-dependent substrates in the presence of FCCP(carbonyl cyanide-*p*-trifluoromethoxyphenylhydrazone) decreased from 70.0 $\pm$ 4.6 to 48.9 $\pm$ 3.2ng-atom O/mg protein x min and the efficiency of oxidative phosphorylation was also lower: the value of the respiratory control rate (RCR) reduced from 2.27 $\pm$ 0.1 in control to 1.70 $\pm$ 0.02 (Table 2). These data are consistent with the literature, since it is known that adverse environmental factors to some extent inhibit respiratory metabolism of the mitochondria and reducing its effectiveness due to partial suppression of oxidative phosphorylation [35].

The incubation medium contained: 0.4M sucrose, 20 mM HEPES-Tris buffer, 5 mM KH<sub>2</sub>PO<sub>4</sub>, 4 mM MgCl<sub>2</sub> and 0.1% BSA, 10 mM malate, 10 mM glutamate. Other additives: 125 $\mu$ M ADP, 10<sup>-6</sup>M FCCP (carbonyl cyanide-*p*-trifluoromethoxyphenylhydrazone). The results of 10 experiments are presented.

The soaking of seeds in a 2 $\times$ 10<sup>-12</sup> M melaphen has prevented by changes in the efficiency of oxidative phosphorylation caused by water deficiency. Also, such treatment had prevented decrease the oxidation rates of NAD-dependent substrates in the presence of ADP or FCCP. It was found out that the treatment of pea seeds with a pyrimidine analog of melaphen – a 10<sup>-14</sup> M pyraphen (which has a minor effect on the LPO activity under conditions of insufficient watering), almost had no effect on the bioenergetic properties of pea seedling mitochondria. The maximum oxidation rates of NAD-dependent substrates in the presence of ADP or in the presence of FCCP differed little from the maximum rates of oxidation of these substrates to mitochondria of the seedlings that were exposed to insufficient watering. However, the efficiency of oxidative phosphorylation increased: the respiratory control rates increased from 1.70 $\pm$ 0.02 to 1.90 $\pm$ 0.02 (Table 2). The changes in

the energy metabolism of mitochondria are, evidently, connected with the physic chemical state of membranes of these organelles. Indeed, soaking seeds with melaphen, protect from peroxide oxidation the unsaturated fatty acids that are part of lipids of membranes, what prevented by the changes in the fatty acid composition of mitochondrial membranes, seedlings grown under conditions of insufficient watering (IWMph) (Table 1). In this case, the relative content of saturated fatty acids such as laurinic, palmitic, and stearic acids by 65, 7.5, and 30% (IWMph), was lower than that of seedlings grown under low moisture (IW). The content of C<sub>18</sub> unsaturated fatty acids, playing a key role in plant tolerance to unfavorable environment [36], remained on the control level. The relative content of C<sub>20</sub> unsaturated fatty acids not also differed from control values. As a result, the ratio of total content of pool unsaturated fatty acids with 20 carbon atoms (20:1 $\omega$ 7 + 20:1 $\omega$ 9 + 20:2 $\omega$ 6) to the content of pool of C<sub>20:0</sub> in the membrane lipids of seedlings treated with melaphen and subjected to water deficit was similar to the control ratio. At the same time as pyraphen almost no impact on the oxidation of C<sub>18</sub> fatty acids under conditions of insufficient watering (IWPyr) (Table 1), but leads to the increase of the relative content of C<sub>20</sub> unsaturated fatty acids in mitochondrial membranes of pea seedlings at 1.4 - 2.7-times (IWPyr) (Table 1), which evidently affected the bioenergetic properties of these organelles.

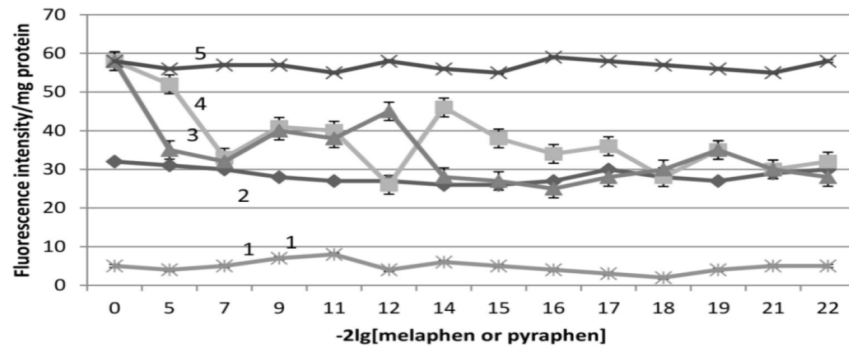
On the basis of presented data, it may be supposed that an increase in the content of unsaturated fatty acids, in particular C<sub>18</sub> and C<sub>20</sub> acids, in the lipid fraction of plant cell membranes might result in improved plant tolerance to water deficit. Really, a close correlation was observed between unsaturation coefficient of C<sub>18</sub> fatty acids in lipid fraction of mitochondrial membranes ( $\Sigma$ unsaturated C<sub>18</sub> fatty acids/ C<sub>18:0</sub>) with the maximum oxidation rates of NAD- dependent substrates ( $r = 0.76489$ ) (Fig. 3).

It may be associated with the content of linoleic acid, which is one of the basic fatty acids that enter into the composition of the cardiolipin, which ensures the effective functioning of the respiratory chain of mitochondria in connection with formation of super complexes of the respiration carriers [37].

Changes of physic chemical properties of mitochondrial membranes leading to changes in energy metabolism also have an effect on

physiological indicators, namely, the growth of seedlings. It is known, that pea seedling are particularly sensitive to drought stress. As has been shown previously, that earlier growth stages were more sensitive to water deficit than subsequent ones [38]. In our experiments, we used the most sensitive to lack of moisture the growth stage of pea seedlings (two-day seedlings). Insufficient moisture inhibits the growth process (Fig. 4), which is consistent with literature data [39-40]. As evident from Fig. 4, soaking of pea seeds with melaphen or pyraphen

under conditions of insufficient watering induce an increase in growth of root at 5 and 1.75 times, respectively. However, pyraphen had almost no effect on the growth of sprouts, whereas melaphen at 3.5-fold stimulated the growth of sprouts. Note that germination of treated and untreated seeds differed significantly. Under drought conditions, the germination of seeds in control had decreased by 46%; when the germination melaphen-treated seeds remained almost unchanged [41].

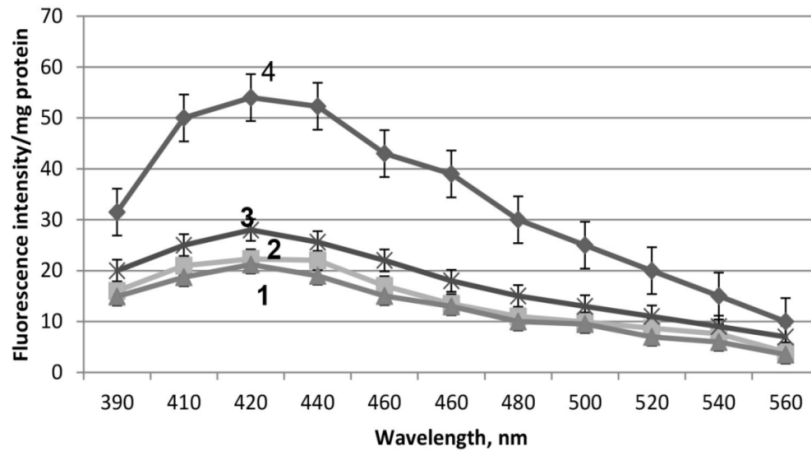


**Fig. 1. The fluorescence intensity of LPO products after introduction of different concentrations of melaphen or pyraphen into the incubation medium of the mitochondria isolated from the sugar-beet storage roots. Y-axis: fluorescence intensity, arbitrary units/mg protein**

X-axis: the concentration of melaphen or pyraphen. 1- fluorescence intensity in samples containing different concentrations of melaphen or pyraphen; 2 -control (without introduction of melaphen or pyraphen into the incubation medium); 3 -introduction of pyraphen in different concentrations into the incubation medium; 4 - introduction of melaphen in different concentrations into the incubation medium; 5- "aged" mitochondria (without introduction of melaphen or pyraphen)

**Table 1. Effects of insufficient watering (IW) and a treatment of pea seeds with melaphen(Mph)or pyraphen (Pyr) upon the relative FA content in the lipids of mitochondrial membrane of pea seedlings, % (The results of 8 experiments)**

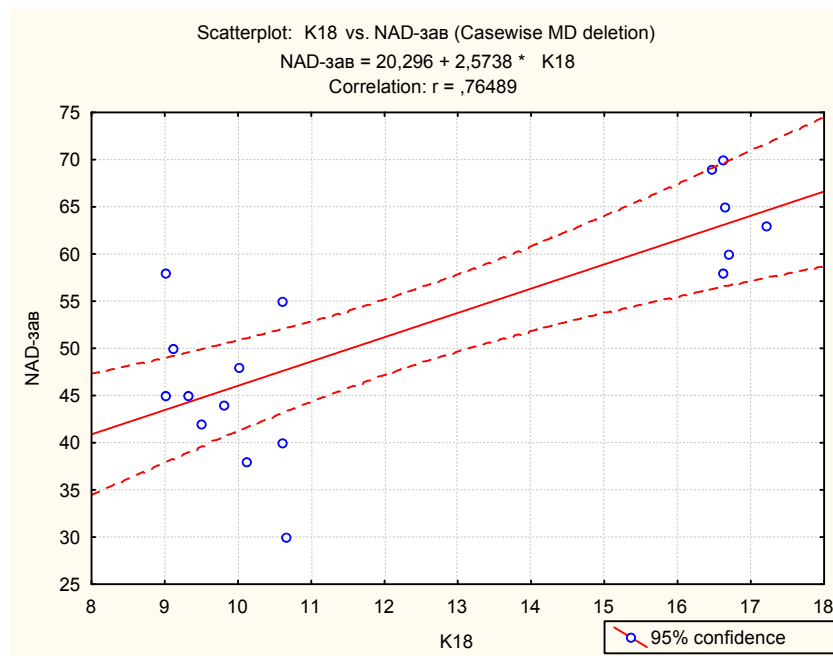
Fattyacid	Control	IW	IWMph	IWPyr
12:0	0.34±0.03	0.94±0.03	0.34±0.02	0.65±0.05
14:0	0.68±0.03	0.67±0.02	0.69±0.02	0.70±0.04
16:1ω7	0.36±0.03	0.47±0.13	0.42±0.05	0.40±0.11
16:0	18.64±0.75	20.74±0.11	18.96±0.50	20.5±0.33
17:0	0.45±0.05	0.66±0.10	0.45±0.16	0.62±0.10
18:2ω6	50.72±0.80	45.22±0.10	50.65±0.01	46.0±0.15
18:3ω3	11.3±0.02	9.18±0.30	10.81±0.09	9.30±0.08
18:1ω9	5.27±0.40	6.77±0.20	5.22±0.01	5.67±0.20
18:1ω7	0.81±0.10	0.61±0.03	0.73±0.05	0.65±0.05
18:0	4.10±0.18	5.83±0.38	4.10±0.15	6.00±0.10
20:2ω6	0.82±0.01	0.30±0.05	0.82±0.01	0.81±0.01
20:1ω 9	2.22±0.01	1.57±0.01	2.6±0.03	2.11±0.02
20:1ω7	1.45±0.01	1.00±0.01	1.52±0.01	1.40±0.01
20:0	1.23±0.03	2.52±0.20	1.30±0.05	2.00±0.03
22:0	1.23±0.11	2.52±0.20	1.04±0.05	2.65±0.10
24:0	0.37±0.02	0.98±0.15	0.35±0.10	0.54±0.15
18:2ω6 + 18:3ω3 + 18:1ω9 + 18:1ω7)/C18:0	16.61±0.30	10.59±0.20	16.44±0.25	10.27±0.40
20:2 ω 6 + 20:1 ω 9 + 20:1 ω 7)/C20:0	3.65±0.10	1.20±0.16	3.80±0.30	2.16±0.20
Index of unsaturation of C <sub>18</sub> fatty acids	1.41±0.02	1.25±0.01	1.40±0.01	1.26±0.02
Index of unsaturation of C <sub>20</sub> fatty acids	0.0531±0.001	0.0317±0.001	0.0576±0.001	0.0513±0.002



**Fig. 2.** Fluorescence spectra of LPO products (Schiff's bases) in mitochondrial membranes of etiolated pea seedlings, exposed to the insufficient watering. *Y-axis: fluorescence intensity, arbitrary units/mg protein; X-axis: wavelength, nm.* 1) Control; 2) IWMph( $2 \times 10^{-12}$  M melaphen 3) IWPyr( $10^{-14}$  M); 4) IW

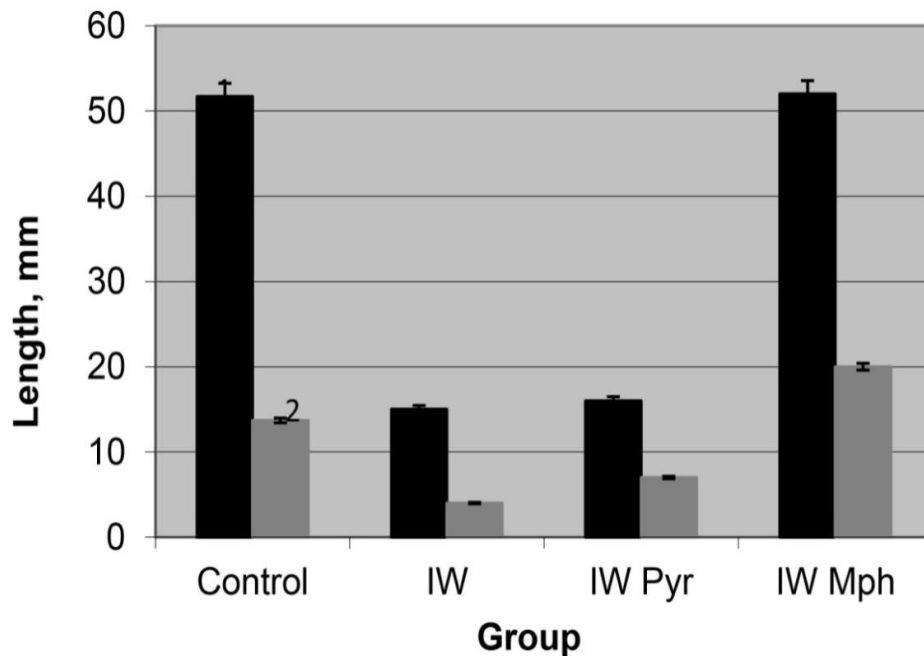
**Table 2.** Effects of insufficient watering (IW) and treatment of pea seeds with the help of melaphen (Mph) or pyraphen (Pyr) on the rate of NAD-dependent substrate oxidation by mitochondria isolated from pea seedlings, ng-atom/ mg proteinx \* min

Group	State 2	State 3	State 4	RCR	FCCP
Control	20.0±1.5	68.0±4.1	30.0±2.0	2.27±0.01	70.0±4.6
IW	12.0±2.0	48.6±3.0	40.2±1.0	1.70±0.02	48.9±3.2
IW+Mph ( $2 \times 10^{-12}$ M)	19.8±3.0	66.0±2.4	27.5±1.3	2.40± 0.02	75.3±5.2
IW+Pyr ( $10^{-14}$ M)	18.5±2.4	50.0±2.1	26.3±1.1	1.90±0.02	52.0±3.4



**Fig. 3.** The correlation between the unsaturation coefficient of C<sub>18</sub> fatty acids and the maximum rates of NAD-dependent substrates oxidation Y-axis shows the maximum rates of NAD-dependent substrates oxidation. X-axis shows the unsaturation coefficient of C<sub>18</sub> fatty acids





**Fig. 4. Effects of the insufficient watering (IW),melaphen (Mph) and pyraphen (Pyr) on the length of shoots and roots of 6-day-old pea seedling; (1) sprout (2) root**

Found stimulation of the growth of roots of seedlings under conditions of water deficiency is of great importance for adaptation to water deficit. The efficiency of melaphen in the protection of plants from water stress is somewhat higher than that of pyraphen, which may be associated with a more efficient protection of membranes with melaphen from lipid peroxidation.

#### 4. CONCLUSION

On the basis of the obtained data it is possible to suggest that tolerance to water stress is determined by the cell antioxidant system protecting unsaturated C<sub>18</sub> fatty acids and unsaturated very-long-chain fatty acids against modifications induced by the oxidative stress, e. g. by activation of free radical processes [21]. The unsaturated coefficient of C<sub>18</sub> fatty acids are closely correlated with the maximum oxidation rates of NAD-dependent substrates (Fig. 3), which primarily depend on the content of linoleic acid in the lipid fraction of the mitochondrial membranes. It should be borne in mind that the complex I of mitochondrial electron transport chain is main point of flux of reducing agents, generated in the mitochondrial matrix. Increasing activity of NAD-dependent dehydrogenase activates the energy processes in the cell, which

increases the resistance of the plant body to the changing environmental conditions. This is particularly important for germinating seeds, which are characterized by a low rate of oxidation of NAD-dependent substrates [35]. In this regard, we can assume that a slight protective effect of complex I mitochondrial respiratory chain with pyraphen obviously associated with low content of unsaturated fatty acids with 18 carbon atoms in the mitochondrial membranes of pea seedlings in condition of insufficient watering (IWPyr) (Table 1). Its protective effect is, evidently, accounted by the prevention of oxidation of fatty acids having 20 carbon atoms under conditions of water deficiency. It was shown that fatty acids having very long chain might participate in the adaptation of plant to stresses, in particular, to osmotic stress induced by salinity. The concentration of very long fatty acids in the vegetative organs of halophytes was 4–64% of total fatty acids and their main components were C<sub>20</sub>, C<sub>21</sub>, C<sub>22</sub>, and C<sub>23</sub> fatty acids ; each of them were included saturated, mono-, and di-unsaturated components, and also C<sub>24</sub>- and C<sub>25</sub> fatty acids [42].

The other PGR – melaphen reduces intensity of lipid peroxidation under conditions of insufficient watering to the control level. In the result of the LPO inhibition have preserved the pool of

unsaturated fatty acids having 18 and 20 carbon atoms at the initial (control) level; that prevents changes in the energetics of mitochondria caused by water deficiency. It should be noted that the maintenance of the high functional activity of mitochondria, which provide the metabolic processes by energy especially important for sprouting seeds, which are in need of the energy resources.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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